Competition in Trinidadian guppies, *Poecilia reticulata*: Effects of competitor-to-resource ratio, sex, resource type, and tempo of predation risk

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Abstract

The monopolization of key resources via intraspecific competition is essential for survival and achieving reproductive success. The best predictor of the patterns of competition for mates is likely the operational sex ratio (OSR), defined as the number of competitors of one sex to the number of ready-to-mate members of the opposite sex, which predicts an increase in intrasexual competition as the availability of mates decreases. OSR theory has been generalized to other resources (e.g. food) under the term competitor-to-resource ratio (CRR), defined as the ratio of individual competitors to the number of resource units available, which predicts the same relationship between competition and resource availability for any type of resource. However, selection pressures are likely to act differently on each sex. Due to their higher reproductive rates, males are usually the most competitive sex in terms of reproduction, whereas females tend to be more aggressive than males for food. In addition, the cost of predation forces prey individuals to trade-off fitness-related activities for antipredator behaviour. As imminent predation risk increases, individuals should invest more energy into avoiding predation at the cost of foraging and mating opportunities. This trade-off is shaped by ambient predation risk as suggested by the risk-allocation model. When imminent predation risk is low, individuals experienced with high ambient predation risk should perform more fitness-related activities to compensate for lost opportunities during period of higher imminent predation risk. Conversely, low ambient predation-risk individuals should respond to imminent risks with an ‘all-or-nothing’ response at the cost of short-term benefits.

These various factors influencing intraspecific competition have been studied, but in isolation from one another in most cases. The aim of this thesis was to explore the potential interactions of these factors in a single species using Trinidadian guppies. To test the above predictions, we observed wild-caught individuals from two populations (i.e. high versus low ambient predation pressures) under different CRRs and imminent risk (i.e. conspecific alarm cue) levels. Surprisingly, there was no difference in aggression rates between sexes overall, independent of the type of resource. The availability of mates had the largest effect on aggression rates with an increase in intrasexual competition as the number of mates decreased. Food was more defendable than mates in our experiment, likely because prospective mates are mobile and can be choosy about their sexual partners. Finally, due to the cost of predation, individuals exposed to high ambient predation risk tended to be less aggressive and more cooperative. This work highlights the complexity of competitive interactions in natural prey populations, and the various selective pressures affecting wild populations.

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Contribution of Authors

We wrote Chapters 1, 2, 3 and Appendix 3 as manuscripts for peer-reviewed publications. To avoid redundancy, Chapter 4 "Materials and Methods" section was simplified for the thesis, but a manuscript version will be written for peer-reviewed publication. PJC Chuard contributed to the conception, planning, collection of data, data analyses and production of all Chapters. GE Brown contributed to the conception, planning, collection of data and editing of all Chapters. JWA Grant contributed to the conception, planning and editing of all Chapters.

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Chuard PJC, Grant JWA, Brown GE The effects of adult sex ratio on mating competition under varying levels of imminent and ambient predation risk

Chuard PJC, Brown GE, Grant JWA Food competition in two populations of wild-caught Trinidadian guppies, *Poecilia reticulata*

Chuard PJC, Grant JWA, Brown GE Mating competition and the range of adult sex ratio in wild guppies

Chuard PJC, Brown GE, Grant JWA Predictors of dominance and foraging success in guppies

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General Introduction

Competition occurs when the use of limited resource reduces its availability to other individuals (Keddy 2001). Competition occurs between species (i.e. interspecific competition) and constitutes one of the main factors shaping communities, for example through competitive exclusion (Darwin 1859; Keddy 2001). Competition also occurs between individuals of the same species (i.e. intraspecific competition) and is the main driver of natural and sexual selection (e.g. Bolnick 2004; Pizzari et al. 2015). Competition can also be defined based on the type of behaviour used. Scramble competition occurs when all individuals have access to a limited resource and use it (van den Berg et al. 2006), potentially to a point when the resource becomes scarce, thus threatening the survival of all individuals (Nicholson 1954). Conversely, when a resource is defendable, individuals can perform contest competition (also called interference competition) through the use of aggression in which the winner of the contest increases its monopolization of the resource (Nicholson 1954), thus increasing the chance of survival of the winner. Competition is costly because individuals spend energy defending or scrambling for a resource instead of actually using it (Whitham 1986). For this reason, individuals optimize their behaviour so that the gains from the outcome of competition are greater than the costs associated with it (Ydenberg and Houston 1986). Animals can use intraspecific aggressive behaviour when competing for food (Magurran and Seghers 1991; Grant and Foam 2002; Kim et al. 2004; Warnock and Rasmussen 2013), mates (Jirtokul 1999b; Grant and Foam 2002; Forsgren et al. 2004; Weir et al. 2011) or territories (Rowland 1989; Chapman and Kramer 1996; Kleiber et al. 2007).

Competitor-to-resource ratio

The term competitor-to-resource ratio (CRR; Grant et al. 2000) was introduced as a measure to allow the comparison of patterns of competition for access to different resources (i.e. food, mates and territories) based on the operational sex ratio theory (OSR, Emlen and Oring 1977) that deals with mating competition. CRR is defined as the ratio of individual competitors to the number of resource units available, whereas OSR is defined as the number of competitors of one sex to the number of ready-to-mate members of the opposite sex. OSR theory predicts an increase in intrasexual competition (e.g. aggression) as OSR increases. The relationship between CRR and aggression rates is expected to be dome-shaped with individuals lowering their aggression rates as resource units (e.g. mates, food) become abundant or scarce per competitor (Fig. 1.1; Grant et al. 2000). When the resource is abundant for each competitor, there is no need to spend energy engaging in competition. However, if the resource is too scarce per competitor, the cost of aggression exceeds the potential gains which results in a decrease in aggression (Grant et al. 2000). Previous work seems to suggest a peak of contest behaviour at a CRR of 2 (Weir et al. 2011).

Predation risk

The non-consumptive effects of predation strongly shape the behaviour of potential prey organisms (Preisser et al. 2005). The non-consumptive effects of predation encompass all alteration of phenotypes adopted by prey to reduce predation risk (Preisser et al. 2005), for example through the use of antipredator behaviour (e.g. vigilance, fleeing, hiding) and habitat choice. In this study, ambient predation risk is defined as the background levels of risk under which prey individuals live within a local habitat, whereas imminent predation is the immediate predation risk perceived by prey individuals. Ambient predation risk can be quantified by the number and effectiveness of predators for a particular prey species or life stage (e.g. Croft et al. 2006). Individuals experiencing high ambient predation risk are not always facing high imminent predation risk as they can select habitats that lower the imminent risk of predation (e.g. Main et al. 1996). Conversely, individuals facing low ambient predation risk can still face high imminent predation risk, such as just prior to a predator attack. Even if predator attacks are rare and infrequent, the risk of predation perceived by the prey in case of such attack is imminent.

The risk of predation represents a cost for individuals engaged in competition. Indeed, a decrease in intraspecific aggression rates under high predation risk (Fig. 1.1), observed in many studies, is probably the result of a trade-off between conspicuously competing for the acquisition and/or defense of limited resources and vigilance towards potential predators (Huntingford 1982). Moreover, aggressive behaviour makes individuals more vulnerable to predation (Toscano et al. 2010). For instance, in populations experiencing high versus low ambient predation pressure, intraspecific aggression rates are lower when competing for food (Magurran and Seghers 1991; Herczeg and Valimaki 2011; Heinen et al. 2013), mates (Farr 1975; Huntingford 1982; Rodd and Sokolowski 1995; Haskins et al. 1997) and territories (Riechert 1993; Riechert and Hedrick 1993; Kemp and Wiklund 2001; Kemp et al. 2006). The same pattern of reduction in aggression occurs in the presence of an imminent predation risk when competing for food (Whitehouse 1997; Elkin and Baker 2000; Kim et al. 2004; Foam et al. 2005) mates (Kelly and Godin 2001; Zhenlong and Jike 2002; Oku and Yano 2008; Leese et al. 2010) and territories (Huntingford 1976; Martel and Dill 1993; Martel 1996). Conversely, the risk allocation model (Lima and Bednekoff 1999; Ferrari et al. 2009) predicts higher rates of behavioural activities (e.g. mating, foraging, fighting) when no imminent risk of predation is present in high compared to low ambient predation-risk populations. Based on the risk allocation hypothesis, prey should compensate for lost opportunities during risky periods by increasing fitness-related behaviour during infrequent 'safe' periods (Lima and Bednekoff 1999; Ferrari et al. 2009).

Sex differences

Males and females often show different intraspecific aggression patterns when competing for mates (e.g. Grant and Foam 2002). Anisogamy, the production of larger gametes by females compared to males, leads to a greater pre-natal investment in reproduction, and lower reproductive rates of females compared to males (Trivers 1972). The higher reproductive rate of males versus females means that females are typically the limiting sex, which leads to higher rates of male-male than female-female competition (Clutton-Brock and Parker 1992). Conversely, female-female aggression is more likely to occur when individuals are competing for food (Nummelin 1988), because body size is often more strongly related to reproductive success in females than in males (Charnov 1993). In many taxa, males choose females based on their body size as an honest signal of fertility (Carpenter 1995; Wootton 1998; Bonduriansky 2001). Based on the asset-protection principle (Clark 1994), the more an individual accumulates reproductive assets, the more it should invest in antipredator behaviour (i.e. adopt risk averse tactics). For instance, male European rollers, *Coracias garrulous*, increase their provisioning rates to nestlings under imminent predation risk, whereas females do not (Exposito-Granados et al. 2016). This difference between males and females is likely due to the accumulation of assets in the form of body mass in females that they will be able to convert into eggs in the future. Increasing provisioning rate to their nestlings under imminent predation risk would decrease females' survival rate, and thus compromise their future reproductive success. However, the high reproductive rates of males (Clutton-Brock and Parker 1992), associated with the production of "cheaper" gametes (Charnov 1982; but see Nakatsuru and Kramer 1982), make them prone to choose continuous mating over antipredator behaviour in order to maximize their reproductive success (Heinen et al. 2013). Predation risk can also affect males and females differently, especially in strongly dimorphic species. Indeed, colourful phenotypes might make individuals more vulnerable to predation (Reichard et al. 2014), and large body sizes might be preferred by predators (Stephens and Krebs 1986) or exceed their limited gapes (Lawrence 1957; Hartman 1958).

General goals

To our knowledge, the patterns of competition for different resources (i.e. mates vs. food) that vary in abundance (i.e. CRR) by both sexes under various levels of both ambient and imminent predation risk remains largely unknown. Most studies in behavioural ecology tend to focus on only one aspect of these issues: competition for food (e.g. Kim et al. 2004) or females (e.g. Jirotkul 1999b), aggression rates under ambient predation (e.g. Magurran and Seghers 1991) or under imminent risk (e.g. Kelly and Godin 2001). Exploring how these various factors interact together to shape intraspecific competitive patterns will improve our understanding of selective pressures in wild prey populations. In this project, we will examine how the nature of the resource (food vs. mates), the number of competitors per resource unit, predation risk, and gender interact to influence intraspecific competitive patterns in two populations of the Trinidadian guppy.

The thesis will be divided into four interrelated chapters to investigate these interactions using the Trinidadian guppy as a model system. Guppies are a well-studied tropical freshwater fish species, which is arguably one of the best model species for field-based studies of evolutionary and behavioural ecology. Their advantages include their small size, abundance, short generation span, ease of breeding in the laboratory, and their number of genetically and environmentally distinct populations over a relatively small geographic area (Amundsen 2003). Guppies are characterized by internal fertilization, viviparity and sexual dimorphism with small, colourful males that do not grow after maturation and large, dull females that keep growing once they are mature (Magurran 2005). Males actively secure copulations by either courting or forcing females to mate (Baerends et al. 1955; Magurran 2005). This species is characterized by a resource-free mating system where polyandry is common (Barbosa et al. 2010). Females will usually reproduce with several males for a given fertilization event. Some of the abovementioned characteristics of guppies are specific to the family Poeciliidae, or even to the species, thus limiting some of the interpretations of our study to species-specific patterns, rather than generalized patterns of prey populations.

Hypothesis statements

In this thesis, we tested predictions of intraspecific competition (Table 1.1) under the effects of CRR with both mates (Chapter 1, 2, 4) and food (Chapter 3) as a resource, high vs. low ambient predation risk (all Chapters), increasing imminent risk of predation (Chapter 2), and sex role (all Chapters). Based on OSR theory, as CRR increases, we predicted that (1) intrasexual aggression rates will increase in both sexes, (2) at the expense of per-capita male mating tactic rates. In populations experiencing high vs. low ambient predation risk, (3) aggression rate, (4) male mating tactic rates, and (5) foraging rates will be lower if the cost of ambient predation risk is high (i.e. risky-competition hypothesis) or higher if the absence of an actual predator represents a 'safe' period (i.e. risk-allocation model, Lima and Bednekoff 1999). As imminent predation risk increases, (6) mating and foraging rates will decrease in favor of antipredator behaviour due to the cost of predation. Finally, as guppies show conventional sex roles (Houde 2001), (7) males will be more aggressive than females in a mating context, and (8) females will be more aggressive than males in a foraging context, (9) as well as more threat-sensitive.

Table 1.1. Predictions of the effects of competitor-to-resource ratio, ambient and imminent predation risk, and sex role on competitive patterns

Explanatory variables	Predictions for competitors	Chapters
As CRR increases	(1) Intrasexual aggression rate increases in both sexes	All
	(2) Per-capita mating tactic rates of males decrease	1, 2, 4
High vs. low ambient predation-risk population	(3) Aggression rate is lower or higher[a]	All
	(4) Male mating tactic rates are lower or higher[a]	1, 2, 4
	(5) Foraging rate is lower or higher[a]	2, 3
As imminent predation risk increases	(6) Mating and foraging rates decrease in favour of antipredator behaviour	2
Sex roles	(7) Males are more aggressive than females in a mating context	1, 2, 4
	(8) Females are more aggressive than males in a foraging context	3
	(9) Females are more threat-sensitive than males	2

[a] Behaviour rate predicted to decrease if the cost of ambient predation risk is high OR increase if the absence of an actual predator signals a ‘safe’ period, as in Lima and Bednekoff’s (1999) risk allocation model.

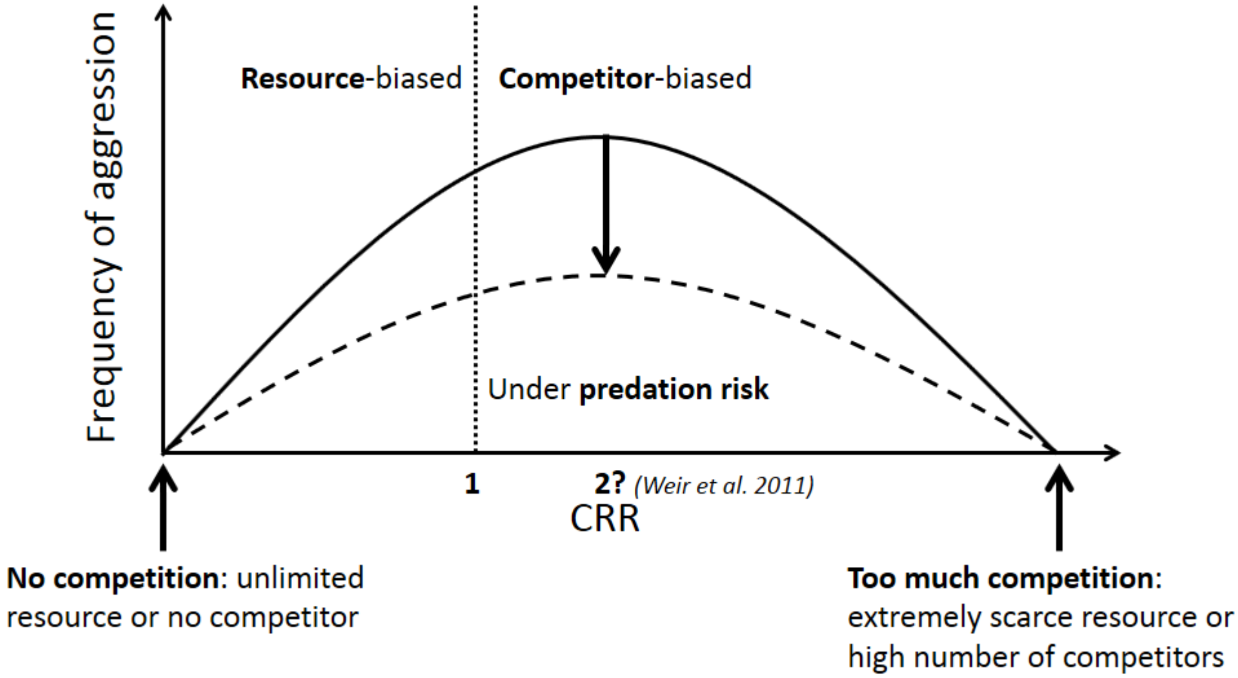


Fig. 1.1. Graphical predictions of the frequency of aggression as a function of competitor-to-resource ratio, with and without predation risk

Chapter 1: The effects of adult sex ratio on mating competition in male and female guppies (*Poecilia reticulata*) in two wild populations

Introduction

Males typically engage in more courtship and competition for mates than females, whereas the former are choosier about their mating partners (Darwin 1871; Bateman 1948; Andersson 1994). These conventional sex roles (sensu Vincent 1994; Forsgren et al. 2004) presumably occur because of the lower potential reproductive rate of females relative to males. As a consequence, females are generally the limiting sex regarding reproduction (Clutton-Brock and Parker 1992), resulting in a male-biased operational sex ratio (OSR, Emlen and Oring 1977), defined here as the number of competitors of one sex/number of ready-to-mate members of the opposite sex (Grant et al. 2000; de Jong et al. 2012). This male-biased OSR leads to more male-male rather than female-female competition over mates.

We are aware of only a few studies that have considered the competitive behaviour of both sexes simultaneously (Kvarnemo et al. 1995; Debuse et al. 1999; Grant and Foam 2002). While males may indeed be the most competitive sex in general, female-female competition in the form of aggression occurs at most OSRs, and females become the most competitive sex when OSR is highly female-biased in several fish species (Grant and Foam 2002; Forsgren et al. 2004; Clark and Grant 2010) and in the European lobster *Homarus gammarus* (Debuse et al. 1999). Furthermore, female-biased OSRs occur in nature (Colwell and Oring 1988; Jirotkul 1999a; Forsgren et al. 2004). Therefore, the focus on describing the patterns of competition in males may have obscured the subtler and less frequent patterns of competition among females. If competition among females for males is more frequent than what has been shown in the literature, then the theoretical predictions of OSR theory can be applied to both sexes.

OSR is the best predictor of the form and frequency of male sexual behaviour (Emlen and Oring 1977; Weir et al. 2011; but see Klug et al. 2010 and Jennions et al. 2012). OSR theory predicts that intrasexual aggression in males, defined as aggression directed towards same-sex individuals, increases as OSR becomes male-biased (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). The same predictions hold for females as OSR becomes female-biased. Alternatively, Weir et al. (2011) suggest that competitive behaviour will decrease in one sex when the OSR becomes highly biased toward that sex (i.e. OSR > ~2) as competition becomes uneconomical due to the low availability of mates compared to the number of competitors. While many studies support the general predictions on aggression rates (Kvarnemo et al. 1995; Grant et al. 2000; Weir et al. 2011), others have investigated the effects of OSR on alternative mating tactics that are used by males. For example, males often switch from energetically costly fighting (and courtship, see below) to less energetically demanding sneaking tactics (e.g. forced mating) when the OSR becomes extremely male biased (Grant et al. 1995; Verrell and Krenz 1998; Kanoh 2000; Mills and Reynolds 2003).While studies of the effects of OSR on the direct competition between males have consistently shown that male aggression increases as the OSR becomes male-biased (Weir et al. 2011), it is more difficult to generalise about male courtship behaviour, a form of indirect competition. OSR theory predicts that male courtship rate will increase as OSR becomes more male-biased (Kvarnemo and Ahnesjö 1996), but most evidence shows the opposite trend (Verrell and Krenz 1998; Weir et al. 2011). Indeed, as OSR becomes more male-biased, the opportunity for male courtship decreases as the relative abundance of females decreases (Jirotkul 1999a). However, when courtship rate is corrected for encounter rate with females, the propensity to court females (i.e. the proportion of encounters with a female during which a male performed a courtship display, sensu de Jong et al. 2010) increases as OSR becomes more male-biased. Regarding alternative mating tactics, most studies suggest that their relative use increases with OSR (Grant et al. 1995; Mills and Reynolds 2003). In guppies, on the other hand, forced mating rates per male, an alternative mating tactic, typically decrease as OSR increases due to fewer encounters with females, combined with the use of interference as a way to interrupt another male's courtship attempt (Jirotkul 1999a). To our knowledge, the effect of OSR on alternative mating tactic propensity remains to be investigated.

Reproductive behaviour is also shaped by the non-consumptive effects of predation (Preisser et al. 2005). While recent studies have focused on the effect of imminent predation risk (i.e. visual or chemical detection of an acute predation risk) on mating behaviour (Cordes et al. 2014; Franklin et al. 2014; Michelangeli and Wong 2014), to our knowledge, cross-population comparisons of the effects of ambient predation risk (i.e. background levels of risk experienced within a local habitat) are rare (Farr 1975; Rodd and Sokolowski 1995; Haskins et al. 1997), despite their relevance to the study of local adaptation. Courtship and aggression can increase vulnerability to predation (Zuk and Kolluru 1998; Toscano et al. 2010), forcing prey to make trade-offs (e.g. Farr 1975; Riechert 1993; Heinen et al. 2013). Thus, the costs of competition are likely greater under conditions of high ambient predation (i.e. risky competition hypothesis), potentially leading to lower rates of intraspecific competition, courtship and mating behaviour relative to low ambient predation risk conditions (e.g. Huntingford 1982; Haskins et al. 1997; Heinen et al. 2013). However, the risk allocation hypothesis (Lima and Bednekoff 1999; Ferrari et al. 2009) predicts a higher rate of fitness-related activities (e.g. mating, foraging) in the absence of an imminent risk of predation (i.e. safe periods as per Lima and Bednekoff 1999) in populations experiencing high versus low ambient predation risk. According to the risk allocation hypothesis, prey may be able to compensate for reduced activity during risky periods by engaging in compensatory behaviour during rare 'safe' periods (Lima and Bednekoff 1999; Ferrari et al. 2009). For instance, in the absence of an imminent risk, male Trinidadian guppies from high predation-risk sites engaged in more forced mating attempts than their low predation-risk counterparts (Magurran and Seghers 1994; see also Baird and Baird 2006). Thus, in the absence of an imminent predation threat, we might expect an increase in competitive interactions among guppies from a high predation population.

To our knowledge, the interacting effects of OSR and ambient predation risk on mating competition in both sexes remains largely unknown. While high ambient predation risk is likely to have a direct effect on the intensity of sexual selection via decreasing the intensity of intrasexual competition, OSR is still expected to shape the patterns of mating competition. However, the costs of strong ambient predation risk might inhibit the effect of OSR on mating competition. Conversely, if the absence of an imminent predation risk represents a 'safe' period to mate, as suggested by the risk allocation hypothesis (Lima and Bednekoff 1999), then a high ambient predation risk may enhance the effect of OSR on mating competition. Evidence of either result would suggest important differences in the strength of sexual selection between populations differing in their ambient risk of predation.

Ever since Emlen and Oring (1977), mating systems theory has focussed on OSR rather than the adult sex ratio (ASR; Kokko and Jennions 2008). ASR is an important component of OSR (Reynolds 1996), and both variables are often manipulated simultaneously in experimental tests of OSR theory (Kokko and Jennions 2008). For example, OSR is typically manipulated via the ASR, assuming that all adults are ready to mate (for examples, see Weir et al. 2011). Hence, ASR can be used as a proxy for OSR (e.g. O'Rourke and Mendelson 2013) when testing mating systems theory, because the directional predictions of OSR theory hold for both OSR and ASR

(Kokko and Jennions 2008; Weir et al. 2011). Because we manipulated ASR in our experiments, we will use ASR rather than OSR hereafter.

Here, we test for the combined effects of ambient predation risk and ASR on mating competition (i.e. intrasexual aggression rates, courtship and alternative mating tactics) in both sexes using wild-caught Trinidadian guppies from two populations (high vs. low ambient predation-risk levels) to explore the following predictions (Table 2.1). As sexually mature female guppies can store sperm for up to 8 months, even when gravid (Constantz 1984), they should show sexual receptivity at all times. This fact supports our use of ASR as a proxy for OSR in our study. We tested the predictions that both (1) males and (2) females will increase their rate of intrasexual aggression as ASR becomes biased toward males or females for each prediction respectively following OSR theory.As ASR becomes more male-biased, (3) male courtship rate will decrease as females become relatively scarce, but (4) male courtship propensity will increase due to competition. As observed in previous studies due to fewer encounters with females (Jirotkul 1999a), (5) the rate of male forced mating (sneaking) will decrease as ASR becomes more male-biased and (6) forced mating propensity will increase along with, but at a faster rate than, courtship propensity. As a consequence of the low availability of mates compared to the number of competitors at high ASRs, aggression rates and mating tactic propensity will decrease at an ASR greater than 2 (Weir et al. 2011). Because courtship behaviour and aggression for access to mates likely increase the risk of predation, individuals will exhibit (7) lower rates of intrasexual aggression and (8) courtship/mating behaviour if they are from high vs. low ambient predation risk populations. Alternatively, in the absence of imminent predation threats, risk allocation theory predicts an increase in aggression and courtship/mating behaviour in populations experiencing high vs. low ambient predation risks (Lima and Bednekoff 1999). As guppies have conventional sex roles (Houde 2001), (9) intrasexual aggression will be greater in males than in females.

Materials and Methods

Study Populations and Fish Collection

To mimic conditions in the wild, where ready-to-mate females are rare (Liley 1966), we used wild-caught females in our experiments, which were both gravid and non-gravid. As a result, we manipulated adult sex ratio (ASR; defined here as the number of adult members of the competing sex/the number of adult members of the opposite sex) in our experiments rather than OSR (see "Materials and Methods: Mating competition"). We collected wild guppies from the Lower and Upper Aripo Rivers, Northern Range Mountains, Republic of Trinidad and Tobago between April 29th and May 30th 2012 for the main experiment (see "Materials and Methods: Mating competition") and on April 22nd 2013 for the addition-of-a-mate experiment (see "Materials and Methods: Competition with and without a mate"). We used seine nets to catch fish and directly placed them in 30-L buckets filled with 10 L of river water. Each bucket contained approximately 30-40 individuals. The duration of the drive from the rivers to the laboratory facilities at the Saint Augustine campus of the University of the West Indies, Republic of Trinidad and Tobago, is approximately 45 min. The Lower Aripo is characterised as a high-predation site (Croft et al. 2006), containing several species that actively prey upon juvenile and adult guppies. Common predators at this site include blue acara cichlids, *Andinoacara pulcher*, black acara cichlids, *Cichlasoma bimaculatum*, and pike cichlids, *Crenicichla sp.*, all of which forage on adult guppies (Croft et al. 2006; Botham et al. 2008; Brown et al. 2009). Conversely, the Upper Aripo is characterised as a low-predation site (Croft et al. 2006; Botham et al. 2008), with fewer predatory species: Hart's rivulus, *Anablepsoides hartii*, which prey primarily on newly-emerged and juveniles but also on small male guppies; and, a freshwater prawn *Macrobrachium crenulatum* (Endler and Houde 1995; Walsh and Reznick 2009). While there are other important differences between these populations, such as higher densities and lower productivity at upstream low-predation sites (Grether et al. 2001), for now, we will refer to these population differences as high versus low ambient predation risk.

We held males and females in separate aerated 100-L aquaria at ~24°C in the laboratory under natural day-light conditions for a maximum of 32 days. We used two holding tanks for each sex. To minimize food-related competition during trials, we fed the fish to satiation, twice daily, with commercial flake food (TetraMin™ provided by Tetra, 3001 Commerce St., Blacksburg, VA) and brine shrimp. We removed the remaining flake residues from the previous feeding period right before providing guppies with more food to avoid bacterial infections. We tested individuals 15 min to 8 h after adding food to their holding tank, with a median of 2 h. We gave the fish at least 24 h before testing to acclimate to laboratory conditions and to promote mating competition as males and females were kept separate from each other. We tested most individuals after a median of 3 days (range = 1 to 7 days). Upon completion of the study, we returned the guppies to their original populations using the same buckets and at the same densities as the collection procedure (see above). Individuals were released into their original rivers using hand nets.

Experimental Protocol

Mating competition

We performed trials in aquaria (45 x 30 x 30 cm) with 6 fish per tank at 5 different ASRs (competitors: mates): 1:5, 2:4, 3:3, 4:2, 5:1, following Jirotkul (1999a) who tested OSR instead by using only virgin females. We used a two-way factorial design that included two populations (Lower vs. Upper Aripo) x 5 ASRs, with 10 replicates per treatment combination. We used each individual fish only once, for a total of 600 individuals.

Trials consisted of 25-min observation periods performed by a single observer (PJC Chuard). We placed males and females from the same unisex holding tank respectively into test tanks, at the required ASR, 1 to 2 hours before testing. Social dominance can be accurately measured after 10 min between two unfamiliar male guppies (Kodric-Brown 1992), hence we expected the social hierarchies within our experimental tanks to have stabilised before observations began because: 1) the fish had at least one hour to acclimate within the test tank; and 2) the fish were somewhat familiar with one another given that they were held in same-sex holding tanks for at least 24h before a trial. In addition, randomisation of trials insured no bias in acclimation time between treatments. The observer recorded behaviour from one side (45 x 30 cm) of the tank while other sides were covered on the outside with white plastic sheets to minimise disturbance. Ten minutes prior to the onset of observations, we dispersed commercial flake food evenly over the surface of the water to satiate all fish without creating defendable food patches (Robb and Grant 1998), minimising food-related aggression during trials. We differentiated males from females by their conspicuous colour patterns and presence of a gonopodium. We quantified aggressive behaviour to measure mating competition (Gorlick 1976; Bruce and White 1995) because the high-energetic costs of aggression make the fish prone to trade-offs with other activities. Additionally, aggression attracts the attention of predators (Toscano et al. 2010). We calculated the per capita rate of intrasexual aggressive acts for individuals of both sexes by adding the frequency of pushing (Magurran and Seghers 1991), tail beating (Liley 1966), biting, chasing, and lateral displaying (Gorlick 1976), which was divided by the number of competitors (i.e. 2 to 5). These aggressive acts are performed by both sexes at similar rates in mixed-sex guppy groups (Gorlick 1976). We recorded two mating tactics for males: sigmoid display and sneaking, which is the most conservative way to measure mating tactics in guppies (Magurran 2005). Previous studies have quantified interference, the interruption of the courtship behaviour of one male by another by either aggressively interacting with the courting male before courting the same female, or disrupting the courtship behaviour of the other (Jirotkul 1999a). Instead, we scored interference as an agonistic behaviour followed by a sigmoid display, or just as a sigmoid display if no agonistic interaction was observed first. Sigmoid display is a courtship behaviour, where males bend their body in a sigmoid shape toward a female while moving up and down and displaying their color pattern (Houde 1987). Sneaking is a form of forced mating without initial courting, when male guppies attempt to insert their gonopodium (modified anal fin) into the female's gonopore (urogenital opening) (Farr 1980a). The total number of each mating tactic observed during a trial was divided by the number of males present (i.e. 1 to 5) to calculate the per capita rate. In the case of courtship and forced mating propensity, we corrected for the availability of females by dividing the respective rates per male by the number of females present in a trial (i.e. 1 to 5). Because we scanned the tank continuously during each observation and recorded the various behaviour patterns that occurred, we could not quantify the number of encounters between males and females, needed to measure propensity (de Jong et al. 2012). However, because all individuals could potentially see each other at all times in our trials, our method of correcting for female availability should be valid (de Jong et al. 2012). The scanning method allowed the observer to record virtually all behaviour of interest that occurred in the tank; the only exceptions were those occurring during the 1-s intervals when behavioural acts were being recorded on the data sheet. Given the low frequency of occurrence of behaviour (see Figs. 2), it is unlikely that we missed much. We performed water changes after each trial and randomised the sequence of trials across all treatment combinations of ASRs and populations.

Competition with and without a mate

As a small amount of food was present in the tank during observations, even though individuals were satiated before trials, we performed an additional set of trials to distinguish between competition with and without the presence of mates. We assumed that aggressive behaviour in the absence of mates might represent competition for food present in the tanks or for general dominance status. In the same tanks that were used for the main experiment, we used the most extreme ASR (5:1, competitors/mates) treatment to promote intense competition (Jirotkul 1999a; 1999b). Trials consisted of two 5-min observation periods, separated by a 3-min interval (see below). Five individuals of one sex from the same holding tank were observed for the first period, and then one individual of the opposite sex was added for the second period. An increase in aggression rate between the first and second period would suggest active competition for access to mates. Or, six individuals were first observed (5 of one sex from the same holding tank and one of the opposite sex), and then the individual of the opposite sex was removed before the second observation period. Removing an individual was used as a control for the addition-of-an-individual experiment. To control for the disturbance caused by removing or adding an individual, a disturbance was applied 3 min before the first observation period by dipping a hand net in the tank for a few seconds. The same acclimation and feeding methods as the main experiment were used. Intrasexual aggressive behaviour was recorded by scanning as in the main experiment, but over a 5- rather than a 25-min period. Both populations were tested separately, and the sequence of trials was randomised. We had a total of 8 treatments (2 sexes x 2 populations x 2 orders) with 10 replicates each, requiring 480 wild individuals.

