Variation in aggressive behaviour in the poeciliid fish *Brachyrhaphis episcopi*: Population and sex differences

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ABSTRACT

Aggression is often positively correlated with other behavioural traits such as boldness and activity levels. Comparisons across populations can help to determine factors that promote the evolution of such traits. We quantified these behaviours by testing the responses of wild-caught poeciliid fish, *Brachyrhaphis epis-copi*, to mirror image stimuli. This species occurs in populations that experience either high or low levels of predation pressure. Previous studies have shown that *B. episcopi* from low predation environments are less bold than those that occur with many predators. We therefore predicted that fish from high predation populations would be more aggressive and more active than fish from low predation populations. However, we found the opposite – low predation fish approached a mirror and a novel object more frequently than high predation fish suggesting that 'boldness' and aggression were higher in low predation populations, and that population-level boldness measures may vary depending on context. When tested individually, low predation fish inspected their mirror image more frequently. Females, but not males, from low predation sites were also more aggressive towards their mirror image. Variation in female aggression may be driven by a trade-off between food availability and predation risk. This suggests that the relationship between aggression and boldness has been shaped by adaptation to environmental conditions, and not genetic constraints.

1. Introduction

The tendency of individuals to be aggressive is an important behavioural axis (Réale et al., 2007; Sih et al., 2004) that often correlates across contexts, as well as with other behavioural axes such as boldness and activity. Combining different axes allows us to describe an individual's temperament or personality. For example, in a landmark study, Huntingford (1976) demonstrated that three-spined sticklebacks, *Gasterosteus aculeatus*, which are more aggressive towards conspecifics are also bolder towards predators. Between population comparisons of temperament have been used to investigate factors that drive the evolution of such traits (reviewed in Réale et al., 2007). Desert spiders, *Agelenopsis aperta*, from arid, resource-limited habitats are aggressive towards both conspecifics and prey, and are bold towards predators, but spiders from food-rich riparian habitats with higher predation risk are timid and less aggressive (summarized in Riechert and Hall, 2000). Intra-species population comparisons of temperament are particularly well studied in freshwater fish, for example in relation to predation risk (e.g. sticklebacks, Bell, 2005; Dingemanse et al., 2007; and poeciliids, Brown et al., 2005; Fraser and Gilliam, 1987). In general, fish from high predation environments are bolder, the opposite effect to that seen in desert spiders.

Mirror image stimulation (MIS) tests have often been used to quantify aggression in fish (Rowland, 1999), for example in Siamese fighting fish, *Betta splendens* (e.g. Lissmann, 1932, cited in Rowland, 1999; Verbeek et al., 2007), salmonids, *Oncorhynchus* spp. (Berejikian et al., 1996; Taylor and Larkin, 1986), cichlids (e.g., *Astatotilapia burtoni*, Desjardins and Fernald, 2010; *Neolamprologus pulcher*, Reddon and Balshine, 2010; *Oreochromis mossambicus,* Ros et al., 2006), rivulus (e.g. *Rivulus marmoratus,* Earley et al., 2000), and zebrafish, *Danio rerio* (Moretz et al., 2007). The assumption is that the mirror presents a view of an unknown conspecific, which mimics the behaviour of the test animal, providing a measure of aggression when an individual encounters a matched opponent (Desjardins and Fernald, 2010). While the context in which the test is performed should be taken into account (Verbeek et al., 2007), MIS tests typically present fewer ethical challenges than pairing live animals in staged encounters, and the responses of the reflected image opponent are better controlled (i.e. they will not assume a subordinate role and avoid aggressive interactions).

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MIS tests can also be used to quantify other types of behaviour relating to animal temperament (Réale et al., 2007). Studies in scurid rodents (e.g. yellow-bellied marmots, *Marmota flaviventris*, Armitage and Van Vuren, 2003; North American red squirrels, *Tamiasciurus hudsonicus*, Boon et al., 2008) have used MIS in combination with open field trials to quantify behaviours that contribute to boldness and activity levels, as well as to aggression.