Statistical Approach

For the main experiment, we measured intrasexual aggression rates in both sexes simultaneously and we calculated an average per individual per sex. We used a generalised linear mixed model (GLMM) with trial number as a random factor and sex, population of origin and ASR as fixed factors. If an interaction involving ASR, sex and population of origin was significant (see “Results”), we performed additional GLM analyses by sex and population to disentangle the effect of ASR on intrasexual aggression rates. Then, we used generalised linear models (GLM) for analyses of each of the four remaining variables measured in males (i.e. courtship rates, courtship propensity, forced mating rates and forced mating propensity). The negative binomial distribution fitted our data best and accounted for most of the over-dispersion (theta values’ mean±SE = 2.71±0.81, N=12). We performed GLM analyses to test for the effect of ASR and population as factors and their interactions on intrasexual aggression for each sex separately and male mating tactic rates and propensities. As ASR had more than two levels, we used planned contrasts to test for our predicted decreases or increases in behavioural rates with ASR (i.e. linear contrast), as well as our dome-shaped relationship predictions (i.e. quadratic contrast). To compare both sexes in terms of intrasexual aggression, we also added sex as a factor in the GLMM along with ASR and population. All analyses were univariate except when testing the prediction on male mating tactic rates between populations (see Table 2.1); for these we included sigmoid and sneaking rates as separate response variables in a single GLM multivariate analysis. The statistics associated with GLM and GLMM were regression coefficient estimates and their associated 95% confidence intervals, and z scores that were respectively reported as: (β, [95% CI], z score, probability). When an interaction between factors was significant (see "Results"), we analysed populations, sexes, or both separately.

For the with-or-without-a-mate competition experiment, we used square root transformed data, which met the assumptions for parametric tests. We performed a repeated-measures ANOVA to test for the effect of the presence of a mate, adding vs. removing a mate, sex and population and their interactions on intrasexual aggression. In the case of a significant interaction including adding or removing a mate and presence of a mate (see "Results"), we analysed addition and removal trials separately.

Each subset of data was based on strong a priori predictions and no multiple-testing was performed on those subsets, thus no statistical correction was applied. Moran (2003) argues that using statistical corrections in ecological studies is more damaging (i.e. failure to reject a false null hypothesis) than beneficial, and that emphasis should be put on biological interpretation and study design, rather than p-values. For each test, we only reported interactive effects when significant. We used the *glm.nb()* function of the *MASS* package (Venables and Ripley 2002) for GLM, the *glmmadmb()* function of the *glmmADMB* package (Fournier et al. 2012) for GLMM, and the *contrast()* function of the *stats* package (Chambers and Hastie 1992) for the planned contrasts in R (3.1.2; R Development Core Team 2015).

Results

Our comparison of intrasexual aggression rates between males and females revealed a 3-way interaction between linear-contrast ASR, population and sex (GLMM: β=-1.96, CI=[-3.78, -

0.13], *z*=-2.01, *P*=0.035). As a result, we analysed each sex by population separately using four different models (i.e. 2 populations x 2 sexes). Consistent with our first and second predictions, the rate of intrasexual aggression increased as ASR increased (Fig. 2.1) in Upper Aripo (low predation) males (GLM: *β*=1.66, CI=[0.77, 2.58], *z*=3.64, *P*<0.001) and in both populations for females (Lower Aripo (high predation) GLM: *β*=2.05, CI=[1.18, 3.30], *z*=3.98, *P*<0.001; Upper Aripo (low predation) GLM: *β*=0.93, CI=[0.22, 1.67], *z*=2.54, *P*=0.011). However, in Lower Aripo (high predation) males, intrasexual aggression rates were not significantly affected by ASR, but the trend was in the predicted direction (Fig. 2.1a; GLM: *β*=0.67, CI=[-0.026, 1.40], *z*=1.84, *P*=0.065). We found no evidence of a decrease in aggression rates at an ASR greater than 2 (Lower Aripo (high predation) GLM: *β*=-0.063, CI=[-0.78, 0.66], *z*=-0.17, *P*=0.86; Upper Aripo (low predation) GLM: *β*=-0.11, CI=[-1.01, 0.78], *z*=-0.25, *P*=0.80), nor in females (Lower Aripo (high predation) GLM: *β*=-0.099, CI=[1.18, 3.30], *z*=-0.22, *P*=0.83; Upper Aripo (low predation) GLM: *β*=-0.61, CI=[-1.30, 0.066], *z*=-1.76, *P*=0.078).

In accordance with the third prediction, the rate of courtship per male decreased as ASR increased (Fig. 2.2a; GLM: *β*=-1.46, CI=[-2.40, -0.53], *z*=-3.12, *P*=0.0018). However, contrary to the fourth prediction, when we corrected for female availability, courtship propensity did not change with ASR (Fig. 2.2b; linear contrast GLM: *β*=-0.23, CI=[-1.04, 0.58], *z*=-0.55, *P*=0.58; quadratic contrast GLM: *β*=-0.57, CI=[-1.36, 0.22], *z*=-1.42, *P*=0.15). Consistent with the fifth, the rate of sneaking per male decreased with ASR (Fig. 2.3a; GLM: *β*=-1.16, CI=[-1.60, -0.72], *z*=-5.15, *P*<0.001). Additionally, sneaking propensity increased, but only at male-biased ASRs (Fig. 2.3b; quadratic contrast GLM: *β*=0.46, CI=[0.022, 0.90], *z*=2.05, *P*=0.040), and not overall (linear contrast GLM: *β*=0.30, CI=[-0.13, 0.73], *z*=1.38, *P*=0.17), whereas courtship propensity remained unaffected (see above). The significant effect of the quadratic contrast of ASR on sneaking propensity was opposite to the prediction that sneaking propensity would decrease at an ASR greater than 2.

The rate of intrasexual aggression was higher in Upper Aripo (low predation) than Lower Aripo (high predation) individuals (Fig. 2.1; GLMM: *β*=0.77, CI=[0.19, 1.35], *z*=2.59, *P*=0.0095). This result was consistent with the seventh prediction based on the risky competition hypothesis: the cost associated with aggression is higher under elevated ambient predation risk. Contrary to the eighth prediction, courtship (Fig. 2.2a) and sneaking rates per male (Fig. 2.3a) did not differ significantly between populations (GLM: *β*=-0.27, CI=[-0.57, 0.037], *z*=-1.73,

P=0.085). If anything, courtship rate tended to be higher in the Lower Aripo population. Finally, our ninth prediction was not supported as aggression rates between males were not significantly higher than in females (Fig. 2.1; GLMM: β=-0.21, CI=[-0.86, 0.45], z=-0.61, P=0.54).

For the with-or-without-a-mate competition experiment, the interaction between presence of a mate and sequence (addition vs. removal of a mate) significantly affected intrasexual aggression rates (Fig. 2.4; repeated measures ANOVA: $F_{1,80}$=10.09, P=0.0018), so we analysed the addition and removal data separately. Indeed, when an individual of the opposite sex was added, intrasexual aggression significantly increased (Fig. 2.4a; repeated measures ANOVA: $F_{1,40}$=6.88, P=0.0057), whereas there was no significant difference in aggression rates when the individual of the opposite sex was removed (Fig. 2.4b; repeated measures ANOVA: $F_{1,40}$=3.21, P=0.078). In addition, sex had a significant overall effect on intrasexual aggression rates (repeated measures ANOVA: $F_{1,80}$=10.36, P=0.0016). Contrary to our ninth prediction, females were significantly more aggressive than males in the with-or-without-a-mate experiment (Fig. 2.4). Moreover, as expected from the first analysis (see above) and our seventh prediction under the risky competition hypothesis, individuals from the Upper Aripo population (low predation) had higher rates of intrasexual agonistic behaviour overall than individuals from the Lower Aripo (high predation) population (Fig. 2.4; repeated measures ANOVA: $F_{1,80}$=51.37, P<0.001).

Discussion

Summary

Overall, we found strong support for six of the nine predictions (Table 2.1). Notably, our results suggested that as ASR increased, aggression rates in both males and females also increased, with the exception of Lower Aripo males. Moreover, courtship and sneaking rates among males decreased as ASR increased, whereas courtship propensity did not change and sneaking propensity increased at male-biased ASRs. Our results also suggested that elevated levels of ambient predation risk resulted in lower rates of aggression in both males and females, but had no effect on male mating patterns. Our most surprising result, however, was related to sex roles in the guppy: males were not more aggressive than females when competing for mates; if anything, the with-and-without-mate experiment indicated the reverse at an extremely high ASR. Taken together, our results suggested that while local ASR strongly shaped mating competition of both male and female guppies, ambient predation risk elicited weaker effects.

Adult Sex Ratio

Despite our use of potentially gravid females, our results on male-male competition were remarkably similar to those of Jirotkul (1999a). In both studies, direct intrasexual competitive behaviours in males increased with sex ratio. However, the range of ASRs in the wild is likely narrower and female-biased compared to our experiment. ASRs for males range between 0.43 and 1.22 in the Upper Aripo, and between 0.18 and 0.54 in the Lower Aripo (Pettersson et al. 2004). Surprisingly, intrasexual aggression rates of Lower Aripo males were not affected by ASR and those rates did not decrease at ASRs greater than 2 in either sex, when aggression is thought to become uneconomical (Weir et al. 2011). The rates of intrasexual aggression in our study were similar to previous findings (e.g. Magurran and Seghers 1991; Kelly and Godin 2001), but were still low compared to other well-studied species (e.g. *Poeciliopsis occidentalis*: Constantz 1975; *Gambusia affinis*: Hughes 1985; *Gobiusculus flavescens*: Forsgren et al. 2004; *Oryzias latipes*: Clark and Grant 2010; *Junco hyemalis*: Cain and Ketterson 2012). The low level of aggression in male guppies may be related to their use of alternative mating tactics (Farr 1980a); they tend to interfere with courting males to increase their mating success, rather than mate guarding (Jirotkul 1999a). Hence, the rates of aggression we observed for both sexes may have been so low that they were economical at any ASR; the one exception to this may be for Lower Aripo males exposed to high predation risk, which were able to use forced mating over intrasexual aggression to access mates. Alternatively, the female-biased ASRs of the Lower Aripo population (Pettersson et al. 2004) might have selected for less aggressive phenotypes in males due to the lower intensity of male-male competition.

More importantly, we demonstrated an increase in female-female aggression with increasing ASR, similar to the well-documented trend in male-male aggression. Though there is less evidence regarding such trends in females (Grant and Foam 2002), they are consistent with OSR theory. Indeed, even among species with conventional sex roles, a decrease in the relative abundance of mates should increase the competitive behaviour in the opposite sex (Emlen and Oring 1977). In the case of guppies, even if males can rapidly inseminate multiple females (Magurran and Seghers 1994), males still express mate choice (Abrahams 1993); thus the number of received inseminations varies among females. As a result, the chance of being inseminated decreases as the ASR becomes female-biased. Female guppies shoal together (Williams 1964) and appear to compete for the highest quality males by chasing away other females. There is always a potential advantage for females to compete for the "best" male as the last male to inseminate a female is likely to father most offspring (Evans and Magurran 2001).

Consistent with the general pattern (Weir et al. 2011), but counter to the prediction of OSR theory, we observed a decrease in the courtship rate per male as ASR increased. This result may reflect a trade-off between investing time/energy for courtship versus aggression to access mates. Alternatively, males might be less likely to use courtship as they are more likely to be interrupted (Jirotkul 1999a). At low ASRs, male intrasexual aggression was low, perhaps because encounters with other males were infrequent while the opportunities to court females were abundant. However, we also found no evidence of an increasing or dome-shaped courtship propensity with ASR (see de Jong et al. 2012). Upper Aripo males may have been increasing their rate of agonistic behaviour as ASR increased as a response to an increase in competition in order to achieve a constant courtship rate whenever they were in close proximity with a female. By contrast, Lower Aripo males may have kept their aggression rates low due to the cost of predation. In addition, and as expected, per capita forced mating rates decreased as ASR increased, likely due to fewer encounters with females at male-biased ASRs (Jirotkul 1999a). In terms of propensity, the use of sneaking provided males with an alternative tactic to courtship to acquire mates (Grant et al. 1995; Mills and Reynolds 2003). As ASR becomes male-biased and risk of being interrupted is high, males seem to favour lower-cost mating tactics over high-cost courtship (de Jong et al. 2009), hence no apparent decrease in sneaking propensity at an ASR greater than 2. In support of this hypothesis, species that do not apparently use alternative mating tactics show an increase in courtship propensity with ASR (threespine sticklebacks, *Gasterosteus aculeatus*: Ward and Fitzgerald 1988; two-spotted gobies, *Globiusculus flavescens*: Forsgren et al. 2004; but see de Jong et al. 2009).

Ambient Predation-Risk Population Differences

Our results suggested that individuals experiencing high ambient predation (Lower Aripo River) in the wild are less aggressive than those from low ambient-predation populations (Upper Aripo River). This trend is consistent with the 'risky competition' hypothesis, which posits that competition under high predation risk is costly. Similar results have been demonstrated in the context of foraging competition among Trinidadian guppies, likely due to the cost of aggression when shoaling (Magurran and Seghers 1991). Presumably, under high ambient predation risk, the costs of competitive aggression would be relatively high, even in the absence of an immediate predation threat. Such non-consumptive effects (Preisser et al. 2005) may lead individuals under high ambient predation risk to trade-off potential benefits associated with competing for limiting resources in favour of increased predator avoidance (Dugatkin and Alfieri 1992). For example, threespine sticklebacks reduce their rates of aggression (Huntingford 1982) allowing them to allocate more energy to foraging and/or reproduction. Threat-sensitive trade-offs seem to be plastic responses rather than fixed population-specific traits (Brown et al. 2006), allowing individuals to adjust their level of competitive aggression to local conditions of risk (Helfman 1989; Brown et al. 2009). Moreover, agonistic behaviour exposes individuals to potential predators (Toscano et al. 2010), further increasing the costs associated with competition.

Low predation streams typically have higher densities of guppies and lower productivity, resulting in higher competition for food (Grether et al. 2001), so we cannot limit our conclusions to the costs of predation risk alone. Stream productivity could, in turn, influence fitness-related trade-offs through adaptations to high vs. low productivity environments (Walsh and Reznick 2010), resulting in differing patterns of energy allocation between predator avoidance and competition. Individuals from low-productivity (and low ambient predation) habitats may invest more energy in intraspecific aggression to access important resources (e.g. food, mates) and less energy to antipredator behaviour than their high-productivity/ambient-predation counterparts (Magurran and Seghers 1991; but see Kolluru et al. 2007 for a counter argument). Our results were consistent with this explanation.

Male mating behaviour did not differ between populations contrary to our predictions (Table 2.1). The Upper Aripo was not one of the four populations in Luyten and Liley's experiment (1985), but was included in Magurran and Seghers' field study (1994), whose results are consistent with the risk allocation hypothesis overall (Lima and Bednekoff 1999). However, the Upper Aripo clustered with the Lower Aripo population in terms of mating tactic rates (Magurran and Seghers 1994). Indeed, even if the hypothesis was not supported in our study (*P*=0.085), males experiencing high ambient predation-risk tended to show a higher mating attempt rate (both courtship and forced mating) than their low ambient predation-risk counterparts. In contrast, but similar to our results, Kolluru et al. (2007) found no difference in courtship and forced mating rates in guppies from low-ambient predation risk sites differing in their food availability. In addition, the Upper Aripo population has been shown to be less behaviourally plastic than other low-ambient predation risk populations (Kolluru et al. 2015). In summary, Upper Aripo males seem to display high rates of mating tactics for a low-risk population, a result that deserves further study (Magurran and Seghers 1994; Kolluru et al. 2015). The Upper Aripo was not included in Grether et al.'s (2001) study, and it could be that this site shows higher productivity than most low-predation sites, resulting in more energy available for males to reproduce.

Sex Roles

Despite conventional sex roles (Houde 2001), our results indicated that male Trinidadian guppies were not more aggressive than females during our observations. Interestingly, at the extremely biased ASRs during the with-and-without a mate experiment, females were even more aggressive than males. These results are surprising, as males are generally expected to exhibit higher rates of intrasexual competition for mates than are females. Conversely, significant female-biased ASRs in the Lower Aripo population (high predation; Pettersson et al. 2004) may have favoured the evolution of aggressive female phenotypes. However, the aggression rates of Upper Aripo females (low predation) followed similar patterns despite no significant female-biased ASRs for this population (Pettersson et al. 2004). Hence, this evolutionary argument for female aggressiveness based on female-biased ASRs is unlikely. Because females can store sperm for long periods of time, they do not need access to a mate to produce a new brood (Constantz 1984); thus females are not expected to fight for males. However, males should be expected to always potentially benefit from inseminating a fertilised female (Liley 1966). While mature females are rarely fully receptive (Houde 1988), even fertilised females may benefit from mating with males with 'attractive phenotypes' (Pilastro et al. 2004), such as large areas of orange coloration (Evans et al. 2004). By doing so, females increase their chance of producing attractive male offspring with a higher future reproductive success out of their next fertilisation. However, a female cannot force a male to reproduce, but she can chase other females away to increase the probability of being courted by a particular male. Female isolation from males likely contributed to similar intrasexual aggression rates as males.

Alternatively, the surprisingly high rates of aggression by females may, partly, reflect competition for the food present in the aquaria during observations (Koops and Abrahams 1999); body size is more strongly related to reproductive success in female than in male guppies (e.g. Reznick and Endler 1982). However, fish were fed to satiation before being tested and both sexes increased their rate of intrasexual aggression after the addition of a mate, so food alone cannot explain the high rates of aggression observed in females. Changes in group composition during the with-and-without-a-mate experiment is not likely to have disrupted the established social hierarchy, resulting in an increase in aggressive behaviour to re-establish dominance status (Ang and Manica 2010); mixed-sex guppy groups are known to form nip-right same-sex social hierarchies (Gorlick 1976). The same author also found that females from pet store populations are also aggressive towards males in the presence of food (~35% of total female aggression directed to males, stable over time, at an unbiased sex-ratio), but rarely the other way around. When looking at the 3:3 ASR treatment in the Upper Aripo population (virtually no predation on females, low predation on males), which is the one most likely to represent Gorlick's (1976) experiment, females directed ~17% (SE = 9%) of their aggression rates towards males. As expected, male-to-female aggression acts were rare; only one such act was recorded in this treatment. Female-to-male aggression rates were approximately twice lower in our experiment than in Gorlick's, thus supporting our hypothesis of female-female aggression towards males rather than food. For those same reasons, the increase in intrasexual rates when adding a mate is not likely due to a small increase in density from 5 to 6 individuals.

Conclusion

In summary, the results of our study demonstrated that ASR, ambient predation risk, and their interaction shape the competitive interactions of both male and female guppies. Females were as aggressive as males on average, which is surprising in a mating competition context given that males are expected to be the most aggressive sex in species with choosy females and courting males (see Balshine-Earn 1996). The observation that aggression rates from high ambient predation risk individuals were lower than among those collected from low ambient predation risk populations suggests a trade-off between vigilance towards potential predators and access to mates. The Upper and Lower Aripo populations tend to have female-biased ASRs (Pettersson et al. 2004), suggesting that it is more likely to observe female than male intrasexual aggression for access to mates in the field, especially in the low-ambient predation risk Upper

Aripo population as they are more aggressive. In this population, females are more likely to fight other females off for better exposure to high-quality males in their surrounding than in the Lower Aripo, thus potentially reinforcing population divergence through the sexual selection of bright coloration in Upper Aripo males (Endler 1995). In light of these results, future research should examine the impact of imminent predation threats on mating competition of male and female guppies from populations differing in ambient predation regimes.

Table 2.1. Predictions and results of the effects of ASR[a], ambient predation-risk population differences and sex on competition for mates

Explanatory variables	Predictions for competitors	Results[b]
As ASR[a] increases	(1) Aggression rate increases in males	True
	(2) Aggression rate increases in females	True
	(3) Courtship rate per male decreases	True
	(4) Courtship propensity in males increases	False
	(5) Sneaking rate per male decreases	True
	(6) Sneaking propensity in males increases at faster rate than courtship propensity	True (at male-biased ASRs only)
High vs. low ambient predation-risk population	(7) Aggression rate is lower or higher[c]	True – lower
	(8) Courtship/forced mating rate per male is lower or higher[c]	False
Sex roles	(9) Males are more aggressive than females	False

[a] ASR is defined here as the number of adult members of the competing sex/ the number of adult members of the opposite sex.

[b] True means the results were consistent with the predictions (i.e. $P<0.05$). False means no significant effect.

[c] Behaviour rate predicted to decrease if the cost of ambient predation risk is high OR increase if the absence of an actual predator signals a ‘safe’ period, as in Lima and Bednekoff’s (1999) risk allocation model.

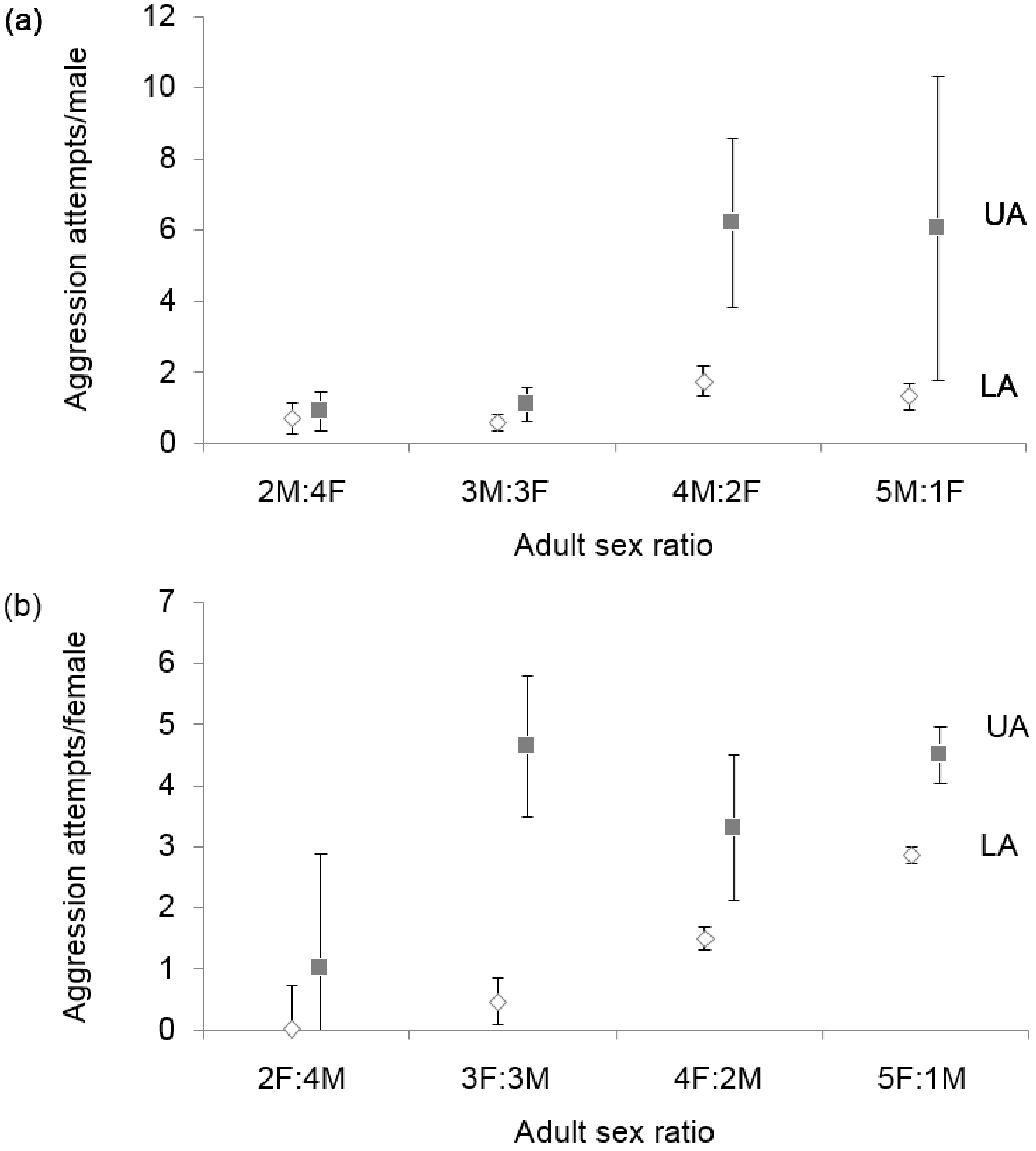


Fig. 2.1 Mean (±SE, N=10) number of intrasexual aggressive acts per individual (a) male (*M*) and (b) female (*F*) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex; i.e. 0.5, 1, 2, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 25-min period

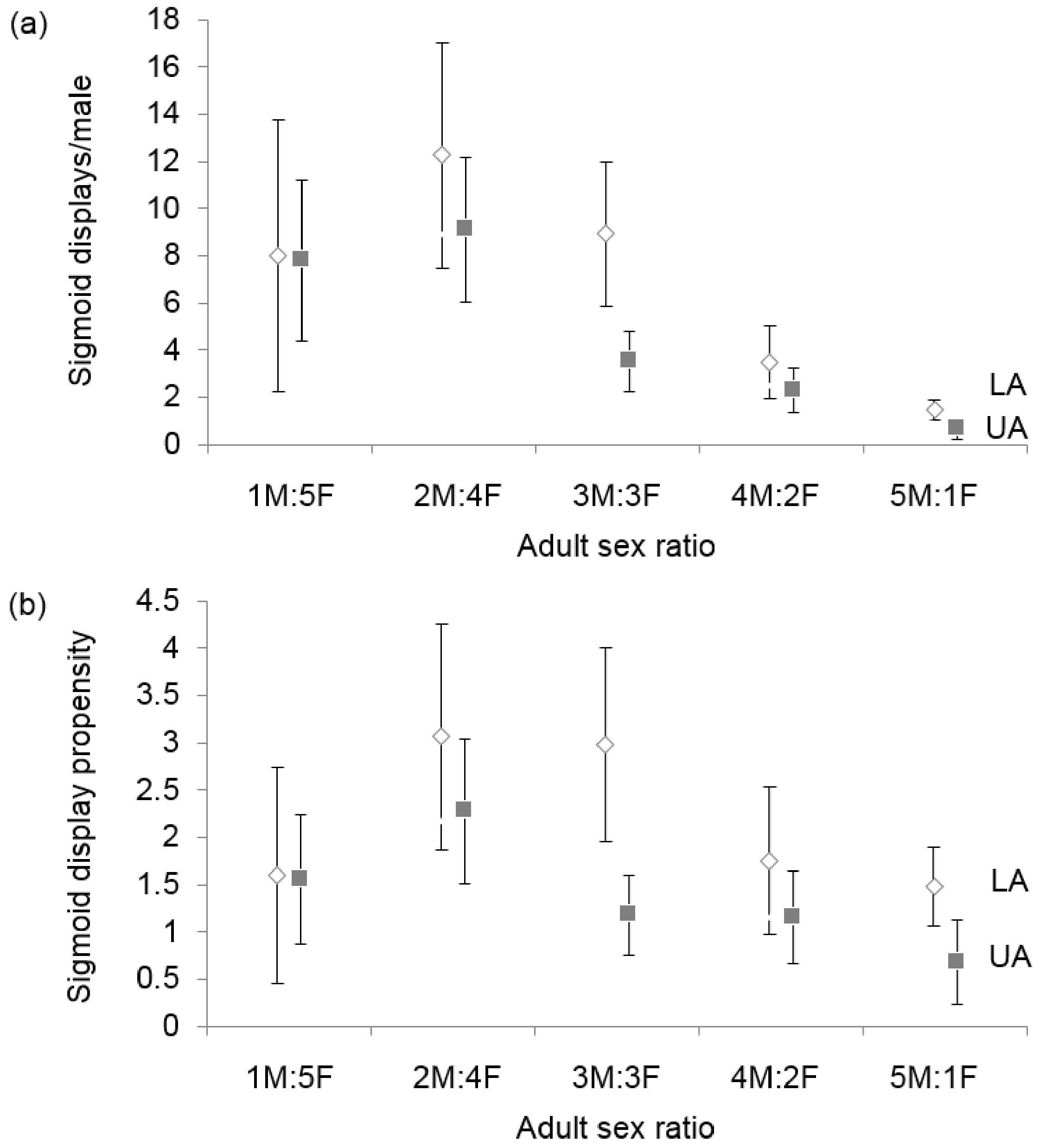


Fig. 2.2 Mean (±SE, N=10) number of sigmoid displays (a) per individual male (*M*) and (b) per individual male corrected by the number of females (*F*) (propensity) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex; i.e. 0.2, 0.5, 1, 2, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 25-min period

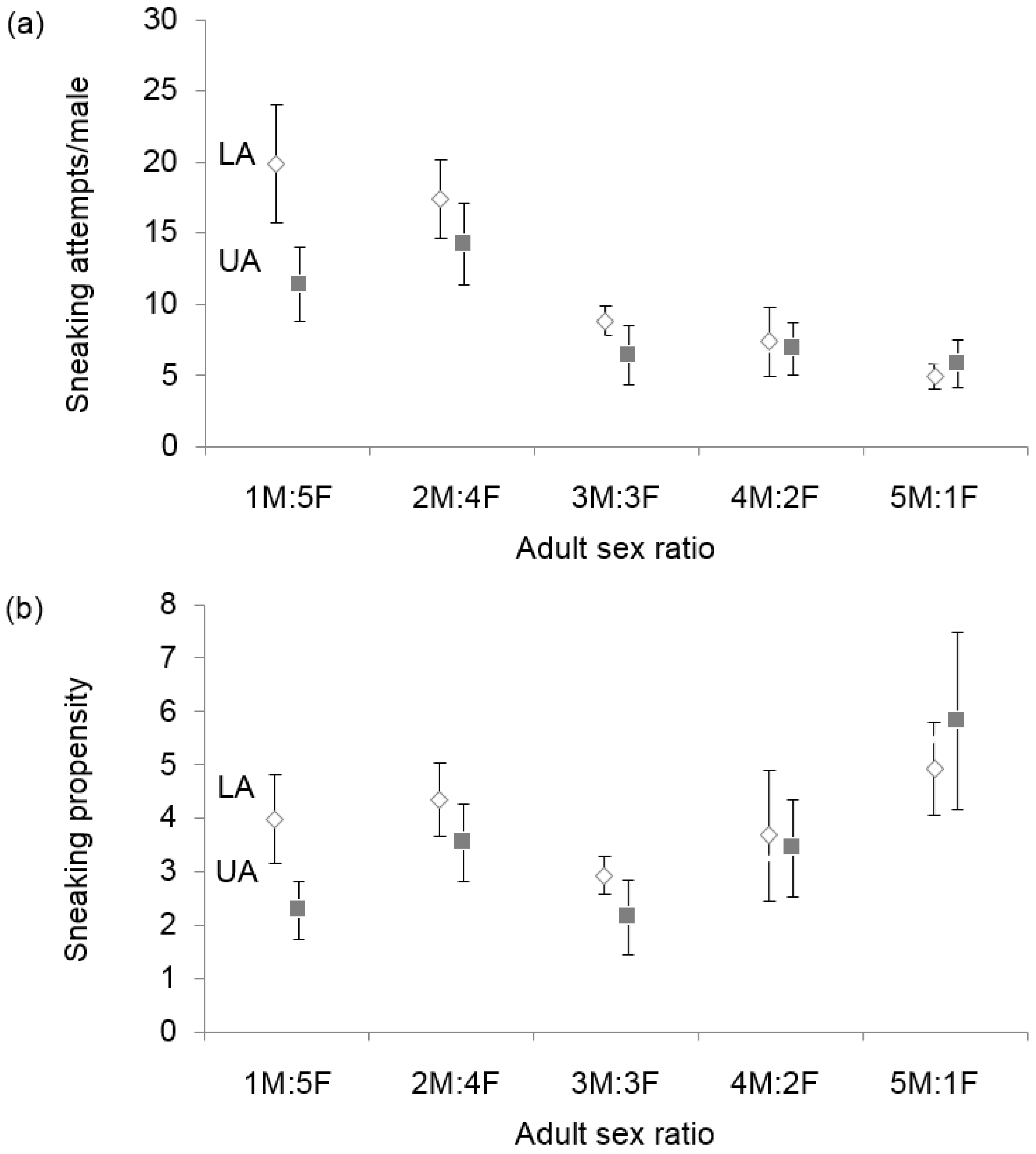


Fig. 2.3 Mean (±SE, N=10) number of forced mating attempts (a) per individual male (*M*) and (b) per individual male corrected by the number of females (*F*) (propensity) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex, i.e. 0.2, 0.5, 1, 2, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 25-min period

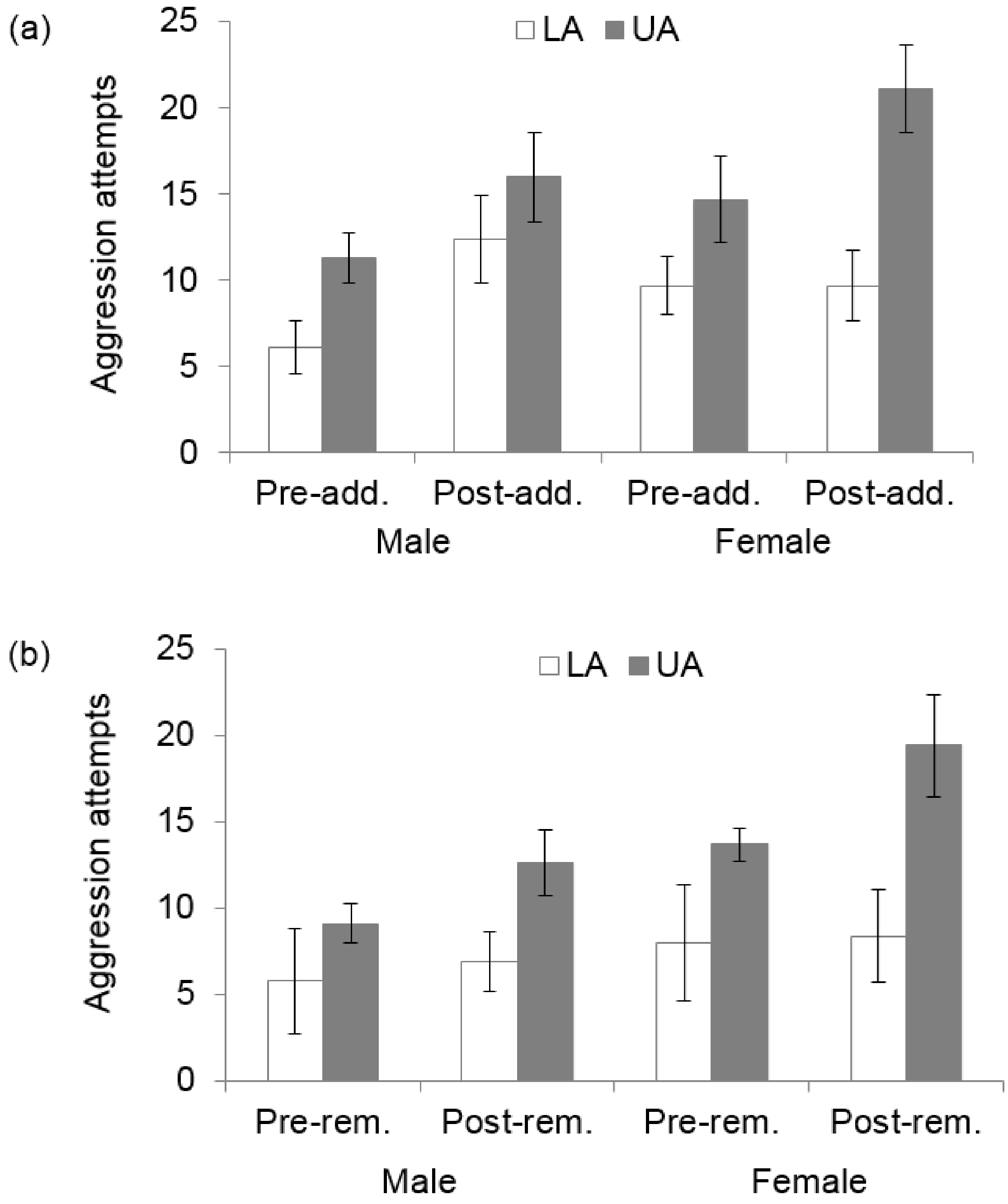


Fig. 2.4 Mean (±SE, N=10) number of intrasexual aggressive acts of 5 individuals of the non-limiting sex (either males or females) for 2 periods of 5 min before (*Pre*) and after (*Post)* (a) the addition (*add.*) or (b) removal (*rem.*) of one individual of the other sex for Lower Aripo (high predation; *LA*; *open bars*) and Upper Aripo (low predation; *UA*; *shaded bars*) guppies

Chapter 2: The effects of adult sex ratio on mating competition under varying levels of imminent and ambient predation risk

Introduction

Predation risk is costly for prey species. Indeed, the non-consumptive effects of predators include costs resulting from trade-offs between fitness-related activities (e.g. reproduction, foraging) and vigilance towards potential predators or antipredator behaviours to minimize the risks of being consumed (Preisser et al. 2005). For instance, under an imminent predation threat, prey individuals may reduce their courtship rates (e.g. Cordes et al. 2014; Michelangeli and Wong 2014), switch to less energetically-costly and conspicuous alternative mating tactics (e.g. Godin 1995), and decrease foraging rates (e.g. Abrahams and Sutterlin 1999). In addition, competitive behaviours (e.g. agonistic interactions) used to obtain resources contributing to fitness (e.g. mates, food) are also reduced under an acute predation threat (e.g. Kim et al. 2004; Foam et al. 2005; Oku and Yano 2008; Leese et al. 2010). These reductions in fitness-related behaviours allow individuals to invest more time and energy in antipredator behaviour (e.g. Brown et al. 2009; Mitchell and McCormick 2013; Franklin et al. 2014) to avoid death, and thus maintain potential future fitness gains. However, in the context of high ambient predation risk, prey individuals have no other option but to forage and reproduce under elevated predation risk. The threat-sensitive predator avoidance hypothesis (Helfman 1989) suggests that prey individuals should be selected to adjust their response to the level of perceived predation risk (i.e. graded response), allowing them to devote time and energy towards important fitness-related activities (e.g. mating, foraging) when imminent predation risk is low. Brown et al. (2009) tested that hypothesis on two guppy populations contrasting in their ambient predation risk. Guppies collected from a high ambient predation population exhibited proportional (i.e. graded) responses to increasing levels of perceived threat through a proportional increase in antipredator behaviour. Conversely, guppies from a low predation population exhibited a non-graded response (i.e. 'all-or-nothing' response) characterized by an intense response (i.e. increase in antipredator behaviour) beyond a certain threshold in perceived risk intensity, consistent with Helfman and Winkelman (1997). When ambient predation risk is low, individuals benefit from a maximal response to imminent predation risk (i.e. hypersensitivity, Helfman and Winkelman 1997) in terms of increased survival without much of a fitness cost since these risky situations are rare (Brown et al. 2009). However, we are not aware of any comparable study that focused on behaviours related to mating (i.e. mating tactics rates, intrasexual aggression rates) to explore if the patterns observed in antipredator behaviour are consistent with the patterns of decrease in mating behaviours under imminent predation risk.

In addition to shaping the response to imminent threats, ambient predation risk may also have a strong effect on its own. Indeed, even in the absence of an imminent predation risk, prey exposed to high levels of ambient predation risk need to invest some energy towards predator vigilance at the expense of important fitness-related behaviours to avoid being consumed by predators (e.g. Farr 1975; Riechert 1993; Heinen et al. 2013; Chapter 1). Moreover, aggressive and courtship behaviours tend to increase detection by potential predators (Tuttle and Ryan 1981; Belwood and Morris 1987; Toscano et al. 2010). Alternatively, the risk allocation hypothesis (RAH, Lima and Bednekoff 1999; Ferrari et al. 2009) suggests that populations experiencing high ambient predation risk should optimize 'safe' periods by increasing their rates of fitness-related activities during periods of low imminent predation risk. However, low-ambient predation risk populations have more 'safe' time to perform these activities, thus they should not show such intensity in behavioural activity in the absence of an imminent risk.

Based on the asset-protection principle (Clark 1994), the more an individual accumulates reproductive assets, the more it should perform antipredator behaviour. For instance, male European rollers, *Coracias garrulous*, increased their provisioning rates to nestlings under imminent predation risk, whereas females did not (Exposito-Granados et al. 2016), likely because females have accumulated assets in the form of body mass that they will be able to convert into eggs during reproduction. However, the 'cheaper' physiological production of gametes in males (Charnov 1982; but see Nakatsuru and Kramer 1982), combined with their higher reproductive rates than females (Clutton-Brock and Parker 1992) make males more likely to attempt mating rather than perform antipredator behaviour (Heinen et al. 2013). In general, males court and fight amongst them more for mates than do females which tend to choose their mates (Darwin 1871; Andersson 1994). While Brown et al. (2009) compared the threat-sensitive response patterns across populations differing in ambient predation pressure, they only examined female guppies as males are less responsive to threat than females (Magurran 2005).

Despite these sex role differences (sensu Vincent 1994; Forsgren et al. 2004), the proportion of one sex relative to the opposite sex, also called operational sex ratio (OSR; Emlen and Oring 1977), is a key factor determining mating competition patterns. As OSR becomes biased toward one sex, that sex will show higher rates of intrasexual aggression (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). Most empirical evidence of this prediction comes from male mating behaviour (Kvarnemo et al. 1995; Grant et al. 2000; Weir et al. 2011). However, consistent with OSR theory (Emlen and Oring 1977), some studies have also shown that females can behave aggressively when OSR is biased toward them (Grant and Foam 2002; Forsgren et al. 2004; Clark and Grant 2010; Chapter 1). OSR is also a good predictor of mating tactic rates but its effects seem to depend on the diversity of mating behaviour used by the species of interest. For instance, in species that use courtship as the only mating tactic, courtship rates per male seem to decrease as OSR becomes biased towards them (e.g. Weir et al. 2011) likely due to the cost of aggression and the lower availability of females (Jirotkul 1999a). By contrast, courtship propensity (courtship rates per male corrected by the number of encounters with females, sensu de Jong et al. 2012) increases as OSR shifts towards a male-bias due to an increase in male-male competition as OSR theory predicts (Emlen and Oring 1977). However, in species that also use alternative sneaky mating tactics, the proportion of courtship seems to decrease as OSR becomes male-biased in favor of an increase in the proportion of alternative mating tactic (Grant et al. 1995; Mills and Reynolds 2003; Chapter 1) likely because of the lower energetic costs of sneaking tactics. Sneaking rates per male decrease as OSR becomes male-biased (Jirotkul 1999a; Chapter 1) for the same reasons as courtship propensity decreases (see above).

Here, we explored for the first time the combined effects of imminent and ambient predation risks, sex role, adult sex ratio (ASR; used as a proxy for OSR; Chapter 1), and their interactions on the mating, foraging and antipredator behaviour of two wild populations of Trinidadian guppies varying in their ambient predation risk. Imminent risk was manipulated using conspecific chemical alarm cue (AC). Damage-released chemical alarm cues are common in aquatic prey species (Chivers and Smith 1998; Ferrari et al. 2010). Within a given species, alarm cue is detected by conspecifics and heterospecifics as an honest and reliable indicator of imminent risk that can elicit a behavioural response (Chivers et al. 2013). We predicted that (Table 3.1) due to the costs of imminent predation risk, (1) individuals will decrease their rates of mating and foraging behaviour in favor of antipredator behaviour in the presence of a perceived risk. In addition, when comparing high- vs. low-ambient predation risk populations, (2) individuals experiencing high ambient predation risk will show a graded response to an increase in imminent risk while low-ambient predation risk individuals will show a non-graded response. When only considering the costs of ambient predation risk, low-ambient predation risk individuals (3) will show higher aggression rates and (4) lower mating tactic rates than their high-ambient predation risk counterparts as suggested in Chapter 1. However, if the costs of ambient predation risk are high, (5) individuals exposed to low ambient predation risk will forage more when imminent risk is absent and show lower antipredator behaviour rates under imminent risk than their high ambient predation risk counterpart but the opposite will occur if individuals under high ambient predation risk take opportunity of a 'safe' period (i.e. absence or low imminent predation risk) to forage as suggested by RAH (Lima and Bednekoff 1999). As males and females usually have different sex roles, females (6) will be more threat-sensitive and (7) less aggressive than males in terms of mating competition. Based on OSR theory, as ASR increases, (8) intrasexual aggression rates will increase in both males and females, (9) both courtship and forced mating rates per male will decrease and (10) courtship propensity will remain unaffected whereas sneaking propensity will increase (as in Chapter 1).

Materials and Methods

Sampling and populations of origin

We sampled individuals between April 24th and May 30th 2012 from two populations located in the Aripo River, Northern Mountain Range, Republic of Trinidad and Tobago; namely the Upper and Lower Aripo Rivers. Despite lower guppy densities and higher stream productivity in the Lower Aripo River compared to the Upper Aripo River (Reznick et al. 2001), we focused on the difference in ambient predation risk between the two populations. This difference led to the natural selection of populations with locally-adapted gene pools to high vs. low ambient predation-risk populations (Fitzpatrick et al. 2015). The selective pressures are strong enough that these gene pools remain resilient to massive gene flow if it were to happen. These two populations are more genetically similar than other Trinidadian guppy populations differing in the ambient predation risk (Carvalho et al. 1991), suggesting more recent geographical isolation of the two populations, or potentially continual gene flow from the Upper

Aripo to the Lower Aripo. The Upper Aripo is known as a low-predation site (Croft et al. 2006, Botham et al. 2008) with few predator species at low densities foraging primarily on newly emerged juvenile guppies. These predator species are Hart's rivulus, *Anablepsoides hartii,* and a small freshwater prawn, *Macrobrachium crenulatum* (Endler and Houde 1995; Walsh and Reznick 2009). By contrast, the Lower Aripo River is described as a high-predation risk site (Croft et al. 2006) with a higher diversity and density of guppy predators than the Upper Aripo River, including pike cichlids, *Crenicichla sp.*, blue acara cichlids, *Aequidens pulcher*, black acara cichlids, *Cichlasoma bimaculatum*, wolf fish, *Hoplias malabaricus*, and two-spot sardine, *Astyanax bimaculatus* (Endler and Houde 1995; Croft et al. 2006; Brown et al. 2009; Walsh and Reznick 2009) preying on adult guppies.

Once captured, we held the fish separately by population of origin and sex in aerated 100-L aquaria at ~24°C in a laboratory at the University of the West Indies, Saint-Augustine, Republic of Trinidad and Tobago. We fed the fish twice a day with brine shrimp and commercial flake food (Tetramin^{TM}) and we let them acclimate a minimum of 24h before observation. After testing, fish were released back to their original population.

Alarm cue extraction

Damage-released alarm cues are known to reliably elicit acute increases in antipredator behaviour in Trinidadian guppies (Brown et al. 2009; 2010). We generated alarm cue from 130 Upper Aripo (mean standard length (± SD) = 21.38 ± 0.34 mm) and 160 Lower Aripo (mean standard length (± SD) = 18.73 ± 0.28 mm) non-gravid females. Donors were euthanized via cervical dislocation. We immediately removed the head and tail (at the caudal peduncle) and manually extruded any remaining visceral tissue. The remaining tissue (skin and underlying skeletal muscle) was placed into 200 mL of aged tape water. We then mechanically homogenized the tissue samples, filtered them through polyester floss and diluted the samples to the desired final stock concentration (~0.10 cm^2 per mL). We combined approximately equal amounts of tissue from both populations to control for population differences in behavioural response to the stimulus (Brown and Godin 1999; Brown et al. 2010). For the 25% AC injections, we diluted the filtrate with the addition of dechlorinated water to reach a concentration of ~0.025 cm^2. We collected a total of 192.16 cm^2 and 195.70 cm^2 of skin tissue for the Lower and Upper Aripo populations respectively (in a final volume of 3879 mL). All the alarm cue filtrate was frozen in 20 mL aliquots at -20°C until needed for testing.

Experimental protocol

Trials were performed in aquaria (40 x 20 x 20 cm) following the standard alarm cue (AC) concentrations used in Brown et al. (2009). This experiment is an extension of Chapter 1's experiment where mating behaviours were observed in shoals of 6 fish from either the Upper (low risk) or Lower (high risk) Aripo populations under 5 different ASR (number of adult members of the competing sex/number of adult members of the opposite sex, i.e. 0.2, 0.5, 1, 2, 5) for 25 min. The selection of ASRs was originally based on Jirotkul's (1999a) study of the effect of OSR on mating competition. For this study, we replicated the two-way factorial experimental design of Chapter 1 into a three-way factorial set up (2 populations x 5 ASRs x 3 AC concentrations) by injecting 10 mL of either 25% or 100% concentration alarm cue or a control consisting of dechlorinated water (data obtained from Chapter 1) with ten replicates each per combination of population and ASR, for a total of 1800 observed individuals. The ~0.1 cm^2 concentration of tissue surface area per mL corresponds to previous guppy studies, from which we expect a strong antipredator response (Brown and Godin 1999; Brown et al. 2009). The 100% alarm cue injection likely represents a nearby imminent danger of predation (i.e. 1 cm^2 of fish tissue damaged) while the 25% concentration could be a slightly injured fish nearby (i.e. 0.25 cm^2 of fish tissue damaged) or the diluted signal of a fish more severely injured further upstream. In both cases, this would represent a weaker risk than the 100% alarm cue concentration.

We placed 6 individuals per test tank and gave them 1 to 2 hours to acclimate before starting an observation trial. A single observer (PJC Chuard) performed all observations. Because social hierarchy usually establishes itself in less than one hour among interacting individuals (Kodric-Brown 1992), aggression rates should remain relatively constant after one hour of acclimation. Moreover, trials were randomized between the 30 treatments so there was no acclimation time bias for any of the treatment. Ten minutes before the start of an observation, we fed the fish commercial flake food uniformly dispersed over the surface to make sure the fish were satiated (Robb and Grant 1998), thus limiting aggression interactions related to foraging. Fish virtually ate all the food during the 10 min following feeding. We distinguished males from females using their conspicuous colouration. Behavioural measurements were recorded over 25 min divided in 5-min periods and the stimulus injection was applied after 10 min. The same behavioural measurements related to mating competition as in Chapter 1 were recorded; namely, (1) intrasexual aggression rates for both males and females by adding up the frequency of biting, chasing, tail beating and pushing for each sex divided by the number of same-sex individuals (i.e. 2 to 5), (2) male mating tactic rates: courtship (i.e. sigmoid display) and forced mating (i.e. sneaking attempt). Male guppies court females by exposing their bright colours to them. To increase the chances of being noticed, males erect their dorsal fin and move up and down while bending their body in a sigmoid shape (Houde 1987). As an alternative mating tactic, male guppies use sneaking attempts where they try to insert their gonopodium inside a female's gonopore without initial courting (Farr 1980a). Then, these rates were individually divided by the number of males present (i.e. 1 to 5) which gave us per-male mating tactic rates; to obtain a measure of propensity, we corrected those rates by the number of females present (i.e. 1 to 5). In addition to those measurements, we also recorded for both sexes together (3) the number of foraging attempts which consisted of an individual pecking at a piece of flake or against the tank, (4) three measures of antipredator behaviour: number of dashing, freezing behaviour and time to resume foraging. Dashing is a sudden burst of swimming, whereas freezing is defined as remaining motionless on the substrate for at least 30 s (Brown et al. 2009). Those two behavioural traits have been shown to increase with predation risk (Brown and Godin 1999; Brown et al. 2009). The time to resume foraging was measured in seconds between the time of stimulus injection and the first individual that was observed foraging. To record those various behaviours, we used the same scanning method as described in Chapter 1, which proved to be accurate since the distribution of the total number of behaviours observed per trial (see Appendix 1.1) had a tail to the right with no sudden cut-off, signalling the unlikeliness of missing data.

Statistical analysis

We only used the 5-min periods pre- and post-stimulus injection to look at the effect of AC on behaviour since acute antipredator behaviours were rarely observed 5 min after injection, suggesting that individuals had resumed their regular activities. We used general linear mixed-models (GLMM) and general linear models (GLM) for the analyses. We fitted our data to the negative binomial distribution for all analyses and tested for over-dispersion. All models were not significantly over-dispersed ($P>0.99$). We used ASR, population of origin and AC as fixed factors in all analyses. As ASR and AC were made of more than two levels, we treated them as planned contrasts to explore the predicted linear relationships (i.e. linear contrasts; see Table 3.1). Quadratic relationships were also considered for ASR, but they were only displayed in the results if significant (because we had no evidence of the dome-shaped relationship of ASR with aggression rates and mating tactic propensities expected in Chapter 1 – see Weir et al. 2011). Except antipredator behaviours, all other behavioural measurements were observed frequently in the pre-stimulus periods. For that reason, we used a repeated measure analysis through GLMM with period (5-min pre- vs. post-injection) as an extra independent variable to test the effect of injection on those behaviours. Since aggression rates were measured in both sexes simultaneously, we added sex as an independent variable and tank ID as a random factor for the repeated measure in the model. Regarding antipredator measurements, we analyzed only the 5-min post-injection period with GLM since freezing and dashing rarely occurred in the pre-injection period and time to resume foraging by definition is measured in the post-injection period. All dependent variable analyzes were univariate with the exception of antipredator behaviours that were analyzed together, as well as mating tactic rates per male and propensities to test the prediction of more male mating attempts in the Lower Aripo (high risk, see Table 3.1). We reported all statistics as follow: (β, [95% CI], z score, probability) where β represents the regression coefficient and CI the confidence intervals. To increase statistical power, we removed non-significant interactions involving at least one quantitative variable (i.e. ASR, AC) when applicable as suggested by Engqvist (2005), except the AC x period interaction since it represented the effect of AC overall (i.e. AC is expected to have an effect once injected during the 5-min period post-injection).

For all analyzes, we only reported unpredicted interactions if significant after the non-significant interactions were removed from the model if necessary. We did not perform statistical corrections (see Moran 2003) as our models were based on a priori predictions and the different data subsets were not multiply tested. We performed all statistical tests in R (3.1.2; R Development Core Team 2015) using the *contrast()* function of the *stats* package (Chambers and Hastie 1992) for planned linear and quadratic contrasts, the *glmmadmb()* function of the *glmmADMB* package (Fournier et al. 2012), and the *glm.nb()* of the *MASS* package (Venables and Ripley 2002) for GLMMs and GLMs respectively.

Results

How does perceived imminent risk influence mating, foraging, and antipredator behaviour?

As expected from the first prediction (Table 3.1), most mating competition behaviours decreased in the presence of AC, including intrasexual aggression rates (Fig. 3.1; GLMM: β=-1.44, CI=[-2.37, -0.51], z=-3.03, P=0.0025), sneaking attempt rates per male (Fig. 3.2a,b; GLMM: β=-0.83, CI=[-1.14, -0.52], z=-5.29, P<0.001), and sneaking attempt propensity (Fig. 3.2c,d; GLMM: β=-0.73, CI=[-1.21, -0.24], z=-2.93, P=0.0033). Foraging rates also decreased when we exposed fish to AC (Fig. 3.3; GLMM: β=-1.09, CI=[-1.33, -0.86], z=-9.05, P<0.001). The exception was courtship rates that were not significantly affected by exposure to AC. including courtship display rates per male (Fig. 3.4a,b; GLMM: β=-0.21, CI=[-0.67, 0.25], z=-0.89, P=0.37), and courtship display propensity (Fig. 3.4c,d; GLMM: β=-0.27, CI=[-0.89, 0.35], z=-0.85, P=0.39). Alarm cue concentrations (i.e. 25% vs. 100%) had no effect on the behavioural response intensity (see below). According to the first prediction, those behaviours decreased in favor of an increase in antipredator behaviour rates under an imminent risk of predation (Fig. 3.5; GLM: β=5.19, CI=[4.50, 5.97], z=14.08, P<0.001).

Contrary to our second prediction, the ambient risk of predation (i.e. Upper Aripo: low risk vs. Lower Aripo: high risk) had no significant effect on the patterns of behavioural change of all recorded behaviours under low- versus high-imminent predation risk (Table 3.2). Lower Aripo (high risk) guppies did not show a graded response to imminent risk compared to Upper Aripo (low risk) individuals (Figs. 3). Moreover, a significant interaction of period with population of origin on foraging rates (Fig. 3.3; GLMM: β=-0.25, CI=[-0.44, -0.052], z=-2.48, P=0.013) and of alarm cue with population of origin on sneaking rates per male (Fig. 3.2; GLMM: β=-0.37, CI=[-0.72, -0.021], z=-2.08, P=0.038) might even suggest an opposite trend with Lower Aripo (high risk) individuals being more sensitive to both low- and high-imminent risk than Upper Aripo (low risk) individuals.

How does ambient predation risk influence mating, foraging, and antipredator behaviour?

When comparing the two populations, according to the third prediction, Upper Aripo (i.e. low-ambient predation risk) individuals were more aggressive overall than their Lower Aripo (i.e. high-ambient predation risk) counterparts (Fig. 3.1, GLMM: β=0.81, CI=[0.47, 1.15], z=4.64, P<0.001). However, contrary to our fourth prediction, but consistent with Chapter 1, Lower Aripo (high risk) males did not perform more reproductive attempts than Upper Aripo (low risk) males, including mating tactic rates per male (Figs. 3.2a,b & 3.4a,b; GLMM: β=-0.12, CI=[-0.36, 0.12], z=-1.02, P=0.31), and mating tactic propensity (Figs. 3.2,c,d & 3.4c,d; GLMM: β=-0.12, CI=[-0.36, 0.12], z=-0.97, P=0.33). As expected from the fifth prediction based on RAH (Lima and Bednekoff 1999), Lower Aripo (high risk) individuals foraged more under no imminent risk than Upper Aripo (low risk) guppies (Fig. 3.3; GLMM: β=-0.25, CI=[-0.47, 0.038], z=-2.31, P=0.021) and showed weaker antipredator response rates under imminent risk (Fig. 3.5; GLM: β=0.67, CI=[0.20, 1.15], z=2.85, P=0.0044).

How do males and females differ in their mating, foraging, and antipredator behavioural patterns?

Regarding sex roles, inconsistent with our sixth prediction, females were not significantly more threat-sensitive than males. They did not show a bigger decrease in intrasexual aggression rates under increasing concentrations of AC than males (Fig. 3.1; GLMM: β=-0.29, CI=[-1.09, 0.50], z=-0.72, P=0.47). In addition, the effect of the interaction of ASR, AC and period (pre- vs. post-injection) was not significant on foraging rates (Fig. 3.3; GLMM: β=0.070, CI=[-0.31, 0.45], z=0.37, P=0.71), but the interaction of ASR (quadratic contrast) and AC was significant on antipredator behaviours (Fig. 3.5; GLM: β= 0.81, CI=[0.12, 1.62], z=2.02, P=0.044), indicating more antipredator behavior at intermediate ASR with the presence of AC. However, these results indicate that females were not more threat-sensitive than males regarding those specific behaviour rates, as ASR had no significant linear interaction with AC and period (i.e. such significant interaction would have indicated that one sex is more threat-sensitive than the other). Contrary to our seventh prediction, females were more aggressive than males overall (Fig. 3.1; GLMM: β=0.36, CI=[0.076, 0.65], z=2.48, P=0.013) while we would have expected the opposite to occur. Females were especially more aggressive than males in the absence of an imminent risk (i.e. during the 5-min period before the injection; Appendix 1.2) as suggested by a significant interaction between sex and period on aggression rates (GLMM: β=-0.49, CI=[-0.47, 1.71], z=-2.08, P=0.037).

How does ASR influence mating competition?

Consistent with our eighth prediction, even under imminent predation risk, ASR had a significant effect on both males and females with an increase in intrasexual aggression rates along with ASR (Fig. 3.1; GLMM: β=1.23, CI=[0.92, 1.54], z=7.74, P<0.001). As expected from the ninth prediction, the opposite relationship with ASR held true for mating tactic rates per male with a decrease in both sneaking attempts as ASR increased (Fig. 3.2a,b; GLMM: β=-0.72, CI=[-0.92, -0.52], z=-6.91, P<0.001) and sigmoid display rates per male (Fig. 3.4a,b; GLMM: β=-1.39, CI=[-2.03, -0.76], z=-4.30, P<0.001). Finally, in accordance with our tenth prediction, sneaking attempt propensity showed a positive correlation with ASR (Fig. 3.2c,d; GLMM: β=0.49, CI=[0.27, 0.71], z=4.41, P<0.001) whereas ASR did not significantly affect sigmoid display propensity (Fig. 3.4c,d; GLMM: β=-0.29, CI=[-0.68, 0.10], z=-1.46, P=0.15).

Discussion

Our results support six of the ten predictions related to the effects of predation risk, sex roles and adult sex ratio on mating competition, foraging and antipredator behaviours (Table 3.1). Notably, individuals decreased their rates of foraging and most mating behaviours in favour of antipredator behaviours in the presence of an imminent risk of predation. Our most surprising result is probably the apparently consistent courtship rates despite the presence of an imminent threat (i.e. alarm cue). However, guppies from the high-predation risk site did not show a graded response to risk in terms of mating, foraging and antipredator behaviour, compared to individuals from the low-predation risk site. In addition, females were unexpectedly more aggressive than males but not more threat-sensitive. As expected from previous studies (e.g. Chapter 1), our results suggest that as ASR increases, intrasexual aggression rates increase at the cost of reproductive attempt rates per male, but along with sneaking attempt propensity. Finally, as expected, the low-ambient risk population was more aggressive, foraged less under no imminent risk, and performed more antipredator behaviours under the presence of alarm cue than the high-ambient risk population. However, the latter did not perform more mating attempts overall than the former, which did not support our prediction. Some of the intriguing effects of alarm cues are likely due to differences in sex roles when both sexes are studied simultaneously, and to the perception of predation risk depending on the information that the cue provides.

How does perceived imminent risk influence mating, foraging, and antipredator behaviour?

Consistent with previous studies, foraging rates (e.g. Abrahams and Sutterlin 1999) and aggression rates (e.g. Kim et al. 2004; Foam et al. 2005; Oku and Yano 2008; Leese et al. 2010) decreased under the presence of conspecific alarm cues in favour of an increase in antipredator behaviours rates (e.g. Brown et al. 2009; Mitchell and McCormick 2013; Franklin et al. 2014). Moreover, our results showed that sneaking attempt rates decreased as well. These reductions in fitness-related behaviours are likely due to the costs of predation risk. Individuals need to balance trade-offs between antipredator behaviours and other important activities in order to avoid death (Preisser et al. 2005). Beauchamp (2009) found similar patterns in birds and mammals in relation to food availability. Antipredator behaviour rates were greater at intermediate ASR under AC likely due to the low availability of mates for one sex at biased ASRs, leading to more risk-taking behaviour from that sex. However, courtship rates did not decrease under imminent predation risk in our experiment suggesting that the proportion of courtship to sneaking increases as imminent predation risk increases. Godin (1995) found the opposite when using a visual cue rather than chemical cues, with an increase in the proportion of sneaking compared to courtship under an imminent risk of predation.

Previous studies have shown that conspecific alarm cues can be interpreted as less risky compared to predator visual cues (e.g. McCormick and Manassa 2008; Marsh-Hunkin et al. 2013) and that response to chemical cues is increased when individuals have been pre-exposed to disturbance cues from conspecifics (Ferrari et al. 2008). In a river ecosystem, when a prey is pursued by a predator such as a pike cichlid (O’Steen et al. 2010), the risk of being killed may be imminent, whereas only detecting a conspecific’s alarm cue in the water could be interpreted as a sign of danger coming from upstream. This difference in predation cue could explain why males did not decrease their rates of courtship under the presence of alarm cues, especially since males are known to be less threat-sensitive than females (Magurran 2005). Under a visual predation risk, male guppies were suspected to take opportunity of the freezing behaviour of females to sneak and copulate (Godin 1995). However, our results suggest that males use courtship rather than sneaking under the presence of conspecific alarm cues. Because predation risk might be perceived as low compared to a visual cue, and also due to the more efficient sperm transfer resulting from courtship rather than forced mating (Pilastro and Bisazza 1999), male guppies may tend to prefer sigmoid display over sneaking, despite the potential of courtship displays to attract predators (Tuttle and Ryan 1981; Belwood and Morris 1987; Toscano et al. 2010), in order to increase their reproductive fitness.

The patterns of response to imminent risk did not significantly differ between the two populations, which seem to show a non-graded response. Brown et al. (2009) used females because they are more threat-sensitive than males, and found a graded response for freezing, area use and shoaling behaviour only. The absence of males in Brown et al. (2009) also may have altered female behaviours since the presence of males leads to behavioural adaptations in females (Darden et al. 2009, Tobler et al. 2011). For freezing behaviour at an ASR of 0.2 (1 male: 5 females), we can graphically see a graded response in Lower Aripo (high risk) individuals (Fig. 3.5c) and a non-graded response in their Upper Aripo (low risk) counterparts (Fig. 3.5d), consistent with Brown et al. (2009). Male harassment might have attenuated the ability of high-ambient predation (i.e. Lower Aripo) females to adjust their behaviour to the intensity of imminent predation risk. For instance, at intermediate imminent risk (i.e. 25% AC), females might have performed as much antipredator behaviours as at high risk (i.e. 100% AC) because foraging would not have been much beneficial due to male harassment.

The fact that high-ambient predation risk females respond more intensely to high imminent predation risk than low-ambient predation risk females (Brown et al. 2009) could explain why Lower Aripo (high risk) individuals seemed to respond in a more hypersensitive way than their Upper Aripo (low risk) counterparts in terms of sneaking rates per male and foraging. Alternatively, since males did not decrease their courtship rates under imminent risk, high-ambient predation females might be aware of the potential of sigmoid display to attract predators, and performed more threat-sensitively under the presence of alarm cues than in the absence of males.

How does ambient predation risk influence mating, foraging, and antipredator behaviour?

Our results on the cross-population comparison of aggression and mating tactic rates match Chapter 1's findings suggesting that despite the costs of imminent predation risk, the costs of ambient predation risk are still affecting the overall behavioural patterns. High-ambient predation risk individuals performed fewer intrasexual aggressive acts than low-ambient predation risk individuals. However, even if the trend was in accordance with the prediction (Figs. 3.2, 3.4), mating attempt rates of Lower Aripo (high risk) males was not significantly higher than their Upper Aripo (low risk) counterparts, likely due to the non-receptivity of the majority of females leading to less mating efforts from males (Chapter 1). According to the risk allocation hypothesis (Lima and Bednekoff 1999; Ferrari et al. 2009), high-ambient predation risk guppies foraged more in the absence of an imminent predation risk than low-ambient predation risk guppies to take opportunity of less frequent safe periods to perform fitness-related activities. For that same reason, Upper Aripo (low risk) individuals performed more antipredator behaviours because they could afford to, as they have more opportunities to forage and reproduce in safe conditions than Lower Aripo (high risk) individuals.

How do males and females differ in their behavioural patterns?