MIS tests have only rarely been used to investigate population-level differences in behaviour. In salmonids, such tests have compared aggression in fish from different habitats (e.g. Chinook salmon *Oncorhynchus tshawytscha*, Taylor and Larkin, 1986), and wild vs. hatchery fish (e.g. steelhead trout, *Oncorhynchus mykiss*, Berejikian et al., 1996). More recently, Moretz et al. (2007) compared the behaviour of three strains of zebrafish in five behaviour tests, including MIS, and found clear behavioural differences between the strains.

We studied the responses of wild-caught poeciliid fish *Brachyrhaphis episcopi* to a novel object and to their mirror image. This allowed us to quantify levels of aggression, boldness and activity in individuals from populations experiencing different levels of predation pressure. *B. episcopi* can behave aggressively to conspecifics, particularly if they are unfamiliar. Females are thought to be naturally territorial (Brown and Braithwaite, 2004; Brown et al., 2005), and in the laboratory they form dominance hierarchies, with larger females trying to maintain physical positions within tanks. Females are aggressive to one another, as well as to males when they persistently display towards and harass them. Males are primarily aggressive to one another (G. Archard, pers. obs.). Preliminary observations demonstrated that *B. episcopi* respond to MIS, with fish initially swimming towards a mirror, turning either left or right when they reach it and then swimming alongside their image. This is similar to the way that they inspect novel objects (G. Archard and V. Braithwaite, unpubl. data). Subsequently, they tend to approach a mirror from the side, swimming repeatedly back and forth parallel to it. In addition, they sometimes display towards their image, with fins stiffened and the body flexed so that the head and tail are closer to the mirror than the main part of the body. Both sexes sometimes bite at their image, and occasionally swipe it with their rear body and tail, in both instances making physical contact with the mirror. These inspections, displays and physical contacts with the mirror are all considered to be involved in aggressive mirror responses in fish (e.g., in the cichlid *A. burtoni*, Desjardins and Fernald, 2010). Biting and tail swiping can both cause damage when inflicted on conspecifics. These aggressive behaviours are also observed in *B. episcopi* when single unknown conspecifics are introduced into a tank with an established hierarchy. In contrast, however, aggressive behaviours are not observed when unfamiliar individuals are introduced into a novel test arena (G. Archard, pers. obs.). Hence, trials investigating aggression in this species need to be carried out in established population tanks.

In the wild, different populations of *B. episcopi* are exposed to varying levels of predation pressure. Temperament tests have previously shown that fish from high predation populations emerge from shelter sooner than those from low predation populations, and so have been described as bolder (e.g. Brown et al., 2005). These differences in temperament have both a heritable and an experiential component (Brown et al., 2007). Here we describe two experiments that were designed to investigate the relationship between boldness, aggression and activity across different populations. Based on results from other species, showing positive correlations between boldness, aggression and activity levels (e.g. sticklebacks, Huntingford, 1976; desert spiders, Riechert and Hall, 2000; great tits, *Parus major*, Verbeek et al., 1996), we predicted that fish from high predation populations would be more aggressive and more active, than fish from low predation populations.

2. Materials and methods

2.1. Animals used

B. episcopi are small, freshwater fish. Like other poeciliids, they are sexually dimorphic (adult standard length: males 20–35 mm, females 20–50 mm). Males may either display to solicit matings, or attempt sneak copulations, and females bear live young. Unlike most other poeciliids, however, they frequently show conspecific aggression.

B. episcopi were wild-caught, using seine and dip netting, from two rivers (the Rio Macho and Rio Quebrada Juan Grande (QJG)) in Panama. Collection sites (two on each river) were selected based on the comparative abundance of fish predators. For each river, one site was upstream, above a barrier waterfall, where *Rivulus brunneus* was the only piscine predator. The other was downstream, below the barrier, where *B. episcopi* exist with a range of characin and cichlid predators (see Brown and Braithwaite, 2004).