Surprisingly, females were not significantly more threat-sensitive than males, even though the trend for aggression seemed to support the prediction (*P*=0.17). As discussed above, this could be due to the presence of males disrupting female antipredator behaviour. The fact that males constantly harass females might prevent females from freezing for instance. By contrast, the decrease in male aggression rates might be indirectly related to imminent risk through female behaviour. Since males might take opportunity of the freezing behaviour of females to court them, they might decrease their aggression rates to focus on courtship under imminent predation risk. As expected from Chapter 1, females were more aggressive than males overall, especially in the absence of AC as imminent risk reduced aggression rates (see above). Female intrasexual aggression behaviour could be due to competition for both food (Nummelin 1988) and mates (Chapter 1) whereas males are more likely to mostly fight for mates (Darwin 1871; Andersson 1994) leading to higher aggression rates between males than females. Alternatively, in species in which males use alternative mating tactics, males are known to switch from aggression to forced mating as competition increases (e.g. Grant et al. 1995; Verrell and Krenz 1998; Kanoh 2000; Mills and Reynolds 2003), whereas females can only use aggression to access mates.

How does ASR influence mating competition?

Finally, our results on ASR support all the predictions following OSR theory (Emlen and Oring 1977) as in Chapter 1 despite the costs of imminent predation risk. Namely, aggression rates increased as ASR increased in both sexes at the expense of mating tactic rates per male. Since forced mating is likely cheaper than courtship display (Grant et al. 1995; Mills and Reynolds 2003), sneaking propensity increased as ASR increased (i.e. competition increased) whereas ASR did not significantly affect sigmoid display propensity. An increase in sneaking propensity allowed males to remain reproductively competitive at male-biased ASR against other males by maximizing their reproductive attempts at each potential encounter with a female.

In summary, the presence of conspecific alarm cues led to a decrease in foraging and most mating behaviours in favor of an increase in antipredator behaviours. The presence of males likely prevented females from being more threat-sensitive, and males seemed to use female awareness for potential predators to catch their attention and court them. In addition, ambient predation risk and ASR shaped mating competitive patterns, even under an imminent risk of predation where some of the energy invested into competition was traded-off for antipredator behaviours. Future studies should investigate the effects of other imminent predation risk cues (i.e. visual cues) on mating, foraging and antipredator behaviours under a combination of ASRs and ambient predation regimes to explore population plasticity in their response to different cues of predation risk.

Table 3.1 Predictions and results of the effects of alarm cue concentration, ambient-predation risk, sex role, and ASR[a] on mating, foraging and antipredator behaviours

Explanatory variables	Predictions	Results[b]
As AC concentration increases	(1) Mating- and foraging-related behaviours decrease in favor of antipredator behaviours	True – except courtship
	(2) High ambient-predation risk individuals show a graded response[c] compared to the non-graded response of low ambient-predation risk individuals[d]	False
Low vs. high ambient-predation risk	(3) Aggression rates are higher	True
	(4) Mating tactics rates are lower	False
	(5) Higher/lower foraging rates at the expense/in favor respectively of antipredator behaviours[e]	True - RAH
Sex roles	(6) Females are more threat-sensitve than males	False
	(7) Males are more aggressive than females	False
As ASR increases	(8) Intrasexual aggression rates increase in both males and females	True
	(9) Mating tactics rates per male decrease	True
	(10) Sneaking attempt propensity increases whereas sigmoid display propensity remains unaffected	True

[a] ASR is defined here as the number of adult members of the observed sex/number of adult members of the opposite sex

[b] True means the results supported the predictions (i.e. $P<0.05$). False means the opposite

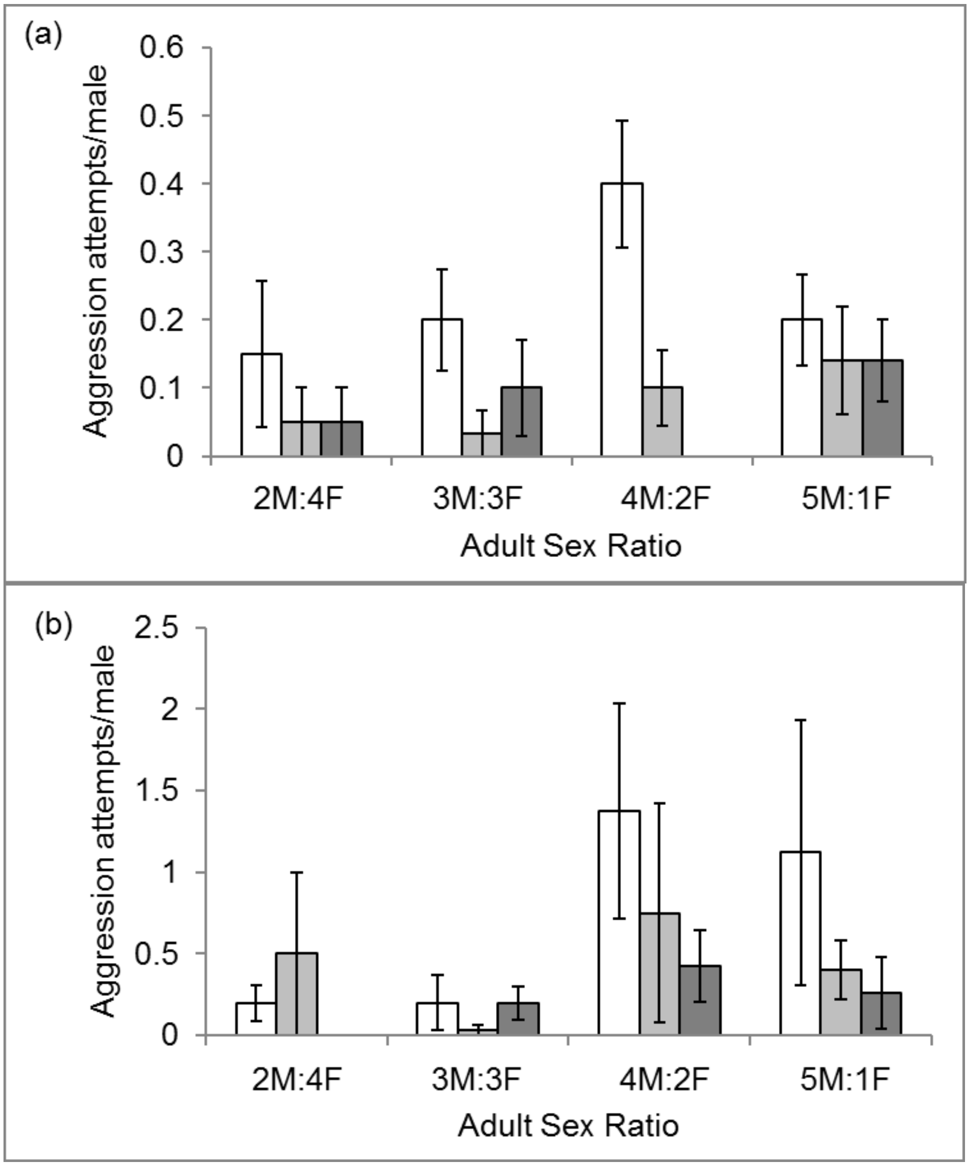
[c] A response is described here as a decrease in competitive, mating, and foraging behaviour, in favor of an increase in antipredator behaviour

[d] A graded response is described here as proportional to the stimulus concentration whereas a non-graded response is constant beyond a certain threshold in AC concentration

[e] Higher foraging and lower antipredator behaviours rates if ambient predation risk is too costly OR lower foraging and higher antipredator behaviours rates if no imminent risk signals a ‘safe’ period following RAH (Lima and Bednekoff 1999)

Table 3.2 Results of GLMMs and a GLM, testing for the effects of the interaction between population of origin (Lower: high risk vs. Upper Aripo: low risk) and presence of alarm cue under different concentrations on the mating, foraging and antipredator behaviours of male and female Trinidadian guppies

Variable	Interaction	Statistical model	Regression coefficient	95% confidence interval	*z*	*P*
Intrasexual aggression rates	AC x Period x Population	GLMM	-0.84	-0.090, 1.78	-1.77	0.078
Sneaking attempt rates per male	AC x Period x Population	GLMM	0.027	-0.44, 0.49	0.11	0.91
Sneaking attempt propensity	AC x Period x Population	GLMM	-0.19	-0.92, 0.55	-0.49	0.62
Foraging rates	AC x Period x Population	GLMM	0.13	-0.21, 0.46	0.74	0.46
Sigmoid display rates per male	AC x Period x Population	GLMM	0.06	-0.62, 0.74	0.19	0.85
Sigmoid display propensity	AC x Period x Population	GLMM	0.13	-0.79, 1.06	0.28	0.78
Antipredator behaviours rates	AC x Population	GLM	-0.63	-1.55, 0.25	-1.40	0.16


(a)
Aggression attempts/male
0.6
0.5
0.4
0.3
0.2
0.1
0
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(b)
Aggression attempts/male
2.5
2
1.5
1
0.5
0
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio

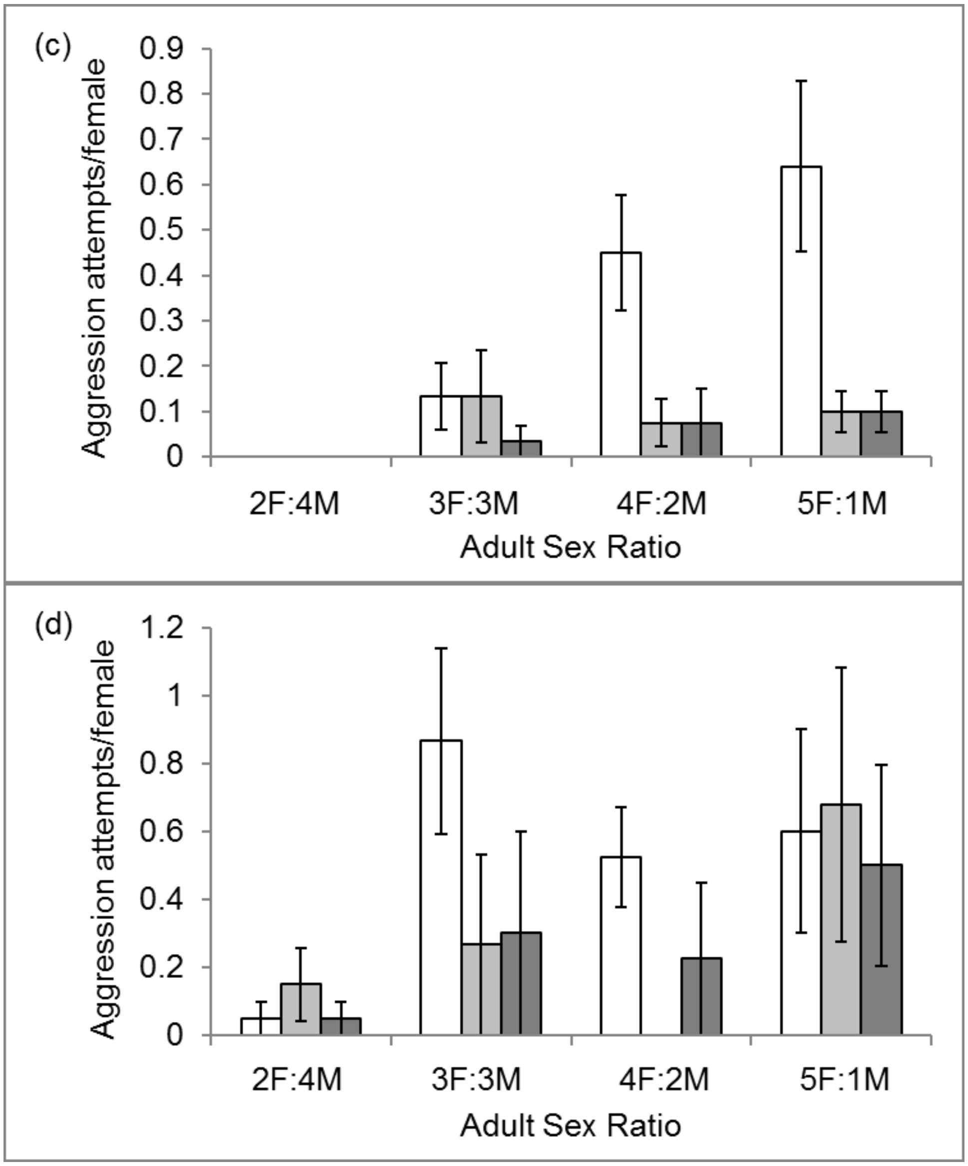
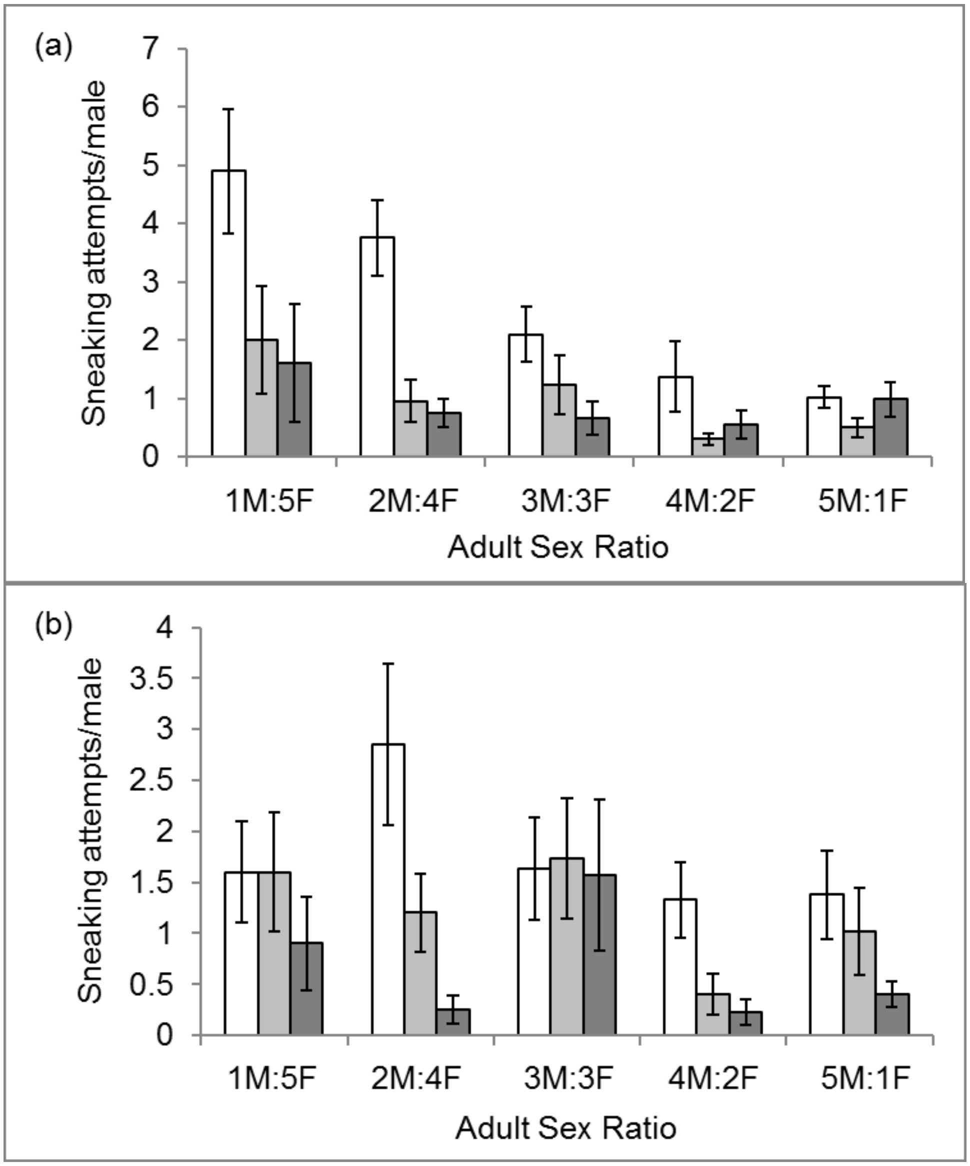


Fig. 3.1 Mean (± SE, *N*=10) frequency of intrasexual aggressive acts per individual male (*M*) from the (a) Lower (high risk) and (b) Upper (low risk) Aripo Rivers, and per individual female (*F*) from the (c) Lower (high risk) and (d) Upper (low risk) Aripo Rivers under different adult sex ratios (number of adult members of the observed sex/number of adult members of the opposite sex) and exposed to either dechlorinated water (*open bars*), 25% diluted (*light gray bars*) or 100% (*dark gray bars*) of the stock concentration of conspecific alarm cue over the 5-min period following stimulus injection


(a)
Sneaking attempts/male
0
1
2
3
4
5
6
7
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(b)
Sneaking attempts/male
0
0.5
1
1.5
2
2.5
3
3.5
4
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio

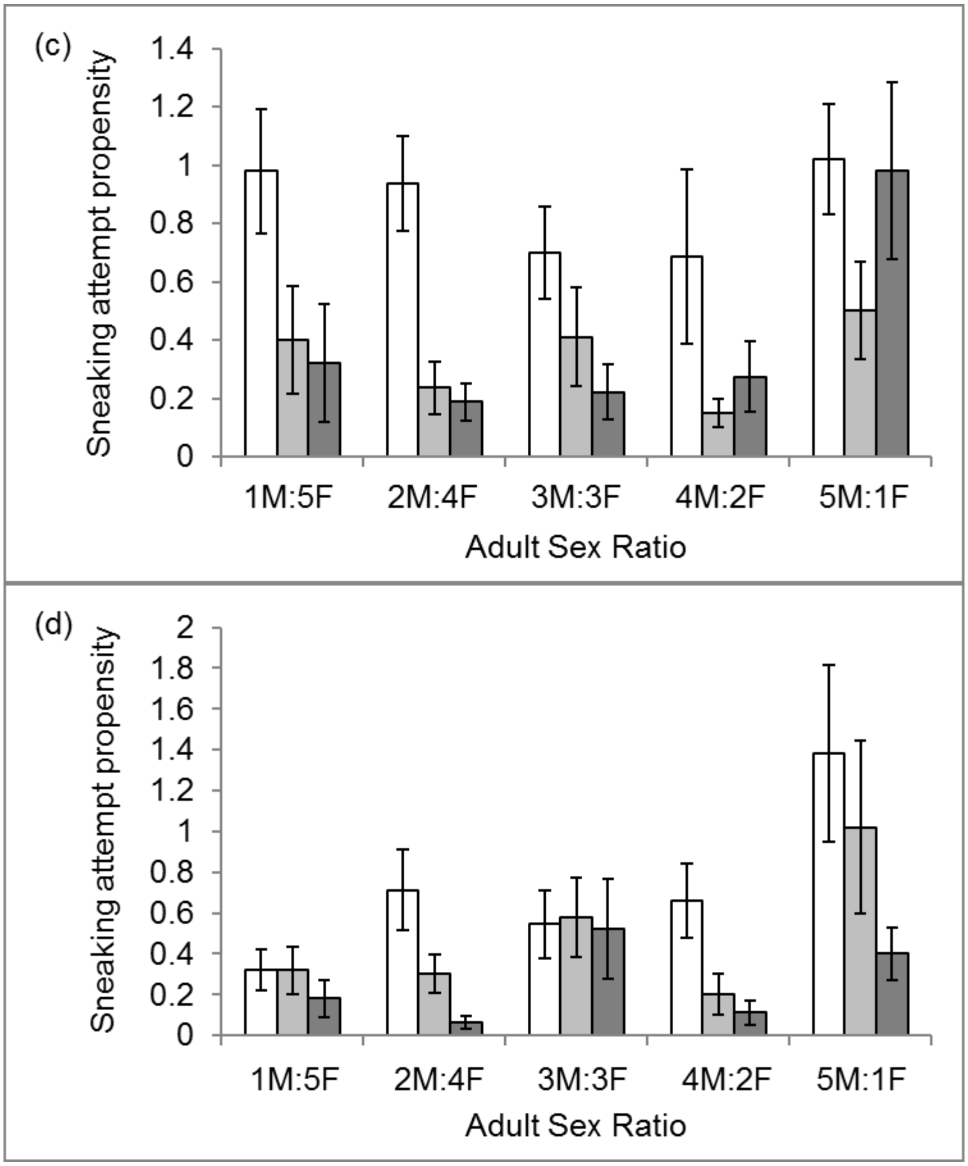


Fig. 3.2 Mean (± SE, *N*=10) frequency of sneaking attempts per individual male (*M*) from the (a) Lower (high risk) and (b) Upper (low risk) Aripo Rivers, and per individual male corrected for the number of females (*F*) present (propensity) from the (c) Lower (high risk) and (d) Upper (low risk) Aripo Rivers under different adult sex ratios (number of adult members of the observed sex/number of adult members of the opposite sex) and exposed to either dechlorinated water (*open bars*), 25% diluted (*light gray bars*) or 100% (*dark gray bars*) of the stock concentration of conspecific alarm cue over the 5-min period following stimulus injection

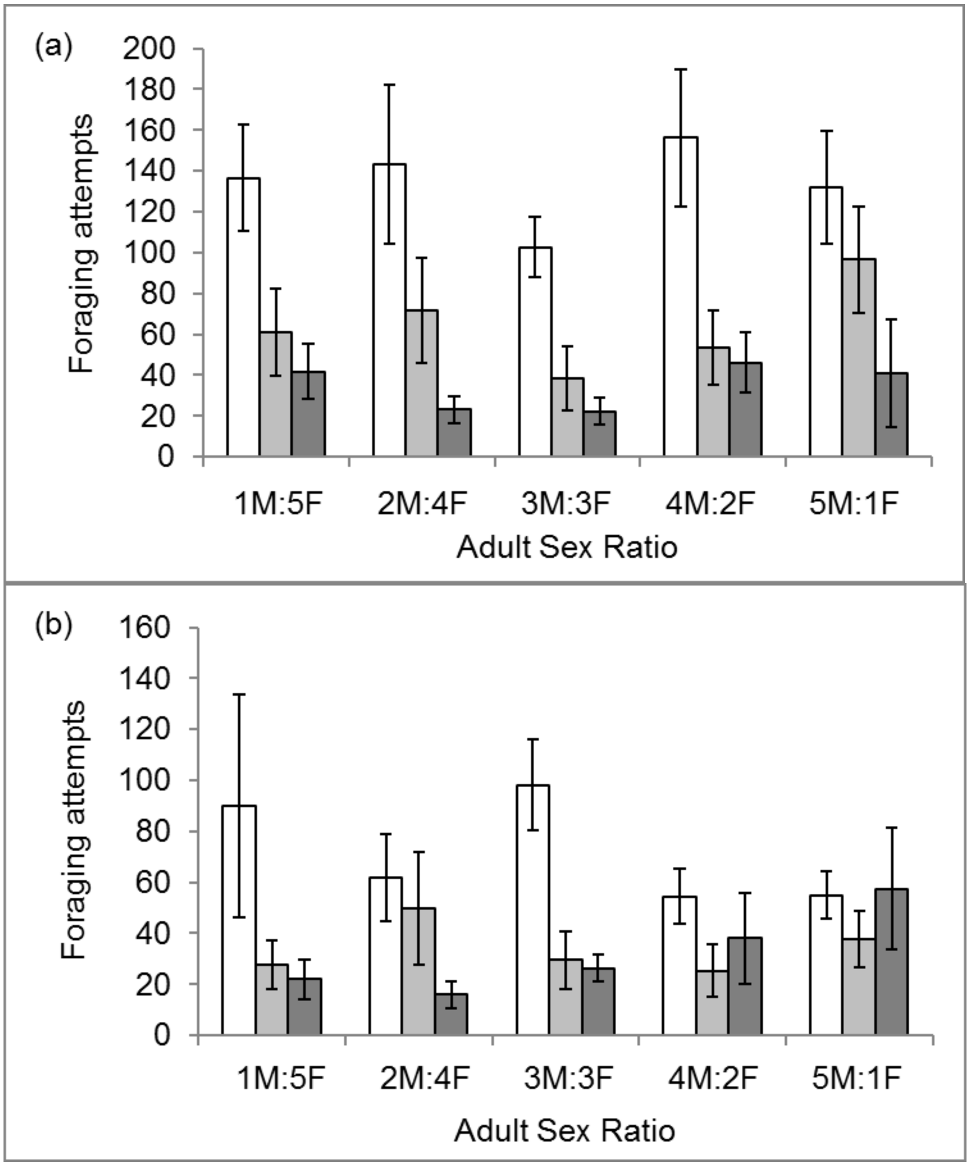
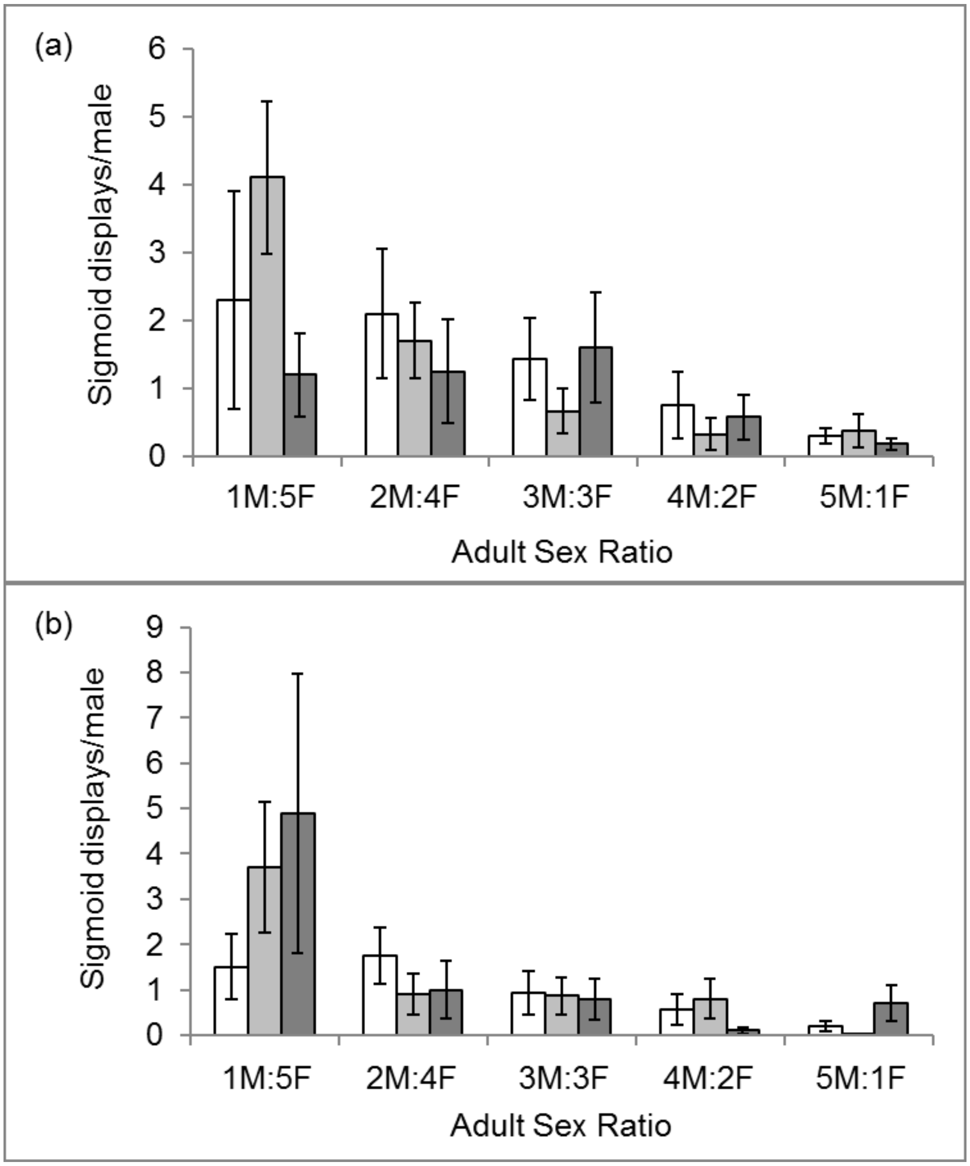


Fig. 3.3 Mean (± SE, *N*=10) frequency of foraging attempts by six individual guppies from the (a) Lower (high risk) and (b) Upper (low risk) Aripo Rivers, under different adult sex ratios (number of male (*M*) adult members/number of female (*F*) adult members) and exposed to either dechlorinated water (*open bars*), 25% diluted (*light gray bars*) or 100% (*dark gray bars*) of the stock concentration of conspecific alarm cue over the 5-min period following stimulus injection


(a)
Sigmoid displays/male
0
1
2
3
4
5
6
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(b)
Sigmoid displays/male
0
1
2
3
4
5
6
7
8
9
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio

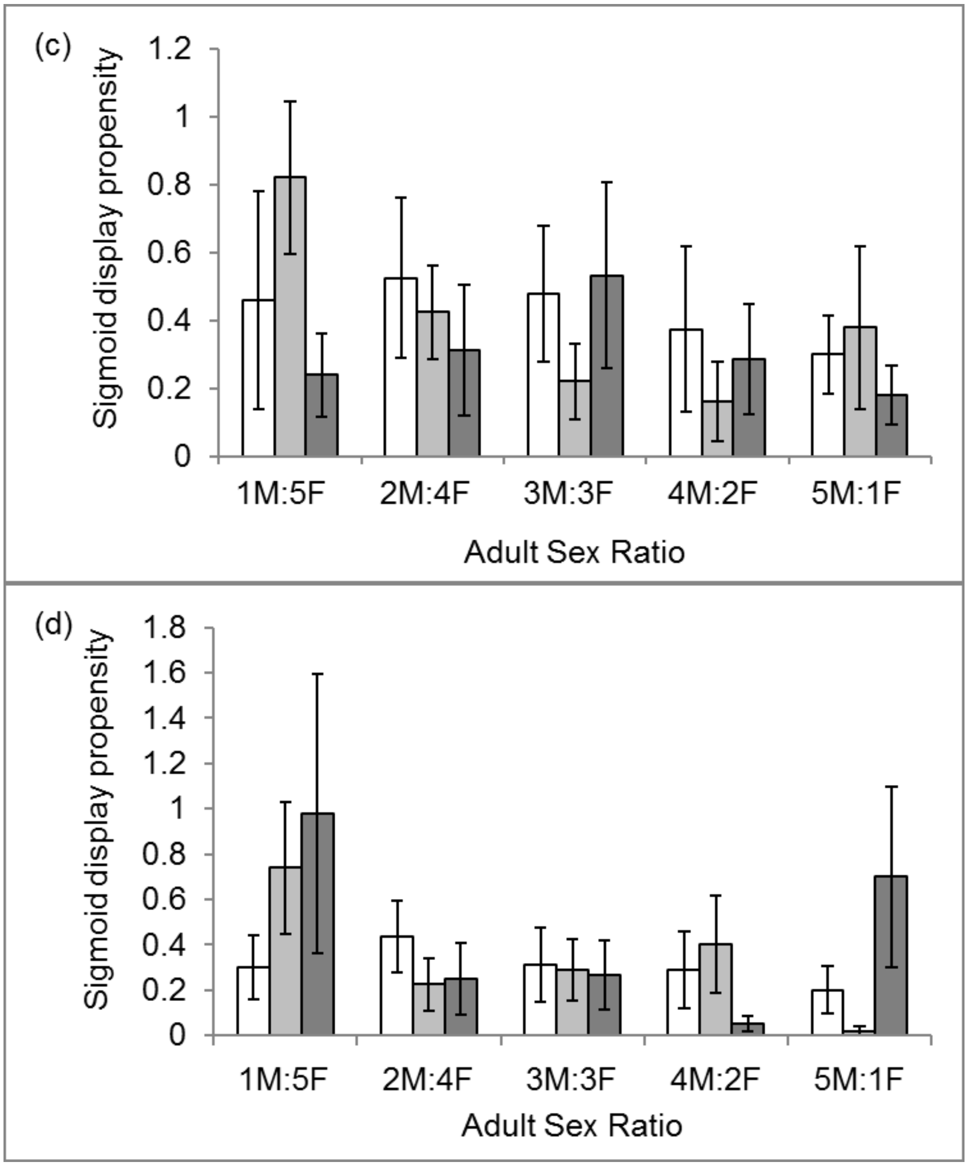
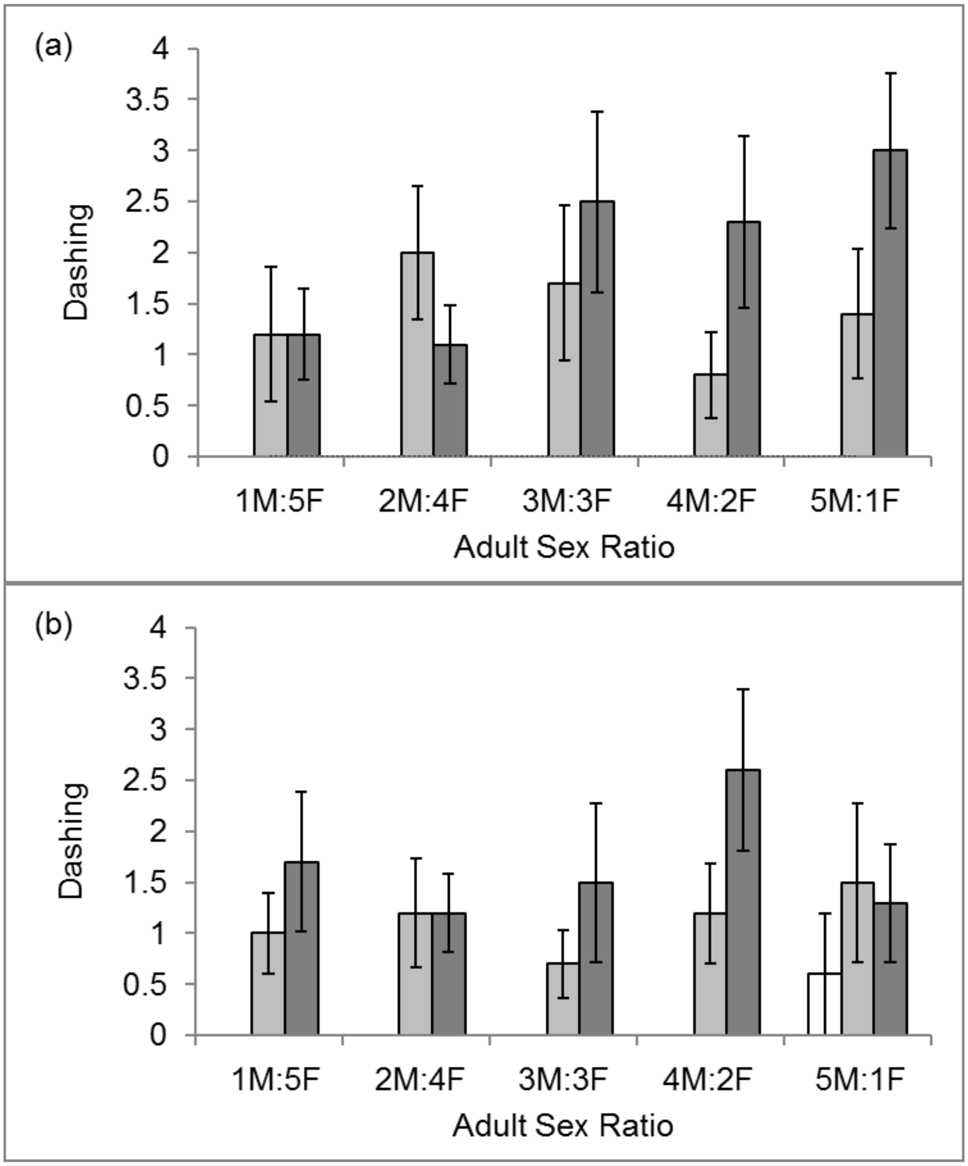
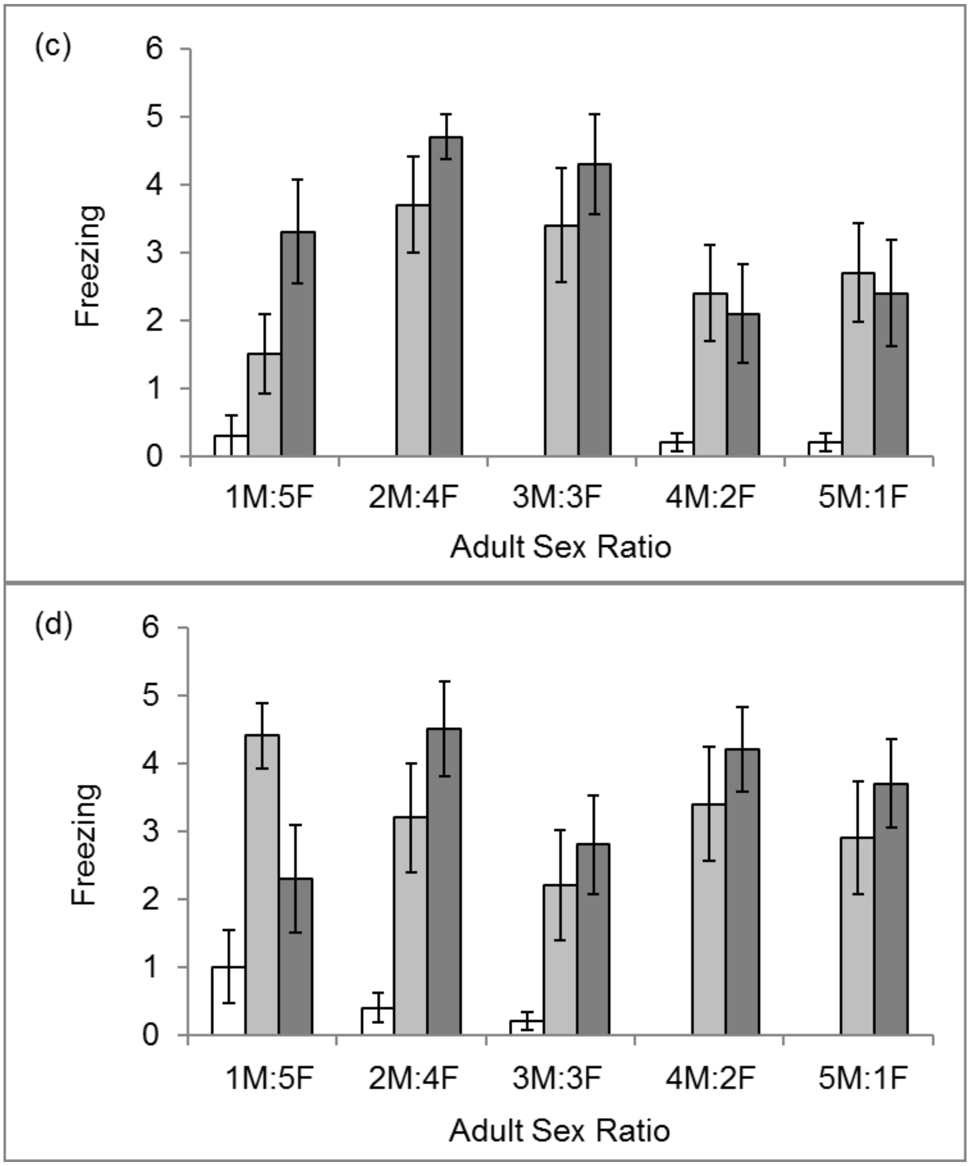


Fig. 3.4 Mean (± SE, *N*=10) frequency of sigmoid displays per individual male (*M*) from the (a) Lower (high risk) and (b) Upper (low risk) Aripo Rivers, and per individual male corrected for the number of females (*F*) present (propensity) from the (c) Lower (high risk) and (d) Upper (low risk) Aripo Rivers under different adult sex ratios (number of adult members of the observed sex/number of adult members of the opposite sex) and exposed to either dechlorinated water (*open bars*), 25% diluted (*light gray bars*) or 100% (*dark gray bars*) of the stock concentration of conspecific alarm cue over the 5-min period following stimulus injection


(a)
Dashing
0
0.5
1
1.5
2
2.5
3
3.5
4
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(b)
Dashing
0
0.5
1
1.5
2
2.5
3
3.5
4
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio


(c)
Freezing
0
1
2
3
4
5
6
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(d)
Freezing
0
1
2
3
4
5
6
1M:5F
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio

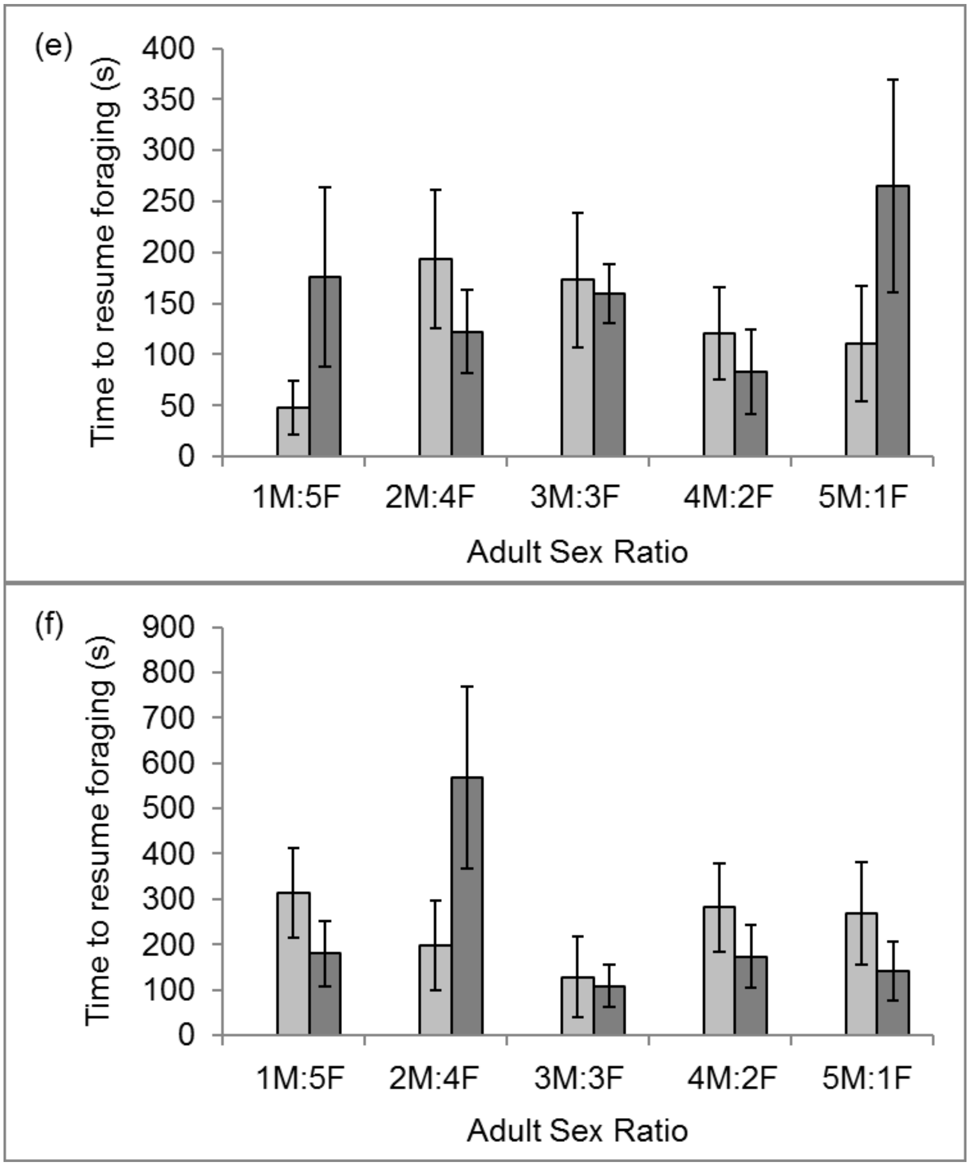


Fig. 3.5 Mean (± SE, *N*=10) frequency of three measures of antipredator behaviour by 6 individual guppies: dashing from (a) Lower (high risk) and (b) Upper (low risk) Aripo individuals, freezing from (c) Lower (high risk) and (d) Upper (low risk) Aripo individuals, and time to resume foraging in seconds from (e) Lower (high risk) and (f) Upper (low risk) Aripo individuals, under different adult sex ratios (number of male (*M*) adult members/number of female (*F*) adult members) and exposed to either dechlorinated water (*open bars*), 25% diluted (*light gray bars*) or 100% (*dark gray bars*) of the stock concentration of conspecific alarm cue over the 5-min period following stimulus injection

Chapter 3: Competition for food in two populations of wild-caught Trinidadian guppies, *Poecilia reticulata*

Introduction

Interference for food is common when it is clumped and predictable in space, and at intermediate levels of abundance (e.g. Schmidt et al. 1988; Grand and Grant 1994; Weir and Grant 2004; Hodge et al. 2009; Tanner et al. 2011; Morandini and Ferrer 2015). Just as operational sex ratio may predict the intensity of competition for mates (Weir et al. 2011), competitor-to-resource ratio (CRR), the ratio of individual competitors to the number of resource units available (Grant et al. 2000), may predict the intensity of competition for food (Noel et al. 2005). The rate of competitive aggression is thought to peak at intermediate values of CRR, approximately 2, with a decrease in aggression rates as resource units become relatively abundant or scarce (Grant et al. 2000; Noel et al. 2005). When the resource is abundant, any time spent on agonistic behaviour is time that could be devoted to foraging. Conversely, if the resource is too scarce, the cost of aggression exceeds the potential gain in foraging opportunities (Brown 1964), resulting in a decrease in aggression rates (Fig. 1.1; Grant et al. 2002; Toobaie and Grant 2013).

The non-consumptive effects of predation strongly affect the behaviour of potential prey organisms (Preisser et al. 2005). The risky competition hypothesis predicts a decrease in intraspecific aggression rates under high predation risk (Chapter 1; Qvarnstrom et al. 2012) as the result of a trade-off between conspicuously competing for limited resources and vigilance towards potential predators (Huntingford 1982). Moreover, aggressive behaviour makes individuals more vulnerable to predation (Toscano et al. 2010). In the case of foraging competition, intraspecific aggression rates are typically lower within populations experiencing high vs. low ambient predation pressures (e.g. Magurran and Seghers 1991; Herczeg and Valimaki 2011; Heinen et al. 2013). Alternatively, the risk-allocation model (Lima and Bednekoff 1999; Ferrari et al. 2009) suggests higher fitness-related activity rates (e.g. foraging, mating) in populations experiencing high vs. low ambient predation risk in the absence of an imminent predation risk. For instance, in the absence of an imminent risk, female sand tilefish *Malacanthus plumieri* from high predation-risk sites have higher foraging rates than their low predation-risk counterparts (Baird and Baird 2006; see also Magurran and Seghers 1994)

Recent findings suggest an interaction between CRR, ambient predation risk, and sex on mating competition in Trinidadian guppies (Chapter 1). Both males and females typically increased their aggression rates towards same-sex individuals as the relative number of mate decreased. We are not aware of any study on the simultaneous effects of CRR and ambient predation risk on foraging competition that directly compares males to females. Here, we explored whether similar patterns of competition were observed in a foraging context. When competing for mates, males are typically more aggressive than females (Clutton-Brock and Parker 1992) due to the higher reproductive rates of males compared to females. Conversely, female-female competition may be more prevalent in a foraging context (e.g. Nummelin 1988; Uccheddu et al. 2015) because body size is usually more strongly related to reproductive success in females than in males (Charnov 1993).

Based on observations of intrasexual competition of wild-caught male and female Trinidadian guppies, from a high vs. low ambient predation-risk population, and under different food CRRs, we tested the following predictions (Table 4.1). (1) Individuals will increase their aggression rates as CRR initially increases (Grant et al. 2000), and will decrease their aggression rates at CRRs greater than 2 due to competition (Weir et al. 2011). Because Trinidadian guppies display conventional sex roles (Houde 2001), (2) females will be more aggressive than males when competing for food. Individuals from the high vs. low ambient predation-risk population will be (3) less aggressive, and (4) forage less. Alternatively, following the risk-allocation model (Lima and Bednekoff 1999), we expect the opposite of predictions 3 and 4 if the absence of an imminent predation risk indicates a ‘safe period’.

Materials and Methods

Collection and Holding of Individuals

To test the effect of ambient predation risk, we used wild-caught individuals from two populations: high vs low levels of background predation risk. The Upper Aripo River, a low-risk population (Croft et al. 2006; Botham et al. 2008), experiences predation from two species which prey upon newborns, juveniles and small male guppies: Hart’s rivulus, *Anablepsoides hartii*, and a freshwater prawn, *Macrobrachium crenulatum* (Endler and Houde 1995; Walsh and Reznick

2009). Further downstream, the Lower Aripo River population has a high-background predation risk (Croft et al. 2006) with species preying upon both adult and juvenile guppies. These predators include, but are not limited to: pike cichlids, *Crenicichla sp.*; blue acara cichlids, *Andinoacara pulcher*; and, brown coscorub cichlids, *Cichlasoma bimaculatum* (Croft et al. 2006; Botham et al. 2008; Brown et al. 2009). While high ambient predation-risk sites tend to correlate with low guppy densities and high stream productivity (Grether et al. 2001), we will refer to the Lower Aripo and Upper Aripo populations as "high predation" and "low predation" sites for now (see Discussion, "Population differences").

We collected guppies using seine nets between April 29th and June 7th 2013 throughout the duration of the experimental trials. We transported fish to the laboratory, a 45-min drive, in 30-L buckets filled with 30-40 guppies and approximately 10 L of water from the individuals' original river. We held the fish under natural day light conditions in 100-L aquaria at ~24°C for a maximum of 40 days. The standard lengths (± SD) of individuals by sex and population were 18.2 ± 1.2 mm for males and 19.1 ± 4.8 mm for females in the Upper Aripo and 14.6 ± 1.1 mm for males and 15.3 ± 3.1 mm for females in the Lower Aripo. Unless being tested in the next 24 h, fish were fed commercial flakes (TetraMin^{TM} provided by Tetra, 3001 Commerce St., Blacksburg, VA) and brine shrimp twice daily. We released guppies back to their original rivers using hand nets, and following the same procedure as their collection.

Experimental Procedure

To enhance foraging competition, we did not feed individuals in the 24 h preceding observations. The day before testing, we made defendable patches of food by dipping standard microscope slides (75 x 25 mm) into unflavoured gelatine (Indulge^{TM}, General Foods Corporation, 250 North Rd, White Plains, NY) using about 20 g gelatine/100 mL water. We applied flake food (Tetramin^{TM}), fragmented into smaller pieces, to a square area (25 x 25 mm) at the centre of one side of the slide and allowed the gelatine to set. Enough food was applied for the patches to last for the entire length of a trial (10 min). We observed males and females separately from each population to avoid any confounding effect of mating competition (Nordell 1998). To manipulate CRR, we used 4 fish exposed to 5, 3 or 1 food patches (i.e. CRRs = 0.8, 1.3, or 4). Thus, we used a 3-way factorial design (i.e. 2 populations x 2 sexes x 3 CRRs) with 30 replicates of each. Fish were only used once, for a total of 1,440 individuals. The sequence of trials was randomized for treatments.

We placed four individuals from the same holding tank in a test tank (45 x 30 x 30 cm) and allowed them one hour to acclimate. The slides were introduced 10 min before the beginning of observations. We removed loose flakes by blowing on slides before introducing them into test tanks. In the one-patch treatment, the single slide was placed on the substrate, in the centre of the tank. For the 3 and 5-patches treatments, slides were placed evenly across the tank, at least 25 mm from any side of the tank (Appendix 2.1). The observer recorded behaviour from the front of the tank; we covered the outside of the remaining sides with white plastic sheets to prevent disturbance. A single observer (PJC Chuard) recorded behaviour for 10 min, divided into two 5-min periods. Guppies were individually identified by a combination of colour patterns, size and shape. For each period, we observed the four fish in a randomised sequence for 75 s, which was summed for the two periods.

We recorded the frequency of agonistic behaviour, performed and received separately, including chasing, biting (Gorlick 1976), pushing (Magurran and Seghers 1991) and tail beating (Liley 1966). In addition, the frequency of foraging was quantified, defined as when an individual pecked directly on a food patch, or pecked within one body length of a patch as food might be found here quickly after the beginning of a trial (i.e. flakes detached from the patch due to foraging).

Statistical Analysis

We performed all analyses using generalised linear mixed-models (GLMM) in the R software (3.1.2; R Development Core Team 2015) with the *glmmadmb()* function of the *glmmADMB* package. We ran each model fitted to the Poisson distribution and tested for over-dispersion. If we detected over-dispersion, we ran the model fitted to the negative binomial distribution, which is known to efficiently handle over-dispersed data (Lindén and Mäntyniemi 2011), and tested the negative binomial model for over-dispersion to validate it (*P*>0.99). We used population, sex and CRR (linear and quadratic contrasts, see Chapter 1) as fixed factors in all analyses, and trial number as a random factor in all GLMM analyses. In addition, due to the predicted sex and population differences (Table 4.1), we analysed each population by sex separately.

First, using GLMM, we tested total aggression rate per fish (given and received aggression combined) fitted to a negative binomial distribution. Second, we analyzed total foraging rates fitted to a negative binomial distribution. We reported GLMM statistical outcomes in the following way: (β, [95% CI], z score, probability) where β represents the regression coefficient and CI the confidence intervals. For those analyses, in the absence of a significant difference between sexes or populations, we reported the statistics of the general model including sex and population as fixed factors. Moreover, we only reported unpredicted interactions if significant. Since we based our tests on a priori predictions, we did not apply any statistical correction to our tests (see Moran 2003).

Results

Contrary to our three first predictions, overall aggression rates (Fig. 4.1) were not significantly affected by CRR (linear contrast GLMM: β=0.12, CI=[-0.17, 0.41], z=0.80, P=0.42; quadratic contrast GLMM: β=-0.081, CI=[-0.37, 0.21], z=-0.54, P=0.59), sex (GLMM: β=-0.0031, CI=[-0.24, 0.24], z=-0.030, P=0.98), nor population of origin (GLMM: β=-0.010, CI=[-0.25, 0.23], z=-0.08, P=0.93). Consistent with our fourth prediction, following the risk-allocation model (Lima and Bednekoff 1999), foraging rates were higher in the Lower Aripo population (high predation) than in the Upper Aripo population (low predation) (Fig. 4.2; GLMM: β=-0.49, CI=[-0.83, -0.14], z=-2.75, P=0.0060). Conversely, CRR (Fig. 4.2; GLMM: β=-0.11, CI=[-0.53, 0.31], z=-0.50, P=0.62) and sex (Fig. 4.2; GLMM: β=0.31, CI=[-0.028, 0.66], z=-1.80, P=0.072) had no significant effects on foraging rate.

Discussion

Overall, our results support one of our four original predictions (Table 4.1). Surprisingly, CRR, sex, and population of origin did not have a significant effect on aggression rates among guppies competing for access to foraging patches. Rather, foraging rates followed the risk-allocation model (Lima and Bednekoff 1999) with higher foraging rates in Lower Aripo (high predation) than in Upper Aripo guppies (low predation). These results suggest that decreasing food availability at constant competitor density does not affect aggression rates in guppies. However, the effect of elevated ambient predation risk seems to select for individuals able to forage more when an imminent risk is absent.

These results contrast with our results from Chapter 1 where both CRR and ambient predation risk had an effect on aggression rates in a mating competition context. Indeed, aggression rates increased as CRR increased, and Upper Aripo guppies were more aggressive than their Lower Aripo counterparts as expected under the risky-competition hypothesis (Chapter 1). However, similar to our findings in a foraging context, males and females did not differ significantly in aggression rates. The most notable difference between the two experiments was the observed rates of aggression, which were more than three times greater in the food- rather than the mating-competition experiment. Perhaps fixed food patches are easier to monopolize and defend than mobile mates, resulting in a greater pay-off for individuals who invest energy in aggressive behaviour when competing for food.

Competitor-to-resource ratio

Unlike Magurran and Seghers (1991), we found no effect of CRR on aggression rate. Note that they manipulated CRR by increasing the number of guppies foraging on a single food patch, whereas we decreased the number of food patches while holding the number of competitors constant, at four. A possible explanation for this discrepancy is that aggression rates increased significantly above a CRR of 4 in Magurran and Seghers' (1991) experiment, well above the range of CRR in our study. In addition, the food patches might have been close enough together in our experiment, so that a dominant fish could defend them against three other competitors, resulting in no decrease in aggression rates at a CRR greater than 2. This explanation is supported by the high rates of aggression in our experiment compared to Chapters 1 and 2. Because an increase in density leads to an increase in aggression rates (Magurran and Seghers 1991) but a decrease in the number of food patches does not, individuals in the wild might be more sensitive to seasonal changes in density (Reznick 1989). By contrast, there is no strong evidence that food availability varies across seasons in Trinidadian streams (Magurran 2005).

Sex roles

Similar to Magurran and Seghers (1991), we found no difference in aggression and foraging rates between males and females. While male guppies forage just enough to satisfy their immediate hunger (Griffiths 1996), females forage longer to produce eggs and provide to embryos (Magurran 2005). Given that individuals fasted for 24 h before testing, it is possible that a 10-min observation period was not sufficient for males to start reducing their foraging rates, and associated aggression rates, compared to females. For example, after at least a 3-hour fast, male guppies switched from primarily feeding to courting after about 10 min (see Fig. 3 in Abrahams (1993)).

The higher foraging rates at intermediate CRR in Upper Aripo females suggested that the dominant individual might have been securing the food patch at the cost of foraging opportunities at high CRR. At intermediate CRR, dominant individuals were likely able to defend patches and still forage, thus providing some opportunities for subordinates to feed when the dominant individuals were foraging as well. On the other hand, at low CRR, individuals that are subordinate at higher CRRs get a chance to monopolize a patch and might chose to remain vigilant towards potential competitors rather than forage. Such attempts from subordinates to forage were not detected in males, which is consistent with the fact that foraging is more important for females than males, given that bigger females are usually more fecund (Charnov 1993).

Population differences

The Upper Aripo population (low predation) was not more aggressive than the Lower Aripo population (high predation). These results do not support the risky competition hypothesis (Chapter 1; see also Magurran and Seghers 1991). In Magurran and Seghers' (1991) study, the Upper Aripo population was not included as a low-risk site. That population has been shown to be less behaviourally plastic than other low ambient predation-risk populations (Kolluru et al. 2015), which might explain the non-significant difference between our two populations. However, those two same populations have been found to differ in aggression rates related to mating competition (Chapter 1). As courtship display has the potential to attract predators (Zuk and Kolluru 1998), being aggressive near individuals performing sigmoid displays might be riskier than in a foraging context, especially for Lower Aripo individuals (high predation), hence why they might have shown similar aggression rates as Upper Aripo individuals (low predation) in a foraging context, but differed in a mating context (Chapter 1).

Foraging rates between populations were consistent with the predictions of RAH (Lima and Bednekoff 1999); Lower Aripo individuals (high predation) foraged more than Upper Aripo guppies (low predation), as in Chapter 2. In the absence of an imminent risk of predation, individuals from a high vs. low ambient predation-risk site tended to compensate for lost foraging opportunities during previous periods of high imminent predation risk. However, due to the cost of predation, high ambient predation-risk guppies seem to spend less time foraging than their low ambient predation-risk counterparts (Magurran and Seghers 1994), suggesting selection for individuals able to forage faster under high ambient predation risk to save more time for antipredator behaviour.

Our conclusions cannot be limited to the effect of ambient predation risk alone, as low ambient predation-risk streams tend to have higher guppy densities (as a direct effect of predation) and lower productivity (Grether et al. 2001), resulting in higher competition for food. Differences in productivity could then act as a factor selecting for high vs. low productivity-adapted behaviours (Walsh and Reznick 2010). These adaptations lead to different energy allocation trade-offs between foraging competition and antipredator behaviour, similar to the effect of ambient predation risk. Indeed, consistent with our results, individuals inhabiting low-productivity streams (low predation) should invest more energy in competing for food instead of foraging, and less energy into antipredator behaviour compared to populations living in high-productivity streams (high predation; Magurran and Seghers 1991; but see Kolluru et al. 2007).

In conclusion, food density did not seem to affect intrasexual aggression rates in guppies. Ambient predation risk reflects, at least in parts, the long-term exposure to imminent predation risk (Brown et al. 2006). Consequently, it would be of great value to explore aggression rates in relation to food under varying risks of imminent predation, in populations that also vary in ambient predation risk.

Table 4.1. Predictions and results based on the effects of CRR[a], sex, and ambient predation-risk population differences on foraging competition

Explanatory variables	Predictions	Results[b]
As CRR increases	(1) Intrasexual aggression rate increases	False
Sex roles	(2) Intrasexual aggression rate is greater in females than in males	False
High vs. low ambient predation-risk population	(3) Intrasexual aggression rate is lower or higher[c]	False
	(4) Foraging rate is lower or higher[c]	True – higher

[a] CRR is defined here as the ratio of individual competitors over the number of food patches available.

[b] True means the results were in accordance with the predictions (i.e. $P<0.05$). False means no evidence of an effect.

[c] Activities expected to decrease if the cost of ambient predation risk is high OR increase in the absence of a perceived imminent predation risk as it would indicate a ‘safe’ period, as predicted by the risk allocation model (Lima and Bednekoff 1999).

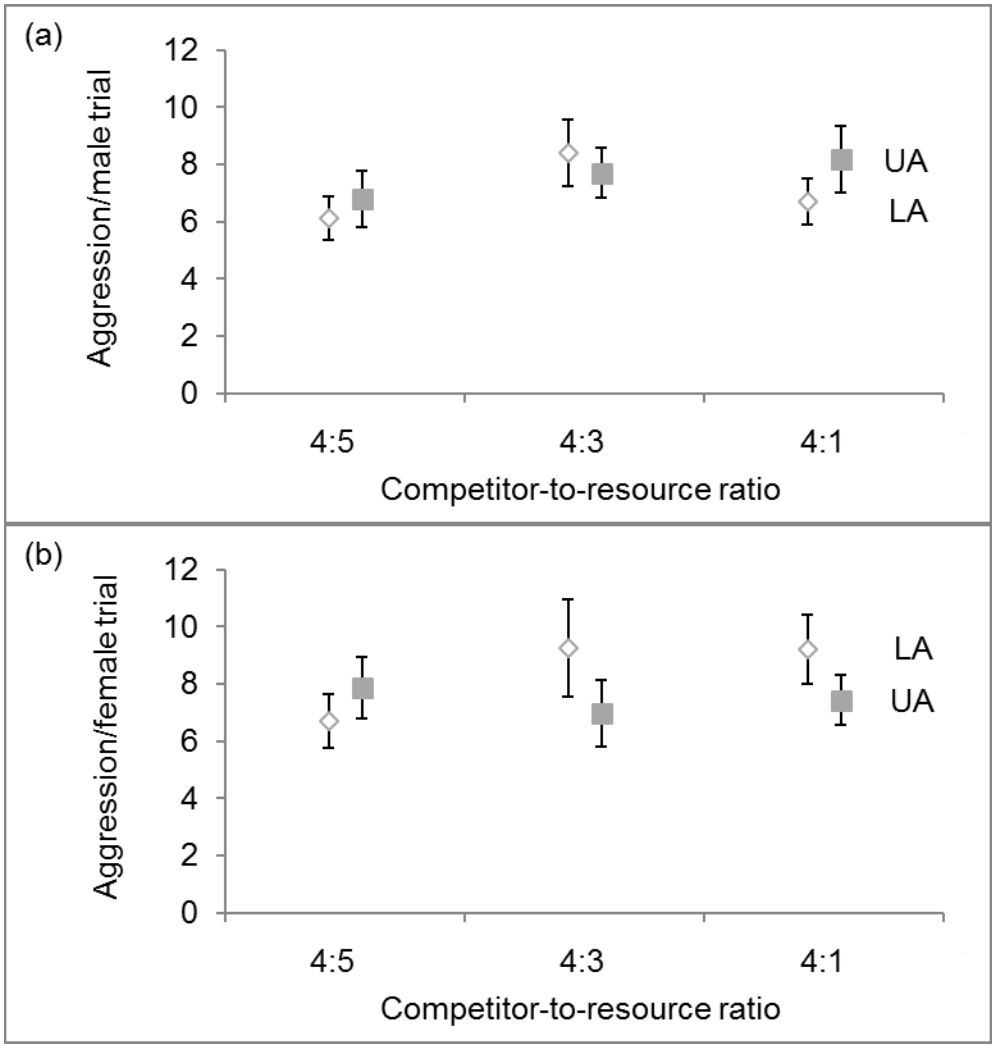


Fig. 4.1. Mean (± SE, N=30) aggression rate, sum of given and received, per trial in relation to three competitor-to-resource ratios (four individuals competing for 5, 3, and 1 food patches, respectively 0.8, 1.33, 4) and two populations of origin: Lower Aripo (*LA*; *open diamonds*; high predation) and Upper Aripo (*UA*; *shaded squares*; low predation) in (a) males and (b) females

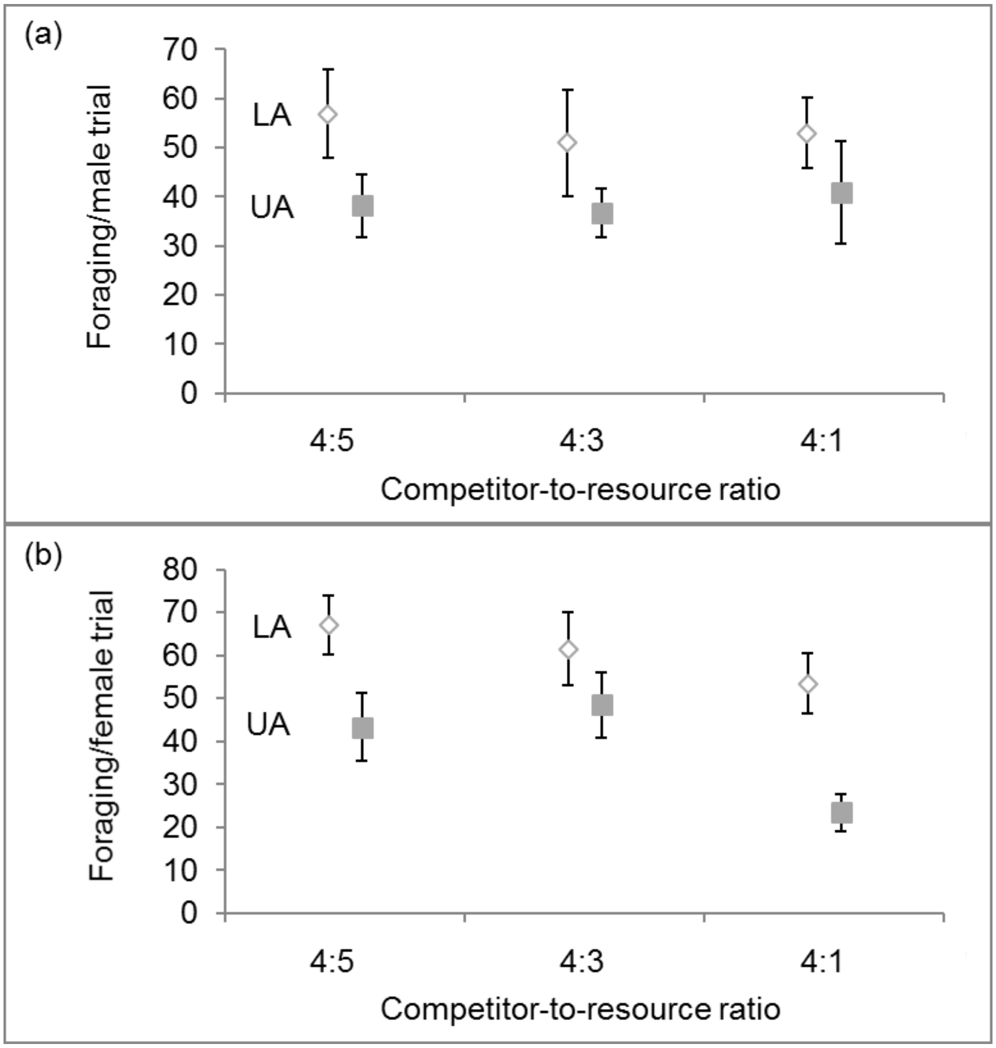


Fig. 4.2. Mean (± SE, N=30) foraging rate per trial in relation to three competitor-to-resource ratios (four individuals competing for 5, 3, and 1 food patches, respectively 0.8, 1.33, 4) and two populations of origin: Lower Aripo (*LA*; *open diamonds*; high predation) and Upper Aripo (*UA*; *shaded squares*; low predation) in (a) males and (b) females

Chapter 4: Mating competition and the range of adult sex ratio in wild guppies

Introduction

Most manipulations of ASR (more specifically OSR) to test mating systems theory have been conducted under laboratory conditions (Weir et al. 2011) using OSRs that may not mimic natural conditions, especially since not all females are receptive in the wild at any given time. For example, a review from Weir et al. (2011) on the effect of OSR on mating competition identified only 7 field studies, compared to 67 in the laboratory. Most laboratory experiments have focused on narrower ranges of OSR than what was found in wild conditions (Weir et al. 2011), thus potentially missing some important behavioural patterns typical of extremely biased OSRs. Moreover, how prey species perceive, integrate and respond to ambient predation risk in the wild is one of the most important unanswered questions in the field of predator-prey dynamics (Lima and Steury 2005). In addition, as behaviour can be influenced by environmental factors not present in the laboratory (Losos and Irschick 1996), such as predation risk, it is important to conduct experiments in the field to identify potential laboratory effects on behaviour. The limitations of having a literature dominated by laboratory studies (e.g. Lima and Steury 2005; Weir et al. 2011) to interpret natural behavioural patterns is the risk of generalizing in-situ patterns potentially resulting from laboratory artifacts.