Groups of 15–20 mixed sex adult fish were housed in glass aquaria (300 mm wide × 300 mm high × 900 mm long), in 65 L water maintained at 25 °C, with a gravel substrate, and internal box filters. Fish were separated by population across four tanks (a total of 16 population tanks) and were fed daily with flaked fish food and live brine shrimp nauplii. Tanks were cleaned once weekly, and were enriched with plastic plants and empty plant pots. Lighting came from overhead fluorescent tubes for 11 h per day, supplemented at lower levels by standard 40 W lamps for an additional half hour at the start and end of each day. Fish were accustomed to routine maintenance and the presence of humans. All trials were carried out between 09:00 and 12:00, by one observer, blind to population.

2.2. Experiment one

To investigate if *B. episcopi* respond to the mirror merely as a novel object, we carried out mirror response trials in the 16 stock tanks. At each end of each tank, we ran a trial where the mirror surface was presented, and a control trial where the non-reflective, grey plastic mirror back was presented, as a standard novel object test (i.e. four trials per tank). At the start of a trial, a mirror (175 × 140 mm) was slowly inserted into the tank. The order of the four trials was random for each tank, and the order of trials between tanks was pseudo-random (because each tank was only ever tested once per day). After the mirror was inserted, the observer remained still 1–2 m from the tank for 2 min before starting observations. Over 5 min, the number of times fish approached within 30 mm (approximately one body length) of the mirror (front or back) was counted. Fish could not be individually identified, and often more than one fish approached the mirror at a time. Hence, counts were converted to numbers of approaches per fish per minute.

2.3. Experiment two

To test individual mirror responses we moved all tank enrichment from within 200 mm of the ends of the tanks, and then allowed the fish to acclimate to the new tank set up for a minimum of three days. For each trial, an opaque, grey PVC barrier was slowly inserted into one end of the tank in such a way as to isolate a single focal fish from the rest of its tank mates. If isolation was not achieved within 2 min, or if the fish in the tank showed any disturbance behaviours (e.g. dashing around the tank, huddling under plants) the trial was abandoned and that tank was left to settle for at least one day before another attempt was made. The barrier was positioned parallel to the end of the tank, and 150 mm away from it.

Lines drawn on the outside of the tank divided the isolation area into three 50 mm sections between the tank end and the barrier. These were further divided into a top and a bottom half giving six equal sized sections. During trials, movement between these areas was recorded, as well as interactions with the mirror stimulus.

Six fish from each tank were tested (total $n = 96$). For each sex, one large, medium and small fish were tested per tank. Sizing was subjective, based on the fish in each tank, and was done solely to avoid retesting individuals. As before, the order of the trials was random for each tank, and the order of trials between tanks was pseudo-random.

Trials consisted of three 5 min observation periods, each preceded by a 2 min settling period. The first and third time periods were controls, when movement and behaviour were recorded, but no mirror was present. At the end of the first control period, the mirror was slowly inserted into the isolated section, and placed against the end wall with the mirror facing into the tank. During this period, response to the mirror and movement were recorded. The following behaviours were measured: (1) fish swims parallel to reflected image with fins extended; (2) fish displays to image by moving parallel to it, with fins extended and body curved away from the mirror (males often also flex their gonopodium, but this could not be reliably quantified); during parallel swimming or display the fish (3) bites at its image; or (4) swipes its image with its tail and rear body. All trials were recorded in real-time using Etholog v2.2.5 (Ottoni, 2000). Data were converted into numbers per minute for the behavioural observations, and rate of movement between the six regions of the tank. Location data were converted into proportions of time.

Across all 96 individual fish observed, biting and swiping at the mirror image occurred less frequently than parallel swimming and displays. Many fish did not bite (38.54%) or swipe (65.62%) at their reflection. Biting and swiping were therefore summed to give an 'aggression' variable where the fish made physical contact with their own image.