All eight existing studies on the effect of ASR on guppies were performed in the laboratory (Jirotkul 1999a; 2000; Head and Brooks 2006; Head et al. 2008; Barbosa and Magurran 2010; Jha 2011; Kiritome et al. 2012; Chapter 1). Among those experiments, only Chapter 1 covered the range of ASR found in wild guppy populations, even if the treatment with an ASR of 5 was too male-biased and outside the range found in natural populations (mean: 0.95; range: 0.20-3.55; Pettersson et al. (2004)). The 7 other studies used ranges of OSR that were too female-biased due to the use of only virgin females and did not cover most of the male-biased part of the natural ASR range (Table 5.1; Jirotkul 1999a; 2000; Head and Brooks 2006; Head et al. 2008; Barbosa and Magurran 2010; Jha 2011; Kiritome et al. 2012). Replicating Chapter 1's experiment in the field will allow for direct comparison of the effects of in-situ vs. ex-situ experimental conditions on behaviour.

In addition, ranges of ASR (used as a proxy for OSR, see Chapter 1) in wild guppy populations have rarely been described (Croft et al. 2003; Pettersson et al. 2004) even though they are likely of primary importance for predicting behaviour occurring in the wild (Rezucha and Reichard 2014). The most recent study by Pettersson et al. (2004) suggest female-biased ASRs in our two study populations, likely due to weaker immune system of male guppies. The authors also suggest that males are more susceptible to predation due to their conspicuous colour and smaller size, and their lower threat-sensitivity compared to females (Magurran 2005), which might also explain female-biased ASRs. Based on Pettersson et al.'s (2004) observations, our range of experimental ASRs used in Chapter 1 should cover the natural range of ASR. Identifying the range of ASR found in natural pools in our study populations is key to interpret the results of our laboratory experiment within a natural and sexual selection framework. In order to better identify the factors influencing competition and describe OSR range in wild guppy populations, this chapter will attempt to replicate a simpler version of Chapter 1's experiments in the field, combined with ASR sampling from wild pools. In addition to the nine predictions in Table 5.2 that are the same as in Chapter 1, we tested the prediction that the range of ASR in the wild should be female-biased overall, and be covered by our range of experimental ASRs.

Materials and Methods

Experimental design

To estimate the range of ASRs within each population, we sampled individual pools using seine nets. If both sexes were present, we stored the sampled individuals for the mating competition experiments (see below) in 30-L buckets filled with 10 L of river water. We placed a maximum of 30-40 fish per bucket and changed the water every 45 min to avoid changes in temperature and oxygen depletion. We used a total of seven pools in the Lower Aripo in 2014, and eight pools in 2015 that we sampled once (Table 5.3). In the Upper Aripo, we collected data on eight pools in 2014, and eight pools that we sampled once in 2015 (Table 5.3). We obtained a total of 20 ASR estimates from the Lower Aripo and 34 from the Upper Aripo in 2014. On April 24th 2015, we obtained an additional 8 ASR estimates from the Lower Aripo and 8 from the Upper Aripo. We estimated ASR in each pool by sampling the whole pool at once from one end of the pool to the next on average 2.5 times (range: 2-5) using 3-mm stretched-mesh seine nets (4 x 0.5 m) until most individuals were sampled, as indicated by a decrease in the number of individuals caught of 90% or more from one sampling attempt to the next.

We performed mating competition experiments in the wild, as described in Chapter 1, at both sites within the Aripo River (i.e. Lower Aripo and Upper Aripo) using transparent rectangular Plexiglas enclosures (40 x 30 x 30 cm) with a bottom placed on the river substrate. The experiment took place during the day between April 19th and May 1st 2014. We used three ASR (as a proxy for OSR, see Chapter 1) treatments (i.e. 1:5, 3:3, 5:1). To be comparable to laboratory data, the amount of water flowing through the enclosures was minimal. In any given trial, we used guppies from the same pool; allowing for higher chances of familiarity between individuals, similar to the laboratory experiment. We gave individuals a 5 min period to acclimate to the test enclosure before the start of observations. We used Go Pro™ cameras placed underwater outside the enclosure to obtain the same side view as the observer in the laboratory experiment. We placed a large, 3-mm stretched-mesh enclosure (6 x 0.5 m) around our Plexiglas enclosures to isolate guppies in our experiment from those swimming freely in the stream. We dispersed flake food as described in Chapter 1 (Tetramin™) uniformly over the surface 5 min before a trial. We measured the same behavioural traits as in Chapter 1 over 10 min. We trained two observers, in addition to PJC Chuard, to record behavioural data from the camera recordings. We tested for the observers' reliability after training by assigning one video recording known to contain all behaviours of interest to all three observers and compared their behavioural records. As we used scanning observation methods without replay, we allowed a maximum 10% difference between observers before considering them reliable. The two observers were trained until they reached that threshold. We performed 30 replicates per treatment.

Statistical analysis

For the range of ASR in the wild, we performed a GLMM factorial analysis as in Chapter 1 (see Statistical approach) under a Poisson distribution (not significantly over-dispersed: $P>0.99$), with population and year as factors, and pool as a random factor. For the behavioural experiment, we analyzed the independent variables as in Chapter 1 with population, sex, and ASR as factors, but with 3 ASR treatments (i.e. 1:5, 3:3, 5:1) instead of 5, under a negative binomial distribution (not significantly over-dispersed: *P*>0.99). However, in addition to trial number (ID) as a random factor, we included an extra random factor to the models: observer (Obs) to account for the 3 different observers who analysed the video recordings. We displayed the statistical outcomes as in Chapter 1: (β, [95% CI], z score, probability). As we found no significant dome-shaped relationship between ASR and aggression rates/mating tactic propensities in Chapter 1, we only reported quadratic contrasts if significant. In addition, unpredicted interactions were only displayed if significant.

Results

As predicted (Table 5.2), ASR in the wild was significantly female-biased overall (Fig. 5.1) in both the Lower Aripo (high predation; 0.46, [0.36, 0.56]) and the Upper Aripo (low predation; 0.54, [0.40, 0.68]). There was no significant difference in ASR between the two populations (Fig. 5.1; GLMM: *β*=0.12, CI=[-0.57, 0.81], *z*=0.34, *P*=0.73) and between years (GLMM: *β*=-0.38, CI=[-1.27, 0.51], *z*=-0.84, *P*=0.40). Eighty-four % of ASRs seem to be found in both populations between 0.2 and 1.0, which are included in our experimental ASR range (i.e. 0.2 to 5.0). Ninety-six % of the ASRs found in the field were 1:1 or lower, while in our experiments in the laboratory and the field, 60% and 67% of the ASRs were female-biased or balanced respectively.

Regarding how ASR affected behavioural rates, aggression rates increased as ASR increased in both sexes (Fig. 5.2; GLMM: *β*=0.73, CI=[0.11, 1.34], *z*=2.33, *P*=0.020), consistent with our first and second predictions and our laboratory findings. However, aggression rates were about 82% lower in the field (Fig. 5.2) than in the laboratory (Fig. 2.1). Contrary to our third prediction and our laboratory findings, courtship rates per male did not decrease as ASR increased (Fig. 5.3a; GLMM: *β*=-0.61, CI=[-1.46, 0.24], *z*=-1.41, *P*=0.16) whereas they did in the laboratory. Similar to our laboratory results, but contrary to our fourth prediction, courtship propensity did not increase with ASR (Fig. 5.3b; GLMM: *β*=0.53, CI=[-0.73, 1.78], *z*=0.83, *P*=0.41). Consistent with our fifth prediction and our laboratory findings, sneaking rates per male decreased as ASR increased (Fig. 5.4a; GLMM: *β*=-0.86, CI=[-1.46, 0.24], *z*=-3.52, *P*<0.001). However, contrary to our sixth prediction and laboratory results, sneaking propensity did not significantly increase with ASR (Fig. 5.4b; GLMM: *β*=0.30, CI=[-0.21, 0.81], *z*=1.17, *P*=0.24).

Regarding population differences, our results did not support the seventh prediction as there was no significant difference in aggression rates between the two populations (Fig. 5.2; GLMM: β=0.52, CI=[-0.24, 1.28], z=1.34, P=0.18). However, our laboratory findings supported the risky-competition hypothesis with lower aggression rates in the Lower Aripo population (high predation) compared to the Upper Aripo population (low predation). Conversely, according to our eighth prediction under the risky-competition hypothesis, reproductive attempt rates per male (i.e. courtship and sneaking combined) were lower in the Lower Aripo (high predation) than in the Upper Aripo (low predation; Figs. 5.3a; 5.4a; GLMM: β=0.62, CI=[0.098, 1.15], z=2.32, P=0.020). We had found no difference in reproductive attempt rates between the two populations in the laboratory experiment. Finally, contrary to our ninth prediction and similar to our laboratory findings, males were not more aggressive than females in a mating competition context (Fig. 5.2; GLMM: β=-0.66, CI=[-1.69, 0.38], z=-1.25, P=0.21).

Discussion

Despite being slightly male-biased compared to what is found in the wild, our range of experimental ASRs, both in the field and in the laboratory, represented the full range of ASRs encountered in the wild for Trinidadian guppies. Previous studies (Jirotkul 1999a; 2000; Head and Brooks 2006; Head et al. 2008; Barbosa and Magurran 2010; Jha 2011; Kiritome et al. 2012) typically tended towards female-biased ASRs. To our knowledge, our experiment is the first to have explored patterns of mating competitive patterns across a natural range of ASRs directly in the field in guppies, although only 2/3 of our experimental ASRs (i.e. 0.2 and 1.0) are likely to represent common ASR conditions. The range of ASR found within each population fell within the confidence interval of the range identified by Pettersson et al. (2004). Both populations were female-biased, likely due to the greater susceptibility of males to predation, and their weaker immune system compared to females (Magurran 2005). Most ASRs were between 0.2 and 1.0, which makes our laboratory experiment relevant for wild behaviour interpretations, especially concerning female-biased ASR treatments. In the wild, females are likely competing for males by being aggressive towards other females, potentially until subordinate females leave the pool.

Our field results were consistent with only four and five of our original nine predictions (see Table 5.2) and nine laboratory findings, respectively (see Table 2.1). These results show mixed support for OSR theory and the risky-competition hypothesis. Notably, as expected, intrasexual aggression rates increased in both sexes as ASR increased in both field and laboratory experiments. This result is consistent with Weir et al.'s (2011) review, which showed an increase in intrasexual aggression with OSR (see Chapter 1), up to a point where competitors are too numerous and aggression rates decrease. This decreasing trend at extremely biased ASRs was not observed in our experiments. In terms of mating tactic rates, as expected in species using alternative mating tactics (de Jong et al. 2009), courtship propensity remained unaffected by ASR, and sneaking rates per male decreased as ASR increased likely due to an increase in competition for the fewer females available in the form of male-male aggression (see Chapter 1), similar to our laboratory results. Moreover, we found no evidence of higher intrasexual aggression rates in males compared to females, contrary to our sex role predictions but similar to our laboratory findings. As discussed in Chapter 1, this might be due to the presence of food in the enclosures, for which females might compete. In addition, female-biased ASRs in the field in both populations might have selected for more competitive females (i.e. more aggressive).

One of our field results supported the original predictions but was not consistent with the laboratory experiment. Indeed, in support of the risky-competition hypothesis, Lower Aripo (high predation) males showed lower reproductive attempts rates than their Upper Aripo (low predation) counterparts, perhaps because the degree of neophobia increased in the Lower Aripo population (Elvidge et al. 2016). Neophobia is an increase in vigilance when individuals are faced with novel environments (e.g. food; Mettke-Hoffman et al. 2013). Due the very short acclimation time in the field compared to the laboratory experiment (see below), Lower Aripo individuals (high predation) might have been more neophobic to the novel context resulting in lower reproductive attempt rates. However, we did not find support for three of the findings of the laboratory experiment. Indeed, we found a lack of support for our sixth prediction for species using alternative mating tactics (see de Jong et al. 2009), as males did not decrease their per capita courtship rate and did not increase their sneaking propensity as ASR increased. Courtship rates were so low in the field (see below) that the effect of ASR might have been undetectable. Regarding sneaking propensity, the absence of an increase with ASR might indicate a trade-off between securing mates as competition increases and survival as mating behaviour might attract the attention of predators (Tuttle and Ryan 1981). Finally, Lower Aripo (high predation) guppies were not less aggressive than their Upper Aripo (lower predation) counterparts, inconsistent with both the risk-allocation model and the risky-competition hypothesis. The absence of a difference in aggression rates could be due to their low frequency (see below), making them economical enough to perform, even under high ambient predation risk.

Behavioural experiments conducted in the laboratory often lead to inconsistent results compared to field conditions (Robinson and Turner 1990; Losos and Irschick 1996; Casas et al. 2004), such as behaviour only observed in the laboratory. These inconsistent results can also be observed through biased behaviour in the laboratory due to the confinement of individuals, and lower behavioural rates in laboratory conditions. In our case, the key difference was that mating competition was 86% lower in the field compared to the laboratory. We can think of two possible explanations for this result. First, in order to enhance mating competition in the laboratory, we separated individual by sex for at least 24h. Individuals are not likely to be in such conditions in the wild as male guppies spend most of their time trying to copulate with females (Magurran 2005). Second, this separation of individuals also allowed them to acclimate to captivity in the laboratory. The lack of long acclimation periods following handling in the field experiment might have led to behavioural consequences (Pickering et al. 1982), such as more vigilance from individuals, resulting in less fitness-related behaviour (e.g. mating, foraging) than in the laboratory experiment.

This study demonstrates the importance of replicating laboratory experiments in the wild to identify the factors shaping behavioural patterns. Indeed, our direct comparison of an experiment conducted both in the laboratory and in the field shows a number of contrasting results, such as lower behavioural rates in the field, as well as lower mating tactic rates in high ambient predation-risk individuals compared to their low ambient-predation risk counterparts. As a consequence, some interpretations are different between laboratory and field experiments. In our case, acclimation periods and conditions are likely to explain most of the differences. However, in general, laboratory stress and artifacts (e.g. separating individuals by sex prior to an experiment to enhance mating competition), might enhance or inhibit natural behaviours, thus potentially undermining our understanding of natural and sexual selection in wild populations. We encourage future studies to perform field experiments in parallel of elaborate laboratory experiments to draw more accurate conclusions on behaviours susceptible to be acting on selective pressures in the wild.

Table 5.1 Means and ranges of ASR[a] and OSR[b] found (a) in the wild and (b) in the nine studies of the effect of ASR[a] on Trinidadian guppies

Studies	ASR[a] (mean; range)	OSR[b] (mean; range)
(a) Chapter 4	0.5[c] (0.01-2.5)	2.5 (0.05-12.5)[d]
(b) Jirotkul 1999a	0.35 (0.04-1)[d]	1.74 (0.2-5)
Jirotkul 2000	0.43 (0.1-1)[d]	2.13 (0.5-5)
Head and Brooks 2006	0.25 (0.1-0.4)[d]	1.25 (0.5-2)
Head et al. 2008	0.23 (0.15-0.3)[d]	1.13 (0.75-1.5)
Barbosa and Magurran 2010	0.62 (0.034-1.2)[d]	3.08 (0.17-6)
Jha 2011	0.23 (0.1-0.4)[d]	1.17 (0.5-2)
Kiritome et al. 2012	0.24 (0.13-0.4)[d]	1.22 (0.67-2)
Chapter 1	1.74 (0.2-5)	8.70 (1-25)[d]
Chapter 4	2.07 (0.2-5)	10.35 (1-25)[d]

[a] ASR is defined here as the number of adult males/number of adult females.

[b] OSR is defined here as the number of adult males/number of ready-to-mate females.

[c] Mean of the Lower and Upper Aripo populations' means

[d] Estimation based on the fact that approximately 20% of female guppies are receptive at any given time in the wild (Magurran 2005)

Table 5.2. Predictions and results of the effects of ASR[a], ambient predation-risk population differences and sex on competition for mates

Explanatory variables	Predictions for competitors	Results[b]
As ASR[a] increases	(1) Aggression rate increases in males	True
	(2) Aggression rate increases in females	True
	(3) Courtship rate per male decreases	False
	(4) Courtship propensity in males increases	False
	(5) Sneaking rate per male decreases	True
	(6) Sneaking propensity in males increases at faster rate than courtship propensity	False
High vs. low ambient predation-risk population	(7) Aggression rate is lower or higher[c]	False
	(8) Courtship/forced mating rate per male is lower or higher[c]	True – lower
Sex roles	(9) Males are more aggressive than females	False

[a] ASR is defined here as the number of adult members of the competing sex/number of adult members of the opposite sex.

[b] True means the results were consistent with the predictions (i.e. $P<0.05$). False means no significant effect.

[c] Behaviour rate predicted to decrease if the cost of ambient predation risk is high OR increase if the absence of an actual predator signals a 'safe' period, consistent with Lima and Bednekoff's (1999) risk allocation model.

Table 5.3. Physical, demographic, and mean characteristics and range of the pools sampled in the Lower (high predation) and Upper (low predation) Aripo populations in 2014 and 2015

Mean characteristics	Lower Aripo 2014 (n=7)	Lower Aripo 2015 (n=8)	Upper Aripo 2014 (n=8)	Upper Aripo 2015 (n=8)
Area (m^2)	10.63 (4.68-32.90)	16.28 (8.85-28.16)	8.60 (3.20-20.02)	8.20 (4.42-14.79)
Depth (m)	0.33 (0.10-0.55)	0.28 (0.12-0.44)	0.44 (0.26-0.73)	0.26 (0.15-0.42)
Velocity (m/s)	0.024 (0.010-0.050)	0.016 (0.10-0.59)	0.096 (0.016-0.210)	0.074 (0.10-0.200)
Guppies per pool	63.25 (13.03-120.00)	45.24 (4.00-141.00)	33.04 (10.80-103.11)	40.38 (8.00-91.00)
Samples per pool	2.71 (1-5)	1.00 (NA)	5.25 (2-5)	1.00 (NA)
Days between sampling	2.54 (2-7)	NA	2.15 (1-5)	NA

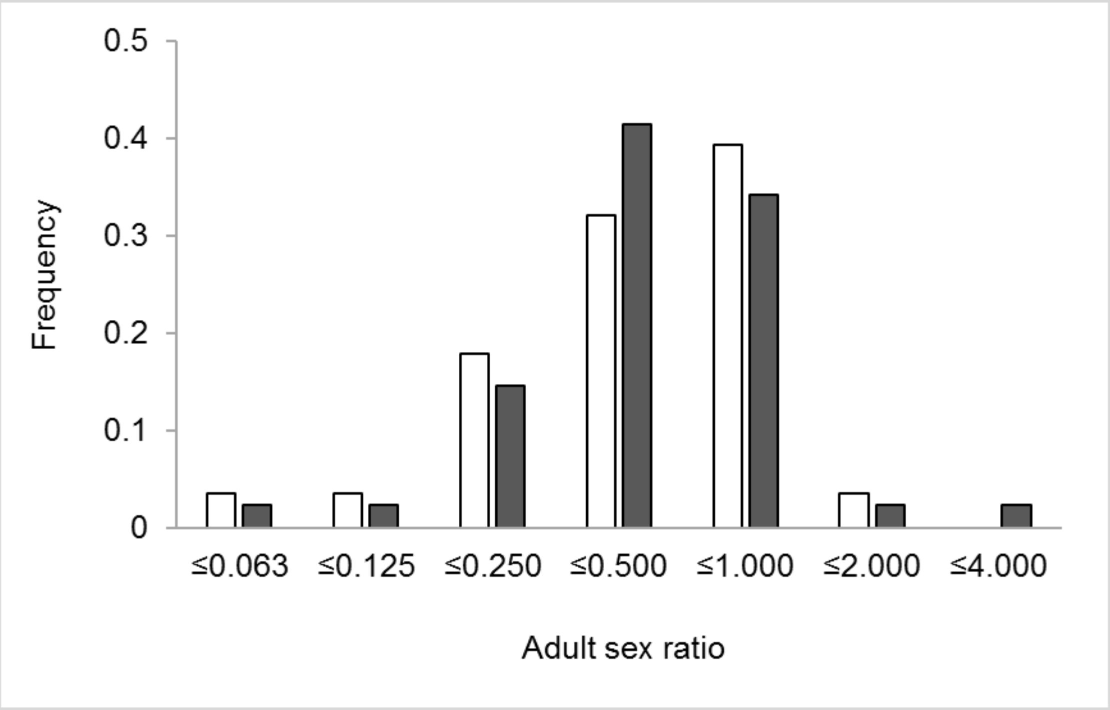


Fig. 5.1 Frequency distribution of the adult sex ratio (number of adult males/number of adult females) in the Lower Aripo (high predation; *open bars*) and Upper Aripo (low predation; *shaded bars*) populations. The x-axis displays the upper limit of each category, the lower limit being the upper limit of the preceding category, if any

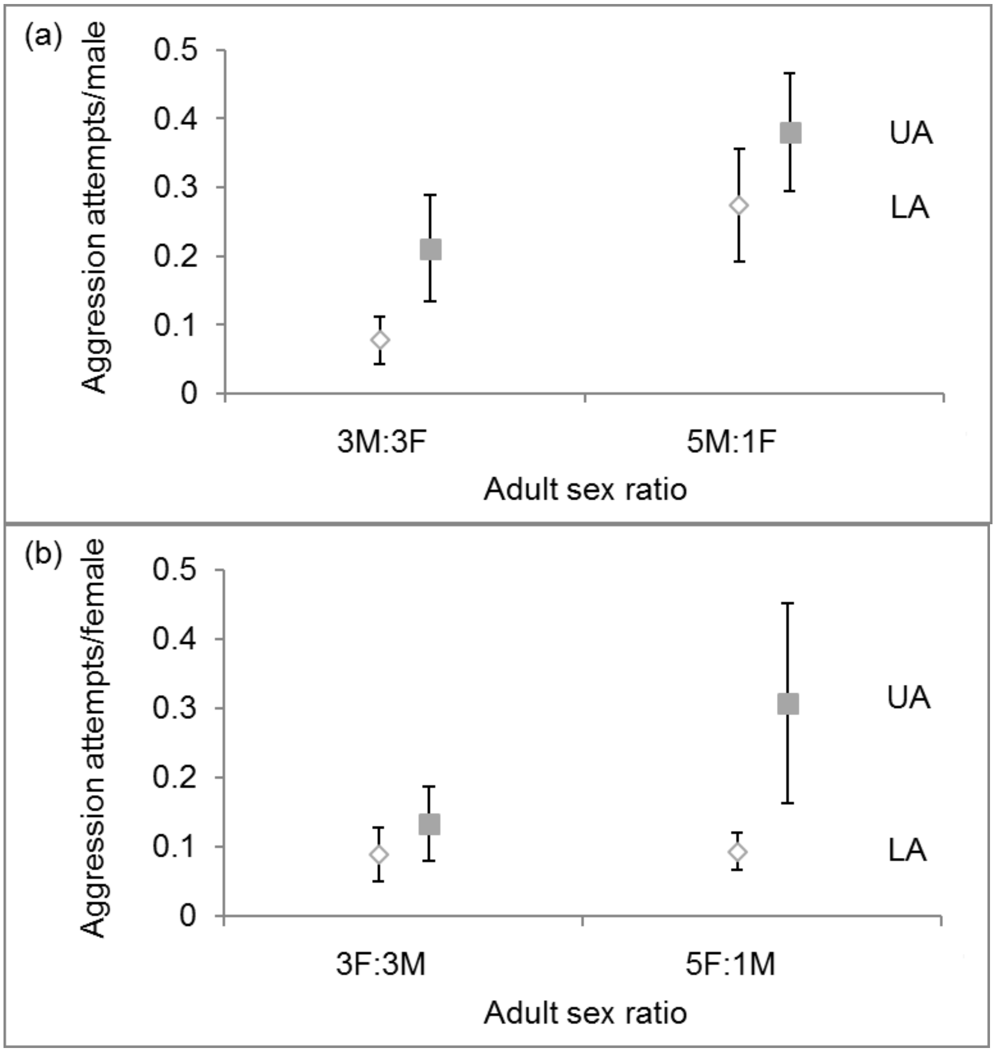


Fig. 5.2 Mean (±SE, N=30) number of intrasexual aggressive acts per individual (a) male (*M*) and (b) female (*F*) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex; i.e. 1, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 10-min period

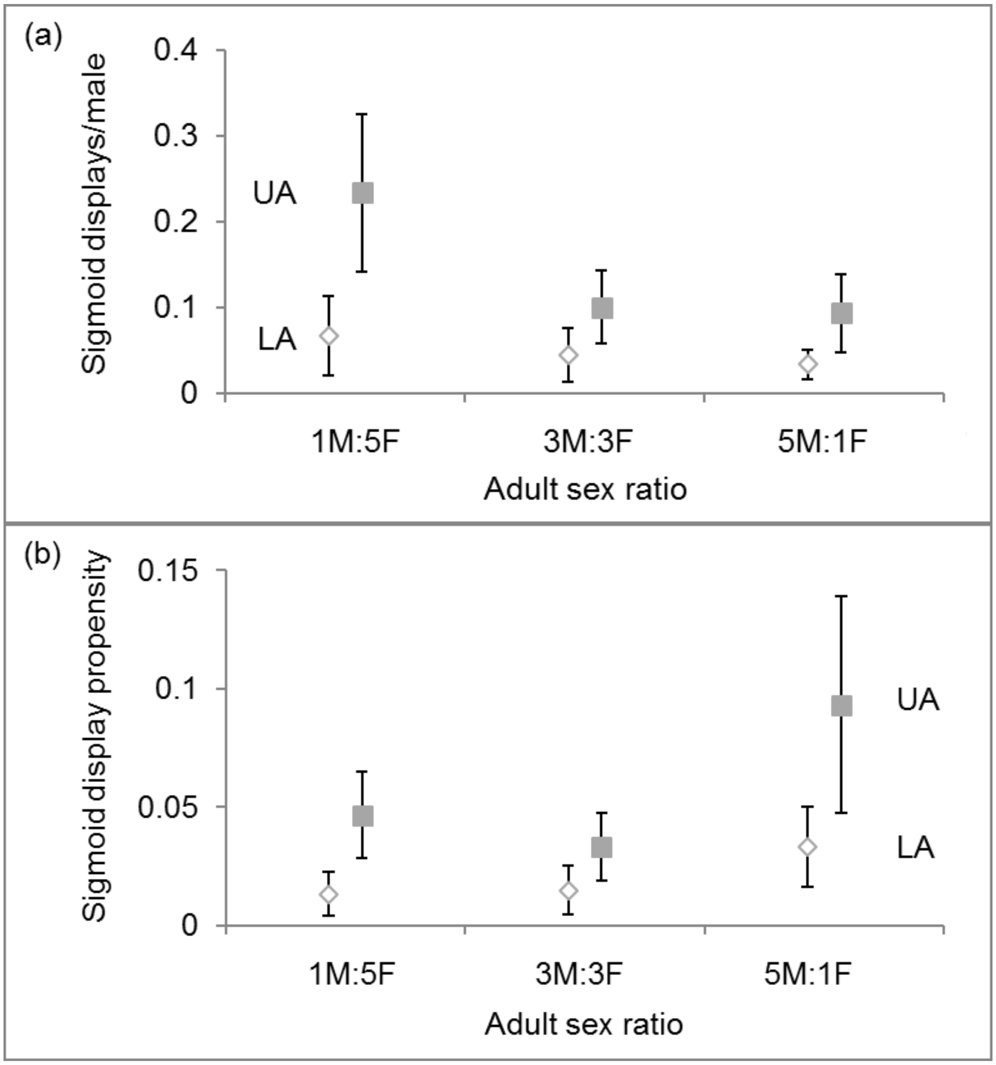


Fig. 5.3 Mean (±SE, N=30) number of sigmoid displays (a) per individual male (*M*) and (b) per individual male corrected by the number of females (*F*) (propensity) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex; i.e. 0.2, 1, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 10-min period

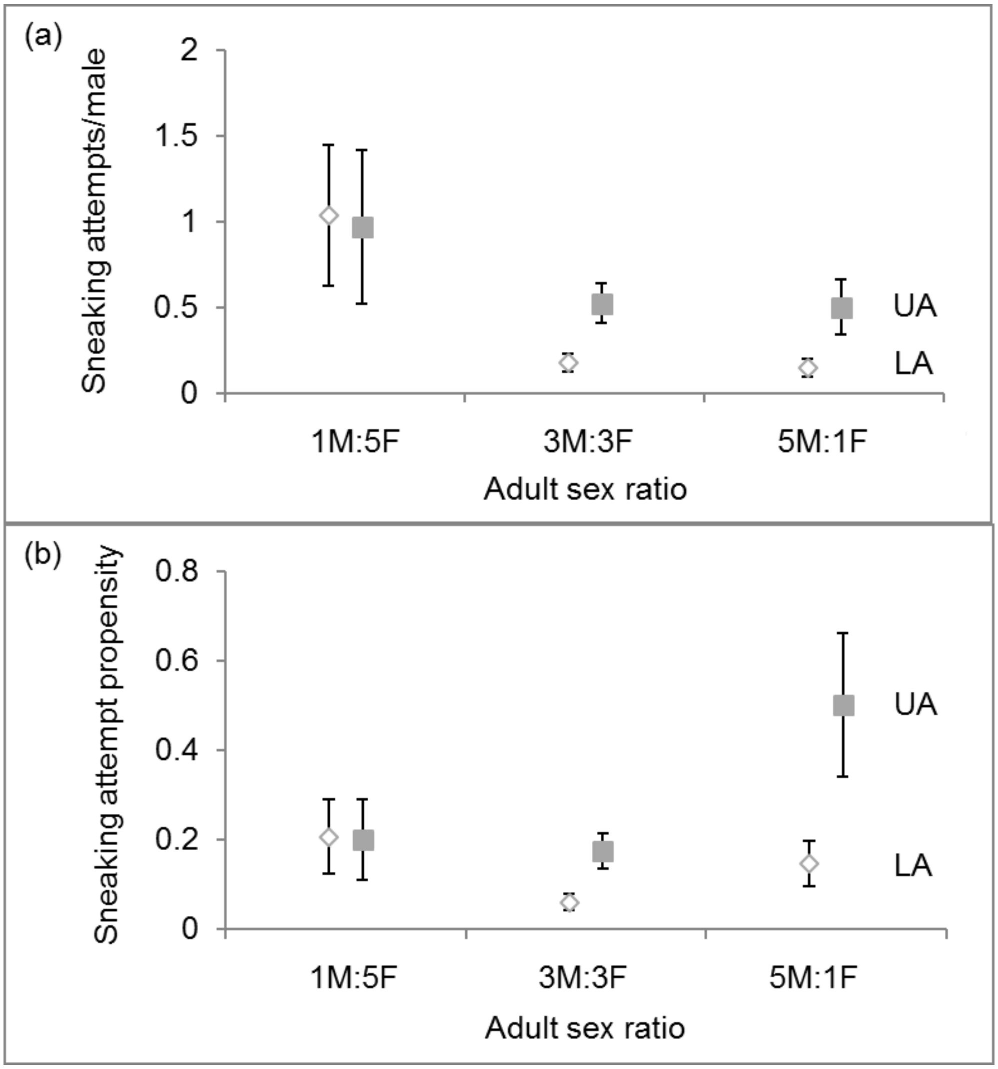


Fig. 5.4 Mean (±SE, N=30) number of forced mating attempts (a) per individual male (*M*) and (b) per individual male corrected by the number of females (*F*) (propensity) in relation to adult sex ratio (number of adult members of the competing sex/number of adult members of the opposite sex, i.e. 0.2, 1, 5) for Lower Aripo (high predation; *LA*; *open diamonds*) and Upper Aripo (low predation; *UA*; *shaded squares*) guppies over a 10-min period

General Discussion

This thesis aimed to explore the effects of key factors influencing intraspecific competition. The goal of this thesis was to explore intraspecific competition in prey populations through the interaction of predation risk, CRR, sex role and type of resource subject to competition. Each of this factor has been extensively studied in relation to competition, but often in isolation from one another. The data presented in this thesis about intraspecific competition demonstrates strong support for OSR theory, especially in the laboratory (Chapter 1 and 2): an increase in the intrasexual aggression rates of both males and females and a decrease in male mating tactic rates as OSR increased. However, when extended to food resources through CRR, our results did not strongly support the predictions of OSR theory: no effect of CRR on aggression rates in a foraging context (Chapter 3). In terms of the effects of predation risk on competition, we found mixed support for the risky-competition hypothesis but only weak support for the risk allocation model. Indeed, in the laboratory, most behaviours decreased under imminent predation risk (Chapter 2), aggression rates were lower in individuals under high vs. low ambient predation risk in a mating context (Chapter 1 and 2), whereas in the field, male mating tactic rates were lower in those same individuals (Chapter 4). Only one result supported the risk allocation model: higher foraging rates in high vs. low ambient predation risk (Chapter 2 and 3). Finally, we found no evidence of the predicted effect of sex roles in a competition context: no difference in intrasexual rates (all chapters) and threat-sensitivity (Chapter 2) between males and females for both mates and food.

Individuals need to secure resources, such as food and mates, in order to increase their fitness (Stockley and Bro-Jorgensen 2011). They should compete for a resource if it is limited and if the gains of that resource are greater than the costs associated with securing that resource (e.g. aggression; Ydenberg and Houston 1986). For that reason, we expected an increase in aggression rates as CRR approaches ~ 2 (Weir et al. 2011). Beyond this value, the cost of aggression would be higher than the gain associated with securing a resource item, thus aggression rates would decrease as CRR increases (Grant et al. 2000). We did not find evidence of such dome-shaped relationship between intrasexual aggression rates and CRR – aggression rates increased as CRR increased in a mating competition context both in the laboratory (Chapter 1 and 2) and in the field (Chapter 4), and did not change with CRR in a foraging context (Chapter 3). The absence of a decrease in aggression rates at high CRRs might be due to the low rates of aggression overall in guppies compared to other well-studied species (e.g. *Junco hyemalis*: Cain and Ketterson 2012), making aggression cheap enough to perform, even at high CRRs. The lack of an effect of CRR on food-related aggression, combined with the high rates of aggression for food compared to when the resource is mate, likely indicates that fixed food is more defendable than mobile mates, and also more valuable/rare in the wild (Ferreira et al. 2016). It is important to note that we did not include the size of individuals as a covariate in our analyses, like many other studies on the effect of OSR on competition (e.g. Jirotkul 1999a; Grant and Foam 2002). As individual size is a good indicator of resource-holding potential (Parker 1974), smaller size differences between individuals might have led to greater aggression rates, and thus increase the variance between trials. To minimize those negative effects, we chose individuals of noticeable size differences within a given trial, especially for females as male guppies are usually more similar in size (Magurran 2005). As we detected a significant effect of ASR on aggression rates in several instances, our selection of individuals based on size for trials seems to have compensated for the non-inclusion of individual size as a covariate in our models.

Aggression appears to be traded-off with mating as we found evidence of a decrease in reproductive attempt rates as ASR increased (Chapter 1, 2, and 4). A male will likely achieve a greater reproductive success if it outcompetes other males before attempting to mate with a female. However, when we replicated the experiment in the field (Chapter 4), the proportion of courtship compared to forced mating was very low compared to an observational study including those two same populations (Magurran and Seghers 1994). The short acclimation period in the field compared to the laboratory experiment might have affected the behaviour of males (Pickering et al. 1982), by making individuals switch from energetically-expensive courtship to less energetically expensive forced mating, as in Godin (1995). Conversely, similar to predation risk, the cost of competition seems to lead to the use of proportionally cheaper mating tactics (i.e. sneaking, Chapter 1 and 2) compared to more expensive ones (i.e. sigmoid display; de Jong et al. 2009).

Our results provide evidence for the risky-competition hypothesis as high-predation risk individuals showed lower intrasexual aggression rates than their low-predation risk counterparts in a mating competition context in the laboratory (Chapter 1 and 2), and lower reproductive attempt rates in the field (Chapter 4). However, aggression rates were not lower when foraging in the high-predation population compared to the low-predation population (Chapter 3). Courtship and aggression have been shown to attract predators (Zuk and Kolluru 1998; Toscano et al. 2010), while foraging seems to be less conspicuous, at least in guppies which mainly feed on benthic algae and aquatic insect larvae (Dussault and Kramer 1981). Thus, guppies exposed to high ambient risk of predation might only reduce conspicuous behaviour to avoid attracting predators. However, we did not find any significant difference in aggression rate between the two populations in the field (Chapter 4) likely because those rates were low compared to the laboratory experiment, probably due to a short acclimation period in the field compared to the laboratory experiment (Pickering et al. 1982). Similarly, there was no difference in reproductive attempt rates between the two populations in the laboratory (Chapter 1 and 2), likely because the fish had been separated by sex at least 24h before the experiment to trigger mating competition. Males from the high ambient predation population probably attempted to reproduce at high rates despite their use to high predation risk as they did not have access to females for a long time.

We also did not find any difference in aggression rates between the two populations in a foraging context for similar reasons (Chapter 3). Indeed, we did not feed the fish for at least 24h before the experiment. Supporting this hypothesis, we found higher rates of foraging in the high ambient-predation risk population compared to their low ambient risk counterpart in the mating competition experiment (Chapter 2) when fish were not food-deprived before the experiment. This result supports the RAH (Lima and Bednekoff 1999), with individuals originally from high ambient predation risk sites foraging more than low predation risk site individuals when imminent risk is absent to compensate for lost opportunities when an imminent predation risk was present. As stated above, foraging is likely less conspicuous to predators than aggression and reproductive attempts, hence the opposite results discussed above.

Even costlier than ambient predation risk is imminent predation risk as it often signals imminent death whereas ambient predation risk indicates the presence of predators in the environment, but not the risk of an imminent attack. Under imminent predation risk, all activity (i.e. aggression, mating, foraging) decreased in favour of antipredator behaviour as the chances of dying increased (Chapter 2). However, the type of imminent predation risk (e.g. chemical vs. visual) seems to play an important role in predicting the patterns of behavioural change under risk. A visual predation threat may be perceived as riskier than a chemical threat, as suggested by a proportional increase in the rates of a cheaper mating tactic (i.e. forced mating) compared to a more expensive mating tactic (i.e. courtship) when individuals were presented with a model predator (Godin 1995), while the opposite occurred in our experiment when individual detected conspecific AC. The results of an increase in visual imminent risk are similar to the results of an increase in competition (i.e. increased in ASR): the switch from expensive to cheap mating tactics. Both visual imminent predation risks and competition impose a cost on mating, respectively through the investment of energy into antipredator behaviour and aggression, resulting in less energy devoted to mating, hence the switch to cheaper mating tactics.

Both populations responded in a non-graded fashion to imminent predation risk, whereas we expected a graded response to AC in the high ambient predation-risk population compared the low ambient predation-risk population based on the RAH (Chapter 2; Lima and Bednekoff 1999). Male guppies are known to sexually harass females (Magurran 2005), which might directly decrease the amount of energy females can devote to fitness-related activities (e.g. foraging) under imminent predation risk. In addition, the potential of male mating behaviour to attract predators (Tuttle and Ryan 1981) might make females more risk-averse when in the presence of males. Finally, two important factors should be considered when interpreting our results. First, there are limitations to explore graded vs. non-graded patterns with only two concentrations of alarm cue as several other studies that showed such responses have used more concentrations (e.g. Dupuch et al. 2004; Brown et al. 2009). Second, the Upper Aripo population has often been shown to be an exceptional low-predation site in the guppy literature (Kolluru et al. 2015), with lower behavioural plasticity, making this population more likely to be similar to high-predation guppy sites (i.e. Lower Aripo). Some of the surprising non-significant results between the two populations in our study could be due to the exceptional population of guppies found in the Upper Aripo River.

Males and females did not show expected behavioural differences based on typical sex role differences (Forsgren et al. 2004). Females were actually more aggressive in a mating context than males in the laboratory (Chapter 1 and 2). Male can use mating tactics in complement of male-male aggression to increase their reproductive success, whereas females can only fight other females away in order to monopolize males' attention, hence why females might use even more intrasexual aggression than males in a mating context.

The absence of a significant difference in aggression rates between males and females in a foraging context (Chapter 3) could be due to the experimental design. The starvation of fish for at least 24h before a trial might have made males more interested in food than in natural conditions. Females are known to forage for long periods than males (Magurran 2005), whereas males are known to forage just enough to be able to reproduce (Griffiths 1996), thus observing individuals for 10 min only might not have been enough to detect a difference in intrasexual aggression rates between males and females. Conversely, we found evidence that males seem to value mating more than females due to their higher reproductive rate (Forsgren et al. 2004) as they kept their courtship rates constant despite an increase in imminent predation risk, whereas females froze (Chapter 2).

Similar to previous work (Pettersson et al. 2004), we found an overall female-biased ASR in the wild (Chapter 4). In species where males perform colourful courtship displays, males are more subject to predation due to their conspicuousness, and thus ASR tends to be female-biased (Reichard et al. 2014). These conditions support our findings of greater intrasexual aggression in females in a mating context compared to males (Chapter 2). Indeed, natural selection might have favored aggressive phenotypes in females, as such females can monopolize the attention of the few males present and thus achieve a greater reproductive success.

General Conclusion

This work is the first to our knowledge to have explored the interactive effects of CRR, predation risk, and sex role in laboratory and field experiments covering a range of conditions likely to be encountered in nature. Those experiments gave us insight into the similar effects that competition and predation risk may have on behavioural patterns as they both represent a cost impacting fitness-related behaviour. Moreover, the ability to defend a resource (e.g. mobile mates vs. fixed food) seems to be key in determining competitive patterns under variation in resource availability. In terms of imminent predation risk, individuals seem to have evolved to adapt their response according to the level of risk associated with a given cue (e.g. chemical vs. visual), to optimize their investments into fitness-related activities. Finally, mates and food are valuable resources to both sexes, as acquiring these resources will significantly increase individuals' fitness. Even if sex role characteristics lead to differences in energy investments to secure one type of resource more than another depending if an individual is male or female, both sexes are expected to actively compete for these resources, especially when they are scarce. Surprisingly, given the fact that guppies are a model species that has been extensively studied in the field of Ecology and Evolution (Magurran 2005), we nonetheless shed light on previously unknown behavioural patterns. As male guppies spend most of their time attempting to achieve copulation, whereas females avoid male harassment and forage, we expected males to compete mostly for mates, and females mostly for food. However, there was no significant difference in intrasexual aggression rates between males and females as they both seem to value these fitness-related resources equally. Similar comprehensive studies should be performed, using other model species, to explore the general patterns and strengthen the theory of intraspecific competition in prey populations.

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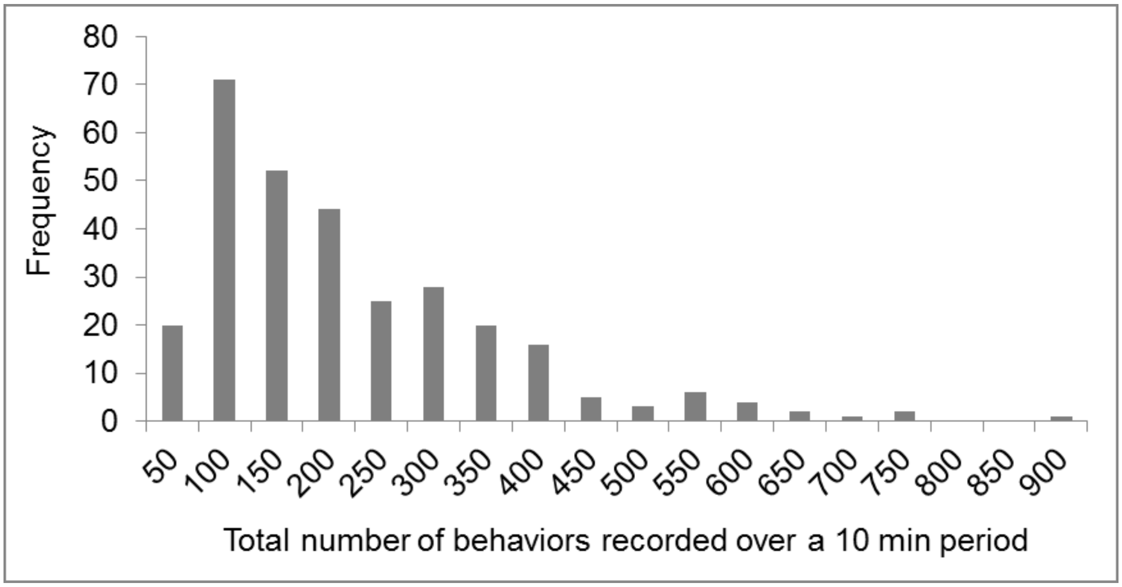
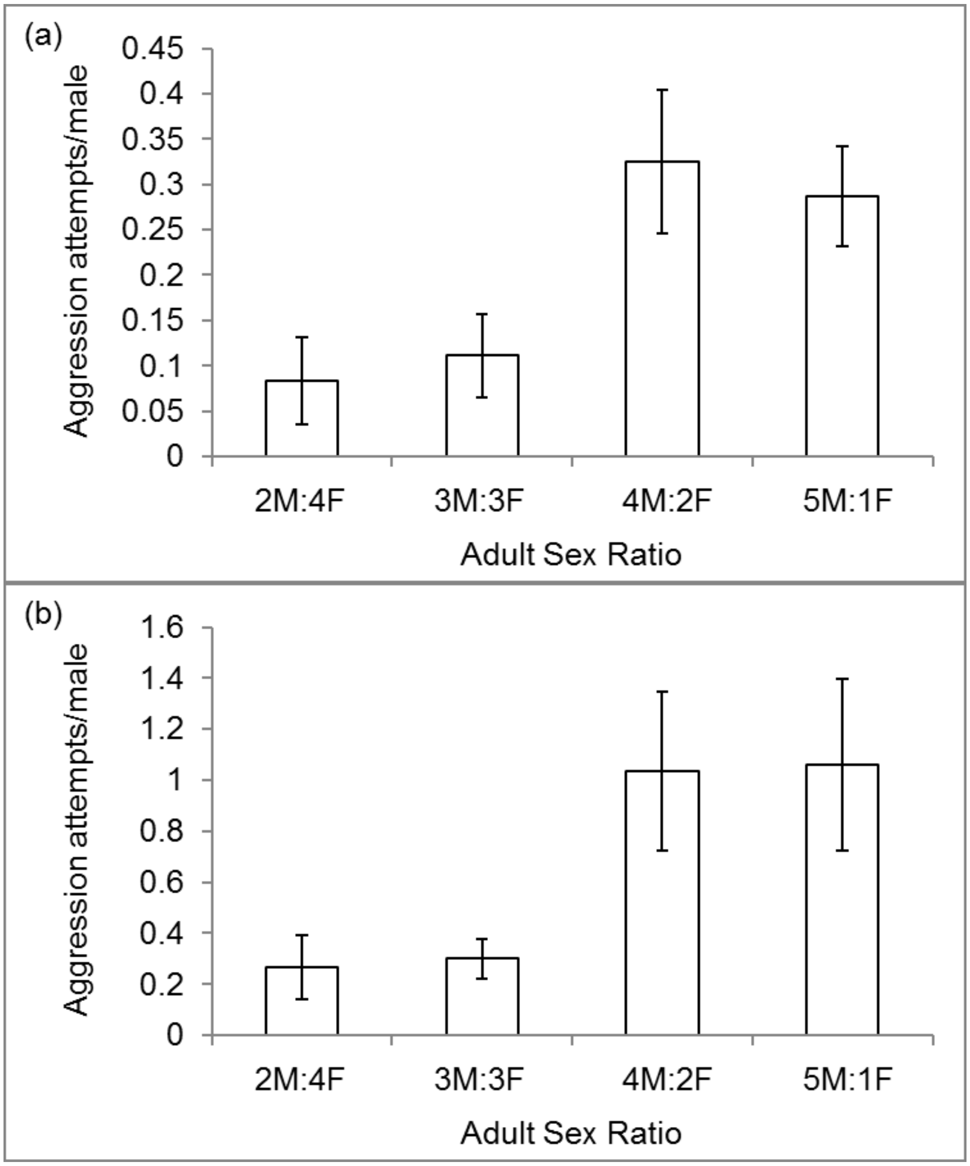
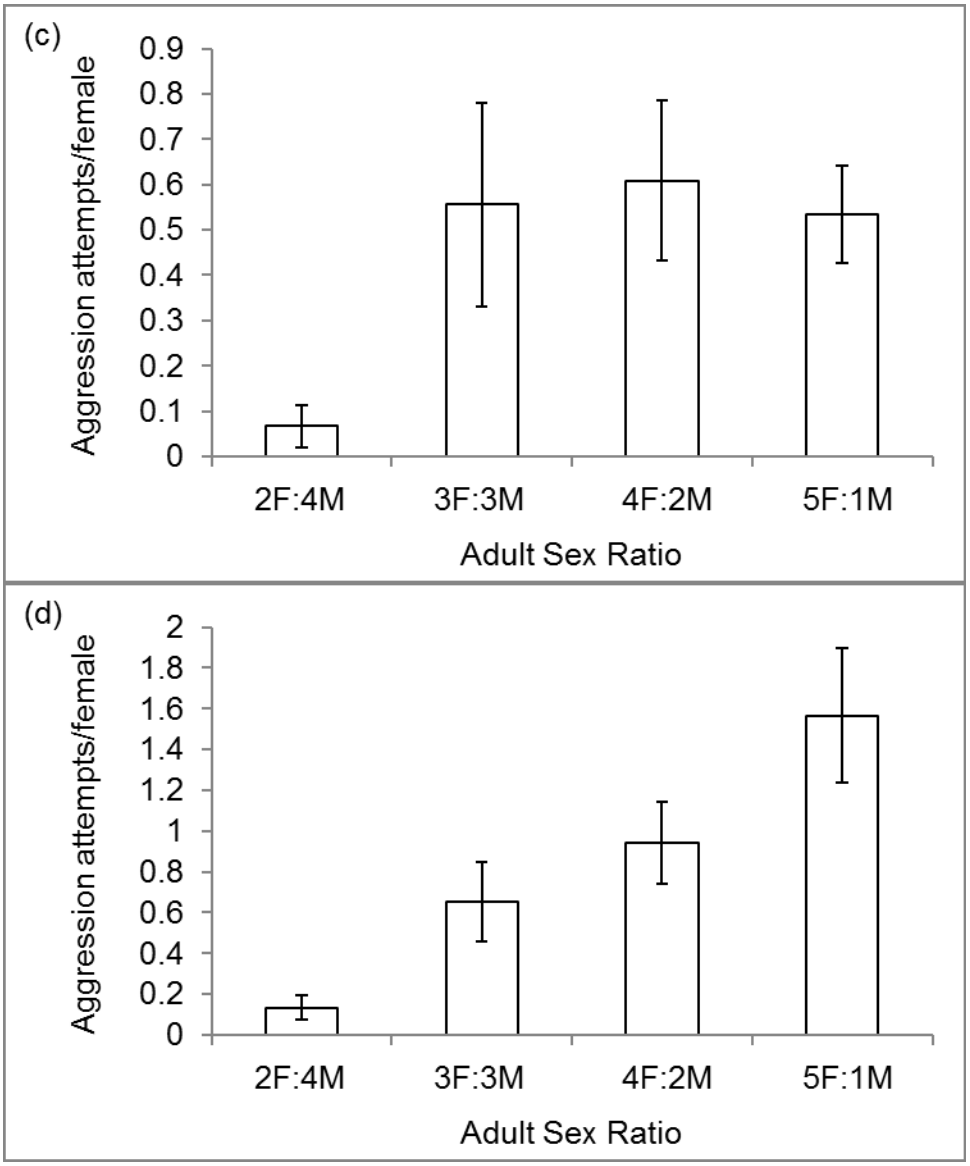
Appendices

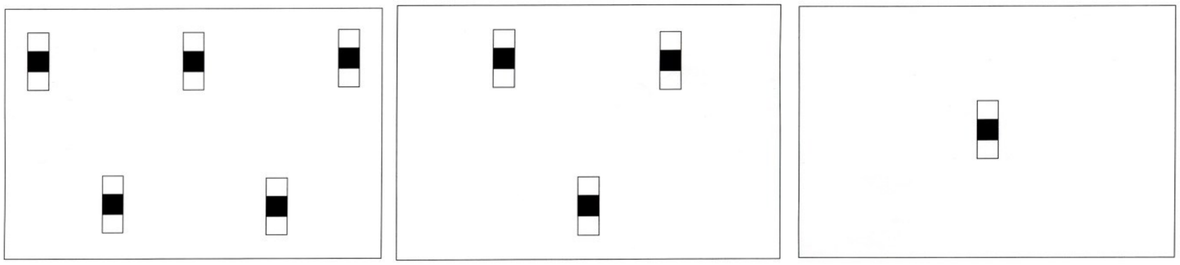


Appendix 1.1 Distribution of the number of behaviours recorded in the 5 min pre- and post-stimulus injection in each trial (N=300) for all treatment combinations


(a)
Aggression attempts/male
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio
(b)
Aggression attempts/male
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2M:4F
3M:3F
4M:2F
5M:1F
Adult Sex Ratio



Appendix 1.2 Mean (± SE, *N*=30) frequency of intrasexual aggressive acts per individual male (*M*) from the (a) Lower (high risk) and (b) Upper (low risk) Aripo Rivers, and per individual female (*F*) from the (c) Lower (high risk) and (d) Upper (low risk) Aripo Rivers under different adult sex ratios (number of adult members of the observed sex/number of adult members of the opposite sex) before stimulus injection



Appendix 2.1. Illustration of the spatial arrangements of the food patches in the three competitor-to-resource ratio (CRR; number of individual competitors over the number of resource units available) treatments as seen from above. From left to right, the CRR are: 4 fish: 5 food patches; 4 fish: 3 food patches; 4 fish: 1 food patch

Appendix 3: Predictors of dominance and foraging success in guppies

Introduction

As Chapter 3 is based on focal observations rather than scanning, we were able to explore the relationship between aggression and foraging success in relation to dominance. This relationship was particularly interesting to investigate as CRR, population and sex had no significant effect on aggression rate in a foraging context (see Chapter 3). Within each sex, individuals might differ in their aggression rates for various reasons. Individual differences in phenotype are good general predictors of resource-holding potential (RHP; Parker 1974). Larger individuals are often dominant to smaller individuals, and hence are more aggressive (Drews 1993; Linderfors and Tullberg 2011) and have higher foraging success (e.g. Eldakar and Gallup 2011), at least when food is defendable (e.g. Weir and Grant 2004; Tanner et al. 2011). If higher foraging success leads to brighter colouration, then colouration may be correlated with RHP (Lyon and Montgomerie 1986; Roulin 2004; Price et al. 2008; Sefc et al. 2014), and used by females as a cue in mate choice (Kodric-Brown and Brown 1984). However, conspicuous colouration might increase predation risk, and thus decrease survivorship (Zuk and Kolluru 1998).

When predation risk is imminent, dominant individuals are more cautious than subordinates, because the latter usually have limited access to resources due to the aggressive monopolization of resources by dominant individuals making exploitation too costly (De Laet 1985; Hegner 1985; Waite and Grubb 1987; Reinhardt 1999). However, in the absence of an imminent risk of predation, dominance structure is expected to be weaker in high vs. low ambient predation-risk populations due to the cost of predation leading to higher rates of antipredator behaviour, resulting in less energy to invest in the establishment of dominance hierarchy (van Schaik 1989). For instance, Magurran and Seghers (1991) found that male and female Trinidadian guppies from low vs. high ambient predation-risk sites showed more food-patch monopolisation.

We wanted to investigate the strength and determinants of dominance structure in low vs. high ambient predation-risk populations using a multi-factor approach, as suggested by Moosa and Ud-Dean (2011). Some animal species have evolved signals of dominance, such as badges in birds, because those signals were cheaper to produce compared to the cost of agonistic contests

(Rohwer 1975). In guppies, dominance could be based on body size, especially in females that keep growing after sexual maturity whereas males do not (Magurran 2005). In males, orange spots are known to be actively selected by females for mating, and the attractiveness of spots (i.e. more red-orange) increase with carotenoid ingestion (Grether 2000; Evans et al. 2004). A dominant male guppy would have a greater foraging success, and thus should have more attractive spots. As a result, the attractiveness of orange spots could be a signal of dominance among males. In addition, some guppies have more dilated pupils (black-eye phenotype) when engaged in aggressive behaviour (*Poecilia reticulata*: Martin and Hengstebeck 1981; Magurran and Seghers 1991), also encountered in other fish species (Eurasian perch, *Perca fluviatilis*: Westerberg et al. 2004), likely indicating aggressive intent to conspecifics, and thus potentially dominance.

We predicted that (Appendix 3.1) the high ambient predation-risk population (1) will show a weaker dominance hierarchy structure than its low ambient predation-risk counterpart. (2) Dominant individuals will have a greater foraging success than subordinates and (3) will be bigger. Finally, dominance will be associated with (4) the black-eye and (5) males with larger relative area, more saturated, and more red (rather than yellow) orange spots.

Materials and Methods

Experimental Procedure

After each behavioural trial (see Chapter 3), during which we also noted the occurrence of the black-eye phenotype (Magurran and Seghers 1991), we measured individuals for weight (± 0.01 g) and standard length, defined as the length between the tip of the snout and the end of the caudal peduncle, using a ruler (± 0.5 mm) for females and using the pictures (± 0.1 mm) for males (see below). Unlike females, we anesthetised males with MS 222 for photography to quantify colour. We photographed males on their left side with a digital 10-megapixel camera (Olympus^{TM}, 3500 Corporate Parkway, Upper Saucon Township, PA) to measure their standard length and the area, hue and saturation of their orange spots using JPEG files in the ImageJ open-access software (http://rsb.info.nih.gov/ij/). Each picture was taken directly after a trial, on a white background with a standard scale to facilitate measuring standard length and the area of orange spots. In addition, a piece of orange tape ($3M^{TM}$, 224 3M Rd, Maplewood, MN) was included in each picture as a reference for hue and saturation, allowing us to correct for small differences in light intensity between pictures. For each picture, we identified all orange spots by eye and measured the sum of their surface area (± 0.1 mm^2) that we then converted into relative area based on the size of the fish. In the "HSB mode" (hue, saturation, brightness) of ImageJ, the software converts hue values expressed as angles, and saturation, into numerical values between 0 and 255. For hue, each number between 0 and 255 corresponds to a specific colour (i.e. 0-22 reds; 23-64 yellows; 65-107 greens; 108-149 cyans; 150-192 blues; 193-234 magentas; 234-255 reds), oranges having values within the reds between 0 and 20. For saturation, the higher the value, the more saturated the patch (i.e. more "colourful", or more different from white). Within each patch, we measured the hue and saturation values of all pigments assessed by eye as coloured (i.e. other than black, grey, and white). For each male, we averaged the highest and lowest value of the range of hue and saturation measured (note that all orange spots are relatively uniform in hue and saturation), and corrected those averages by the corresponding average of the orange tape from the same picture by subtracting the orange spot values from the tape values. The orange tape had a standard hue of 12 to 15 and a saturation of 238 to 255.

Statistical Analysis

We explored the structure of the dominance hierarchy through the coefficient of variation (CV; standard deviation/mean for each trial) of net aggression (difference between given and received aggression per fish; Gorlick 1976) using a GLM fitted to a Poisson distribution (not significantly over-dispersed: $P>0.99$) after making all CVs positive by adding the lowest CV value found in our data set to all CVs to avoid having a mean dominance of 0 in trials. In a despotic hierarchy with one aggressive fish, the group CV would be high, whereas in a more egalitarian hierarchy with no apparent dominant, the group CV would approach 0. Second, we analysed individual differences in foraging rates in relation to predictor variables (i.e. dominance rank, body size, black eye, and male relative area, hue and saturation of their orange spots) by population for each sex separately. We ranked individuals within trials based on dominance (i.e. 1 to 4, 1 being the alpha individual) to explore how dominance translates into foraging success. The higher the difference between given minus received attacks, the higher the rank. In the case of a tie between 2 or more individuals, we established dominance using given aggression. For all rankings thereafter, in the rare event of remaining a tie, we attributed the tied ranks at random.

For males, because of the high number of individual variables measured, we used a PCA in each population to reduce the number of predictor variables. We made all measurements positive by adding a constant to all data points of a predictor variable if necessary. That addition allowed for logarithmic transformation of the variables to reduce skewness and magnitude of the data which might negatively affect the outcome of PCA (Venables and Ripley 2002). To control for magnitude, we also standardized the data before PCA by centring it to 0 and scaling it to have a unit variance. We could not include the rare males with no orange spot in the PCA (i.e. 28 out of 720). We picked a threshold coefficient value between 0.3 and 0.4 to insure a minimum of two representative variables per PC as those values are commonly used in the selection of PCs in behavioural studies (e.g. Boon et al. 2007; Martin and Réale 2008). We only retained the PCs that explained most of the variance of the data based on the Kaiser-Guttman criterion (Kaiser 1991). Those PCs were individually used to rank individuals within each trial (i.e. 1 to 4, 1 being the individual that corresponded the most to the predictor variables selected for the PC). We ranked males that could not be included in the PCA (i.e. no orange spots) based on the most important predictor variable of the PC (i.e. highest coefficient) to start, and then going down the list of coefficients by decreasing order of importance in case of a tie. We used the ranking of individuals for each PC as a fixed factor (linear contrast) along with CRR (linear and quadratic contrast, see Chapter 1) and dominance rank (linear contrast) in the analysis of foraging rates using both GLMs and GLMMs fitted to a negative binomial distribution. All possible models were computed followed by Akaike's corrected information criterion (AICc) model selection (Burnham et al. 2011; see Appendix 3.2).

Regarding females, we ranked them based on their body size (linear contrast) and used that as a fixed factor in the analyses along with dominance rank (linear contrast) and the occurrence of a black-eye phenotype as a covariate (i.e. 0, 1, and 2). We applied the same analyses and model selection as the ones for males.

Finally, to explore the predictors of dominance, we performed the same analyses as just described in males and females but this time on net aggression fitted to a Poisson distribution (not significantly over-dispersed: $P>0.99$), and without dominance rank as a fixed factor.

Results

Regarding social status, contrary to our first prediction, dominance structure was not weaker in Lower Aripo individuals (high predation) compared to Upper Aripo individuals (low predation) as indicated by no significant difference in the CVs of net aggression between the two populations (Appendix 3.3; GLM: β=0.25,CI=[-0.40, 0.92], z=0.75, P=0.45). In accordance with our second prediction, high dominance was associated with foraging success in both populations for males (Appendix 3.4a, b) as suggested by the selection of the GLMM model including dominance rank only (Appendix 3.5a, b). Contrary to our second prediction and unlike males, dominance was not a good predictor of foraging success in females. Indeed, in Lower Aripo females (high predation; Appendix 3.4c), the GLM intercept model was selected because of parsimony (Appendix 3.5c). In Upper Aripo females (low predation), the GLMM model including CRR only was selected with more foraging attempts at intermediate CRR compared to extreme CRRs (Appendices 3.4d; 3.5d; see chapter 3). The selection of those models regarding foraging success excluded all other predictors (i.e. size, black-eye phenotype, colour of male orange spots) as potential predictor variables in both populations. However, similar to males (see above), some females had significantly greater foraging success than others females, as suggested by no significant difference in the CVs of foraging rates between both sexes (Appendix 3.6; GLM: β=-0.0060, CI=[-0.31, 0.30], z=-0.039, P=0.97), even if dominance rank did not correlate with that success in females.

The PCAs based on male phenotype led to the selection of 3 out of 5 PCs for each population explaining 64% of total variance in the Lower Aripo population (high predation) and 67% of the total variance in the Upper Aripo population (low predation). In the Lower Aripo (high predation), in decreasing order of variance explained, the PCs were (Appendix 3.2a): the association of orange colour and high saturation of the orange spots, referred as LA-Hue hereafter; large guppies with small relative area of orange spot (i.e. LA-Size); and individuals displaying a black-eye phenotype and high saturation of their orange spots (i.e. LA-Eye). Regarding Upper Aripo males (low predation; Appendix 3.2b), following the same order, the PCs were: high saturation of their orange spot, displaying no black-eye phenotype and small in size (i.e. UA-Saturation); large relative area and saturation of orange spots (i.e. UA-Area); and orange colour of the orange spots and large individuals (i.e. UA-Hue).

Contrary to our third, fourth and fifth predictions, the intercept model was the best predictor of dominance in Lower Aripo males (high predation; Appendix 3.7a), indicating that body size, black-eye phenotype and orange spots were not good predictors of dominance despite the fact that dominance rank predicted foraging success in males (see above). Conversely, according to our third and fourth predictions, larger Upper Aripo males (low predation) with a black-eye phenotype were more likely to be dominant (Appendix 3.8) as the best model contained only the individual ranking based on UA-Saturation (Appendix 3.7b). Surprisingly, and opposite to our fifth prediction, the selection of this model also suggested that males with highly saturated orange spots tended to be subordinate compared to males with dull orange spots. The two other phenotypes based mostly on relative area and hue of the orange spots were not selected in the final model (Appendix 3.7b). Regarding Lower Aripo females (high predation), contrary to our third prediction and comparable to their male counterparts, size was not a good predictor of dominance (Appendices 3.7c). However, bigger females were more likely to be dominant in the Upper Aripo population (low predation; Appendix 3.9) similar to males from the same population even if dominance did not contribute to foraging success in females as opposed to males (see above). The dominance of bigger females was indicated by the best model which included only body size (Appendix 3.7d). Contrary to our fourth prediction, the black-eye phenotype was not included in the best model (Appendix 3.7d).

Discussion

Contrary to our first prediction, dominance structure was not significantly weaker in the Lower Aripo population (high predation) compared to the Upper Aripo population (low predation). However, males that initiated the most aggression and received the least had the highest foraging success as expected under our second prediction, whereas dominance did not translate into foraging success in females. In Upper Aripo individuals (low predation), bigger individuals were dominant as expected by our third prediction. Consistent with our fourth prediction, black-eye phenotype was the best predictor in Upper Aripo males. Conversely, phenotypical predictors of dominance seem to be too costly to have evolved in the Lower Aripo population (high predation), as indicated by smaller individuals and less colourful males in high-predation guppy populations (Magurran 2005). Finally, our most surprising result is likely the observation that Upper Aripo males (low predation) with less saturated orange spots were dominant, opposite to our fifth prediction, whereas the relative area of the spots and their hue had no significant effect.

We have some evidence that competition for food is more important in females than in males (see Chapter 3) when ambient predation-risk is low. Indeed, in females, dominance rank was not a good predictor of foraging success. Subordinate individuals foraged at the cost of receiving aggression from dominant individuals. This strategy is beneficial as some females, independent of their dominance rank, likely get more foraging success than others. This result suggests that the fitness gains of foraging (e.g. increased reproductive success) outweigh the cost of receiving aggression in females likely because eggs require more energy than sperm to be produced (Beissinger 1987).

In terms of dominance structure, size seems to be the only predictor of dominance in Upper Aripo (low predation) females, whereas size, black-eye phenotype and orange spot saturation are good indicators of dominance in males from the same population. As female guppies are bigger and more variable in size than males (Magurran 2005), size alone should be a valuable-enough indicator of RHP (Parker 1974) to establish dominance ranks. In Upper Aripo males (low predation), however, because size does not vary that much between individual male guppies (Magurran 2005), they seem to also rely on other available cues as indicator of RHP. These cues do not only indicate physical superiority (i.e. size), but also motivation (i.e. black-eye phenotype; Martin and Hengstebeck 1981), as well as foraging success and female preference (i.e. colouration; Evans et al. 2004; Eldakar and Gallup 2011). Female guppies tend to select males based on their colouration, used as an honest signal of high quality genes (Kodric-Brown and Brown 1984).

Contrary to our prediction, dominance hierarchy was not weaker in the Lower Aripo population (high predation) compared to the Upper Aripo population (low predation). However, there was no strong predictors of dominance in the Lower Aripo population (high predation), indicating a potential cost of ambient predation risk resulting in the non-recognition of indicators of RHP (i.e. too costly to have evolved or be maintained). Indeed, predation risk has been shown to impair cognition in sticklebacks (*Gasterosteus* sp.) with females being less choosy towards conspecifics (Kozak and Boughman 2015). The absence of recognition of such indicators is likely the result of a trade-off between monopolising fitness-related resources (e.g. through dominance) and investing energy toward cognitive abilities related to antipredator behaviour.

Out of the three measures of male orange spots (i.e. area, hue, and saturation), only saturation had an effect on predicting dominance structure. The relative area of orange spots being genetically inherited in guppies (Magurran 2005), it is not surprising that it had no effect in predicting dominance. Indeed, a dominant individual, with a greater foraging success, would not see its relative orange spot area increase with time. Contrary to the good genes hypothesis (Arnold 1983), our results showed that female selection of males with larger area of orange colouration (Magurran 2005) does not provide genes for higher competitive ability to their male offspring in relation to foraging. Hue of the orange spots did not seem to affect dominance rank in male guppies either. Given that both males feeding on carotenoid-rich and carotenoid-absent food develop red and orange spots (Kodric-Brown 1989) suggests that hue should not be a good indicator of RHP as even males with a poor diet and/or low foraging success are likely to develop red/orange rather than yellow spots. Surprisingly however, males with less saturated orange spots tended to be dominant. Unlike area, saturation of the orange spots in guppies is controlled by both genes and diet (Magurran 2005); more carotenoid consumption leads to more saturated spots (Kodric-Brown 1989). Once males have reached a high concentration of pigments in their orange spots (i.e. more saturated spots), they might reduce their aggression rates as high male-male aggression rates have been shown to reduce reproductive success (Farr 1980b). Moreover, such reduction in aggression rates could allow males to save energy for courtship, as bright orange spots stand strong chances of being selected by females (Magurran 2005). Males with duller spots might maintain high aggression rates to increase their foraging success, and thus indirectly improve their reproductive success in the future. This hypothesis is supported by the high foraging success of dominant males (e.g. dull spots).

While aggression rates in Lower Aripo individuals (high predation), due to the costs of predation, seemed to be more related to inherited personality (Farr 1983) rather than to physical phenotypes, Upper Aripo (low predation) individuals established their dominance hierarchy based on phenotypical traits. Individuals ranked each other based mostly on size, but males also relied on eye and scale colour to assess other male conspecifics' competitive abilities. Because the determinants of dominance in males differed from the predictors of female mate choice, our study underlines one of the mechanisms maintaining high phenotypical diversity in males from low vs. high ambient predation-risk populations (Magurran 2005).

Appendix 3.1. Predictions and results based on the effects of individual differences on foraging competition

Explanatory variables	Predictions	Results[a]
High vs. low ambient predation-risk population	(1) Dominance structure is weaker	False
Individual differences	(2) Dominant individuals have a greater foraging success than their subordinates	True – only males
	(3) Bigger individuals are dominant	True – only low predation guppies
	(4) Black-eye phenotype indicates dominance	True – only low predation males
	(5) Males with larger relative area, more saturated, and more red (rather than yellow) orange spots are dominant	False

[a] True means the results were in accordance with the predictions (i.e. $P<0.05$). False means no evidence of an effect.

Appendix 3.2. Summary of the principal component analysis of male phenotype for the (a) Lower Aripo and (b) Upper Aripo populations

(a) Lower Aripo

Explanatory variables	Components **1**	**2**	**3**	4	5
Corrected hue[a]	**-0.74**	0.08	0.13	0.27	-0.59
Corrected saturation[b]	**0.44**	-0.32	**0.62**	-0.16	-0.54
Relative area	-0.33	**-0.57**	-0.28	-0.70	-0.04
Black eye phenotype	0.33	-0.34	**-0.67**	0.41	-0.41
Size	0.18	**0.67**	-0.29	-0.49	-0.43

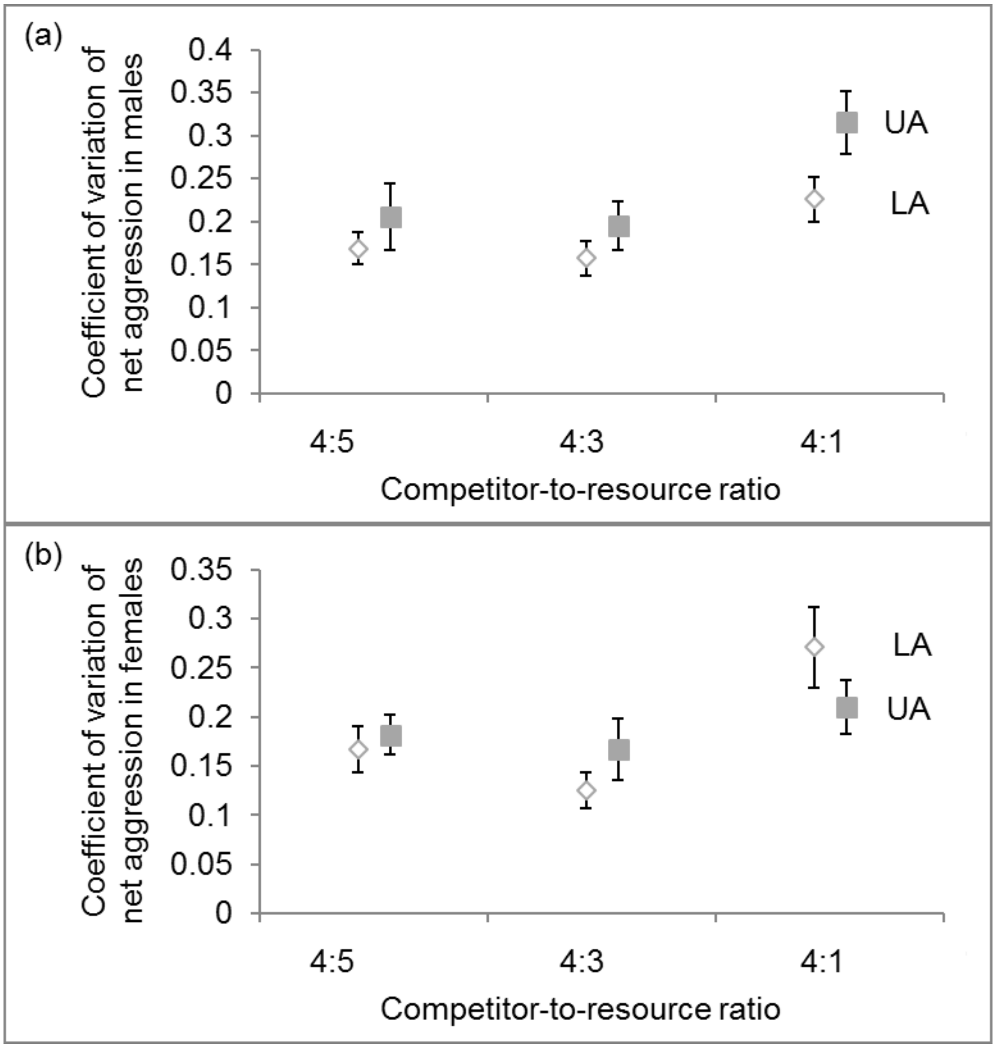
(b) Upper Aripo

Explanatory variables	Components **1**	**2**	**3**	4	5
Corrected hue	0.34	0.37	**-0.70**	-0.26	0.43
Corrected saturation	**-0.59**	**0.38**	0.04	0.49	0.52
Relative area	-0.10	**-0.80**	-0.09	-0.10	0.57
Black eye phenotype	**-0.57**	0.15	0.09	-0.80	-0.01
Size	**-0.45**	-0.23	**-0.70**	0.20	-0.47

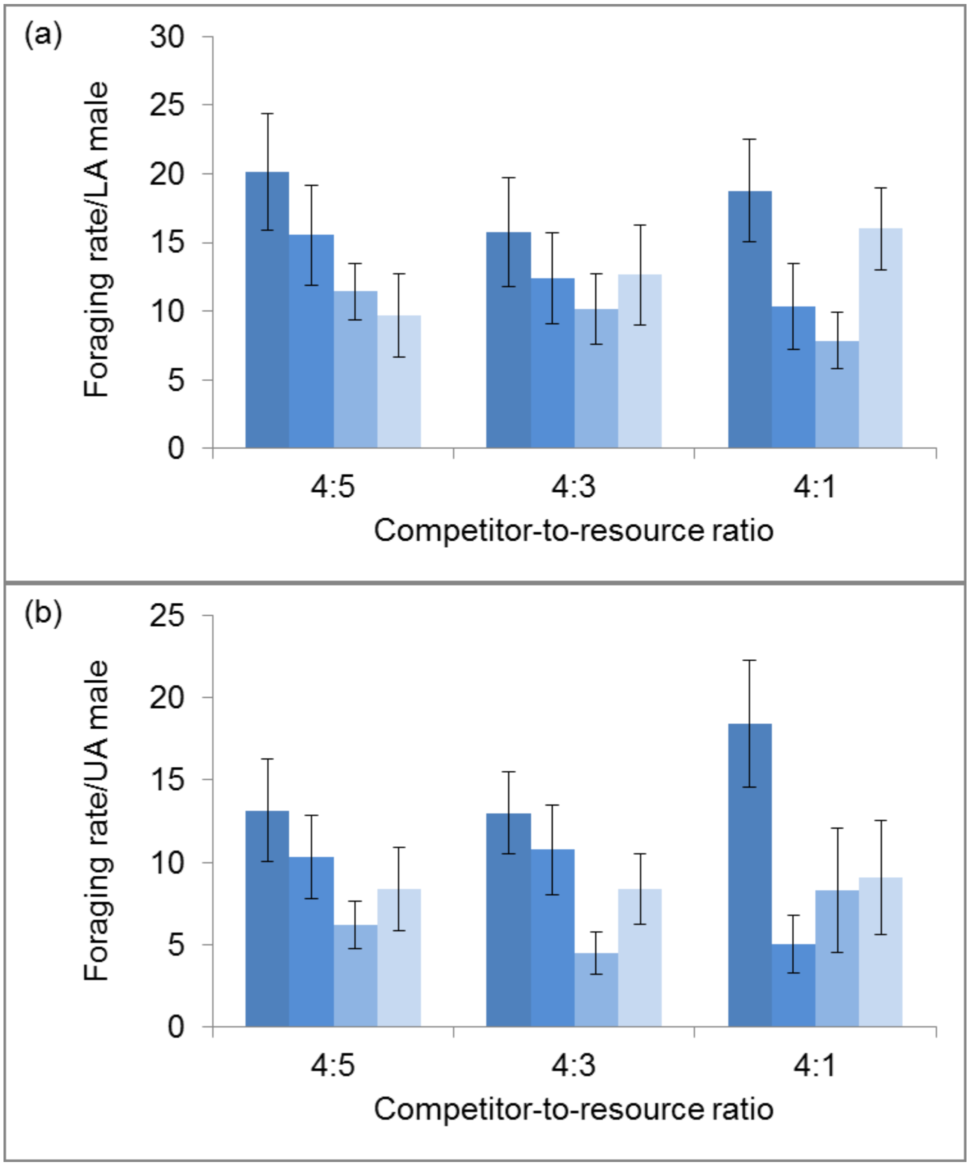
Components retained for ranking of individuals (using Kaiser-Guttman criterion) and their associated main coefficients (≥0.38) are in bold

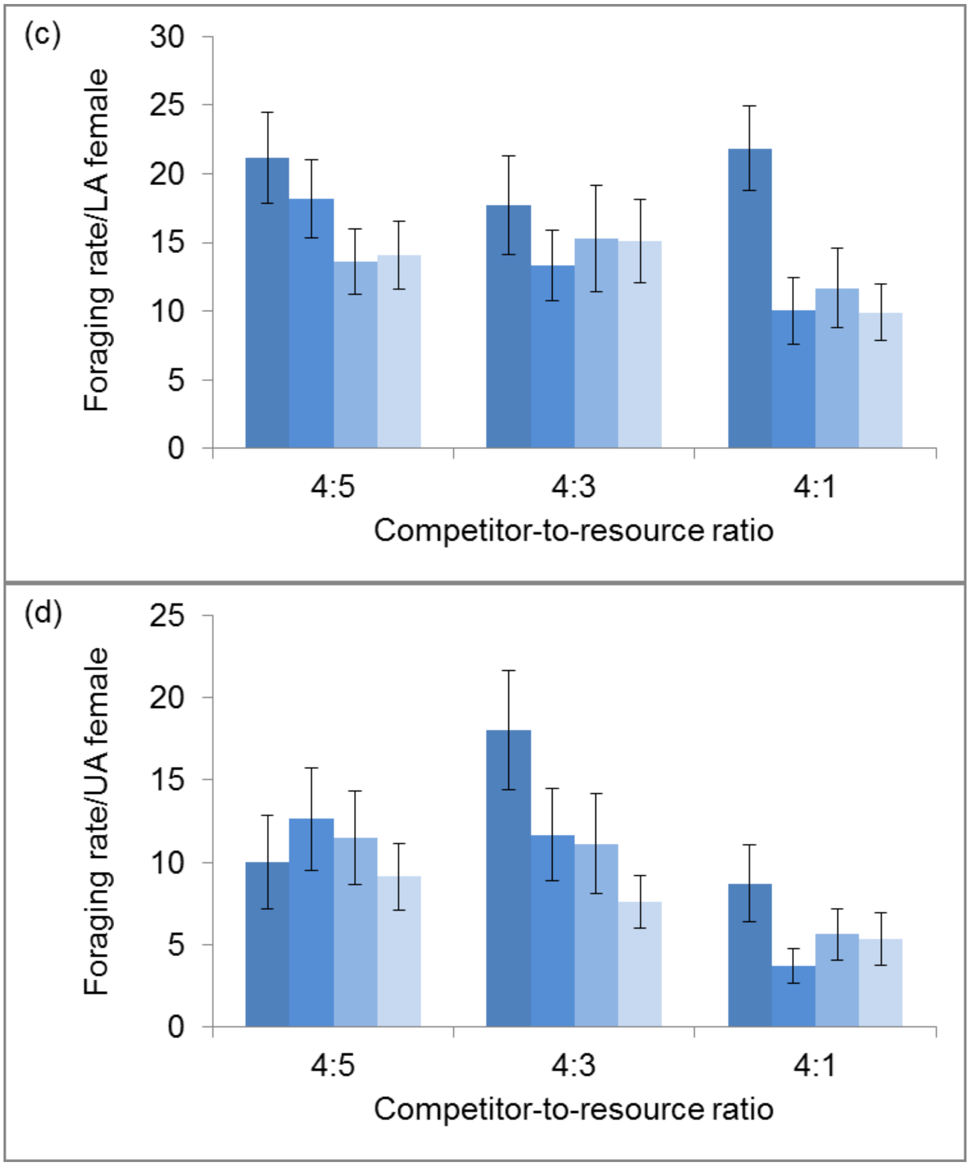
[a] High corrected hue indicate orange rather than yellow spots

[b] Low corrected saturation indicate highly saturated orange spots



Appendix 3.3. Mean (± SE, N=30) coefficient of variation of net aggression per trial of focal individuals in relation to three competitor-to-resource ratios (four individuals competing for 5, 3, and 1 food patches, respectively 0.8, 1.33, 4) and two populations of origin: Lower Aripo (*LA*; *open diamonds*; high predation) and Upper Aripo (*UA*; *shaded squares*; low predation) in (a) males and (b) females


(a)
Foraging rate/LA male
30
25
20
15
10
5
0
4:5
4:3
4:1
Competitor-to-resource ratio
(b)
Foraging rate/UA male
25
20
15
10
5
0
4:5
4:3
4:1
Competitor-to-resource ratio



Appendix 3.4. Mean (± SE, N=30) foraging rate performed by focal guppies ranked by dominance (*shaded bars*: the darker the more dominant) in relation to three competitor-to-resource ratios (four individuals competing for 5, 3, and 1 food patches, respectively 0.8, 1.33, 4) in Lower Aripo (*LA*; high predation) (a) males and (b) females, and Upper Aripo (*UA*; low predation) (c) males and (d) females

Appendix 3.5. Summary of the eight best models predicting foraging success based on the AICc criterion in (a) Lower Aripo males (high predation), (b) Upper Aripo males (low predation), (c) Lower Aripo females (high predation), and (d) Upper Aripo females (low predation)

(a) Lower Aripo males

Candidate models	k[a]	AICc	ΔAICc
1 GLMM intercept + Dominance[b]	2	2473.43	
2 GLMM intercept	1	2479.00	5.57
3 GLMM intercept + PC-LA-Size[c]	2	2482.06	8.63
4 GLMM intercept + CRR[d]	2	2482.28	8.85
5 GLMM intercept + PC-LA-Hue[e]	2	2483.06	9.63
6 GLMM intercept + PC-LA-BE[f]	2	2483.42	9.99
7 GLMM intercept + CRR*Dominance	4	2483.73	10.30
8 GLMM intercept + CRR*PC-LA-Size	4	2485.98	10.55

(b) Upper Aripo males

Candidate models	k	AICc	ΔAICc
1 GLMM intercept + Dominance	2	2158.62	
2 GLMM intercept + CRR*Dominance	4	2159.26	0.64
3 GLMM intercept + Dominance*PC-UA-Sat[g]	4	2165.44	6.82
4 GLMM intercept + Dominance*PC-UA-Hue	4	2166.45	7.83
5 GLMM intercept + Dominance*PC-UA-Area[h]	4	2170.04	11.42
6 GLMM intercept + PC-UA-Sat	2	2170.24	11.62
7 GLMM intercept	1	2173.36	14.74
8 GLM intercept + Dominance	2	2174.04	15.42

(c) Lower Aripo females

Candidate models	k	AICc	ΔAICc
1 GLM intercept + Dominance	2	2662.72	
2 GLM intercept	1	2663.76	1.04

3 GLM intercept + Dominance + BE[i]	3	2664.73	2.01
4 GLMM intercept	1	2665.56	2.84
5 GLM intercept + BE	2	2665.77	3.05
6 GLMM intercept + Dominance + BE	3	2665.96	3.24
7 GLM intercept + CRR	2	2666.02	3.30
8 GLM intercept + CRR*Dominance + BE	5	2667.57	4.85

(d) Upper Aripo females

Candidate models	*k*	AICc	ΔAICc
1 GLMM intercept + CRR*Dominance	4	2196.80	
2 GLMM intercept + CRR	2	2197.06	0.26
3 GLMM intercept + Dominance	2	2198.44	1.64
4 GLMM intercept + CRR*Dominance + BE	5	2198.90	2.10
5 GLMM intercept + CRR + BE	3	2199.11	2.31
6 GLMM intercept	1	2199.58	2.78
7 GLMM intercept + Dominance + BE	3	2200.49	3.69
8 GLMM intercept + BE	2	2201.63	4.83

[a] Number of fitted parameters

[b] Ranking of individuals based on their net aggression (difference between given and received aggression)

[c] Ranking of males based on a principal component primarily described by their size

[d] Competitor-to-resource ratio defined here as the number of individuals to the number of food patches made up of three levels (i.e. 0.8, 1.33, 4)

[e] Ranking of males based on a principal component primarily described by the hue of their orange spots

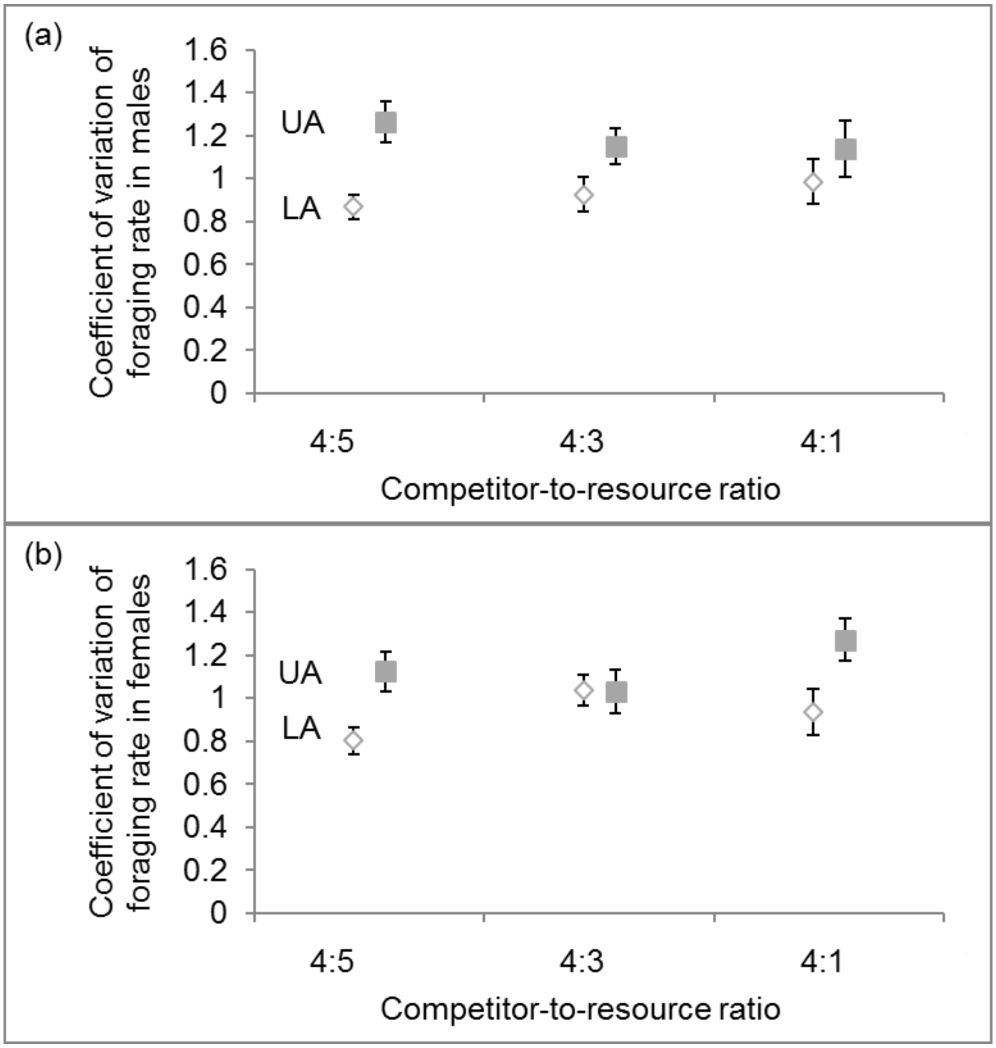
[f] Ranking of males based on a principal component primarily described by the black eye phenotype

[g] Ranking of males based on a principal component primarily described by the saturation of their orange spots

[h] Ranking of males based on a principal component primarily described by the relative area of

their orange spots

[i]Occurence of the black-eye phenotype



Appendix 3.6 Mean (± SE, N=30) coefficient of variation of foraging rate per trial of focal individuals in relation to three competitor-to-resource ratios (four individuals competing for 5, 3, and 1 food patches, respectively 0.8, 1.33, 4) and two populations of origin: Lower Aripo (*LA*; *open diamonds*; high predation) and Upper Aripo (*UA*; *shaded squares*; low predation) in (a) males and (b) females

Appendix 3.7. Summary of the eight best models predicting dominance (i.e. net aggression: difference between given and received aggression) based on the AICc criterion in (a) Lower Aripo males (high predation), (b) Upper Aripo males (low predation), (c) Lower Aripo females (high predation), and (d) Upper Aripo females (low predation)

(a) Lower Aripo males

Candidate models	*k*[a]	AICc	ΔAICc
1 GLM intercept	1	1743.23	
2 GLMM intercept	1	1745.25	2.02
3 GLM intercept + CRR[b]	2	1746.65	3.42
4 GLM intercept + PC-LA-BE[c]	2	1746.82	3.59
5 GLM intercept + PC-LA-Size[d]	2	1747.93	3.70
6 GLM intercept + PC-LA-Hue[e]	2	1748.57	4.34
7 GLMM intercept + CRR	2	1748.69	4.46
8 GLMM intercept + PC-LA-BE	2	1748.88	4.65

(b) Upper Aripo males

Candidate models	*k*	AICc	ΔAICc
1 GLM intercept + PC-UA-Sat[f]	2	1885.55	
2 GLMM intercept + PC-UA-Sat	2	1887.61	2.06
3 GLM intercept + CRR*PC-UA-Sat	4	1893.28	7.73
4 GLMM intercept + CRR*PC-UA-Sat	4	1895.43	9.88
5 GLM intercept + PC-UA-Hue	2	1901.85	16.30
6 GLM intercept	1	1902.94	17.39
7 GLMM intercept + PC-UA-Hue	2	1903.90	18.35
8 GLMM intercept	1	1904.96	19.41

(c) Lower Aripo females

Candidate models	*k*	AICc	ΔAICc
1 GLM intercept	1	1815.34	
2 GLM intercept + BE[g]	2	1817.14	1.80

3 GLMM intercept	1	1817.36	2.02
4 GLM intercept + CRR	2	1817.49	2.15
5 GLMM intercept + BE	2	1819.17	3.83
6 GLM intercept + CRR + BE	3	1819.22	3.88
7 GLMM intercept + CRR	2	1819.54	4.20
8 GLM intercept + Size[h]	2	1820.98	5.64

(d) Upper Aripo females

Candidate models	*k*	AICc	ΔAICc
1 GLM intercept + Size	2	1754.62	
2 GLM intercept + Size + BE	3	1756.56	1.94
3 GLMM intercept + Size	2	1756.68	2.06
4 GLM intercept	1	1757.76	3.14
5 GLMM intercept + Size + BE	3	1758.63	4.01
6 GLM intercept + BE	2	1759.68	5.06
7 GLMM intercept	1	1759.78	5.16
8 GLM intercept + CRR	2	1761.62	7.00

[a] Number of fitted parameters

[b] Competitor-to-resource ratio defined here as the number of individuals to the number of food patches made up of three levels (i.e. 0.8, 1.33, 4)

[c] Ranking of males based on a principal component primarily described by the black eye phenotype

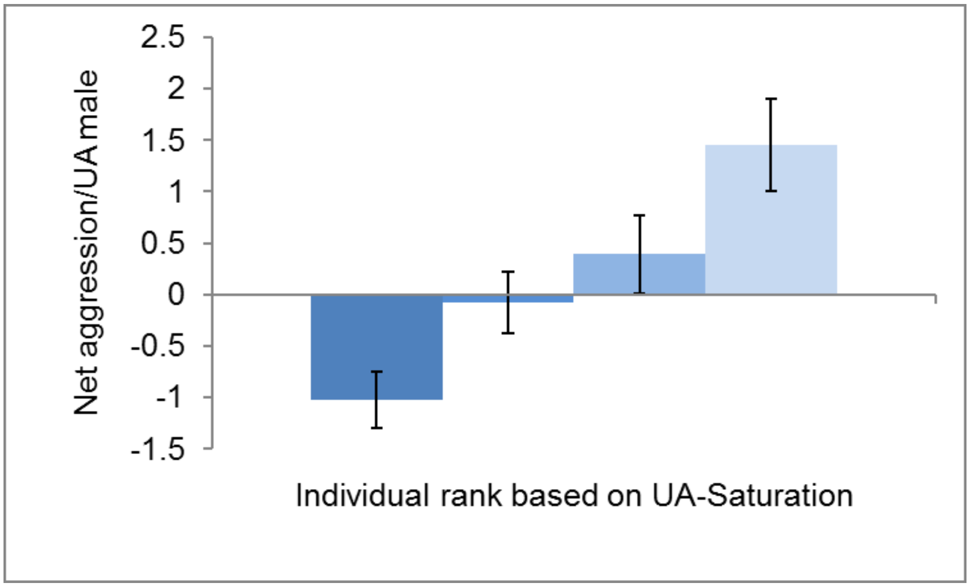
[d] Ranking of males based on a principal component primarily described by their size

[e] Ranking of males based on a principal component primarily described by the hue of their orange spots

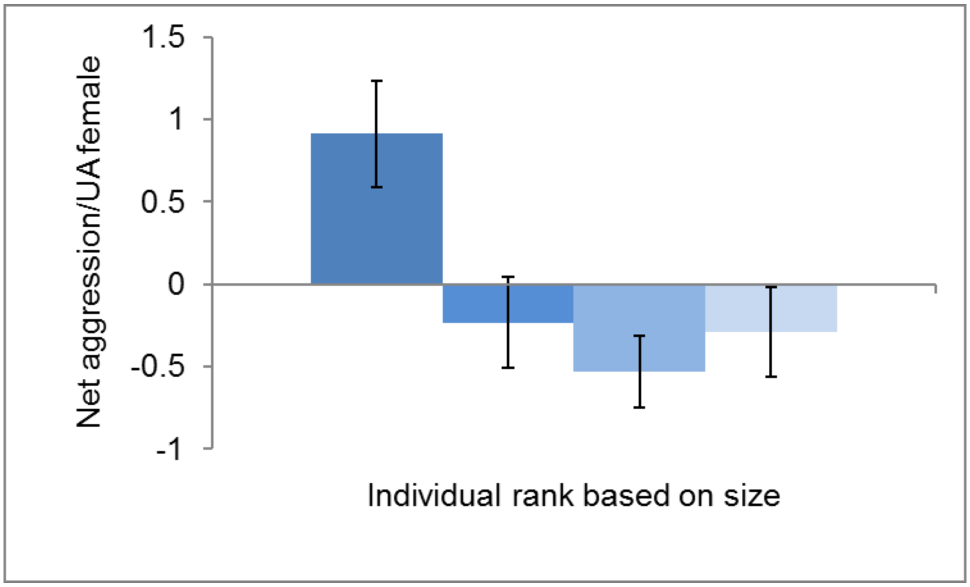
[f] Ranking of males based on a principal component primarily described by the saturation of their orange spots

[g] Occurence of the black-eye phenotype

[h] Ranking of females based on their size



Appendix 3.8 Mean (± SE, N=90) net aggression (difference between given and received aggression) of focal male guppies from the Upper Aripo (*UA*; low predation) ranked based on principal components characterized by (*shaded bars*: the darker the more the individuals correspond to the predictor variables selected for the PC) saturated orange spots, no black-eye phenotype and small in size (UA-Saturation)



Appendix 3.9. Mean (± SE, N=90) net aggression (difference between given and received aggression) of focal female guppies ranked by size (*shaded bars*: the darker the bigger) in the Upper Aripo population (*UA*; low predation)