2.4. Statistical analyses

All averages presented are means ± SE. Data were analysed using SPSS v17.0. Data were checked for homogeneity of variance and normality of residuals as required. Some variables for Experiment two required transformation prior to analyses (see below).

The number of responses per fish per minute from Experiment one were analysed using repeated measures ANOVAs with tank as subject, mirror image vs. control (novel object) as a within subjects factor, and predation regime and river as between subjects factors.

Rates of response to the mirror from Experiment two were analysed using linear mixed models. Specifically, data were analysed after log-transformation using a split-plot ANOVA design with the restricted maximum likelihood method. Predation regime and river of origin are treatment factors with tank as the experimental unit. Sex is a completely randomized subplot within tank. Movement rates and proportion of time in the mirror zone were analysed similarly, but with trial phase (control one, mirror presentation, control two) as a within subjects factor incorporated into the split-plot design. Proportions were arcsine square-root transformed prior to analyses. For movement rates, a compound symmetry repeated covariance type gave the lowest AIC value. For proportion of time in the mirror zone, an ante-dependent first order repeated covariance type gave the lowest AIC value.

Correlations between variables were performed on values aggregated separately for females and males within each tank. While this reduces sample size for these analyses, it controls for the non-independence of individuals within tanks. The sexes are separated because we predict sex differences in behaviour. The relationships are therefore analysed conservatively, because cor-

relations cannot be incorporated within the repeated measures, split-plot model framework.

2.5. Ethical note

Fish were captured in August 2008, under Authoridad National del Ambiente (Republic of Panama) permit SE/A-84-08. After capture, the fish were exported to the Pennsylvania State University (under ANAM permit SEX/A-101-08) to create stock populations for behavioural studies. All work was carried out under IACUC 28642. At the time of trials, fish had been in the laboratory for 5–6 months. Experiment one was conducted in January 2009, Experiment two in February 2009. All fish had previously taken part in other assays of temperament. We do not predict, and have not observed, that these trials affect reproduction or survival, or impose undue suffering. After the experiments the fish were maintained in the laboratory for further behavioural observations.

3. Results

3.1. Experiment one

The mean number of approaches per individual per minute was greater towards the reflective side of the mirror than the opaque back (reflective side = 0.44 ± 0.05, opaque back = 0.12 ± 0.02; ANOVA: $F_{12,15} = 41.41$, $P < 0.001$). Upstream, low predation fish approached the stimuli more than fish from downstream, high predation sites (upstream = 0.34 ± 0.05, downstream = 0.22 ± 0.04; $F_{1,12} = 5.17$, $P = 0.042$). There was no interaction between the type of stimulus (reflective side or opaque back) and predation regime ($F_{12,15} = 2.44$, $P = 0.144$), so fish from upstream populations were generally more responsive to novel stimuli, including mirror images. There was no difference between the approach rates of fish from the two different rivers (Rio Macho and Rio QJG, $F_{1,12} = 2.91$, $P = 0.114$), and none of the remaining interactions were significant (all $P > 0.202$).

3.2. Experiment two

Fish from low predation sites performed more parallel behaviours than fish from high predation sites, and Rio QJG fish performed more parallel behaviours than Rio Macho fish, but the interaction term, along with all other effects in the model, was not significant (Fig. 1a, Table 1). Males performed more display behaviours than females, but there were no other significant effects (Fig. 1b, Table 1).

Overall, Rio QJG fish were more aggressive towards their mirror image than Rio Macho fish (Fig. 1c, Table 1). Neither predation regime nor sex affected levels of aggression during the mirror trials,

Table 1

Effect of predation regime, river of origin and sex on the rate of behaviours performed in front of the mirror. Behaviours are swimming parallel to the mirror, displaying to the mirror, and an aggregate variable for aggression, that is the sum of biting and swiping. Data were log transformed prior to analyses.

Effect	Displays		Parallels		'Aggression'	
	F	P	F	P	F	P
Predation	1.68	0.219	8.02	**0.015**	3.58	0.083
River	0.03	0.871	8.57	**0.013**	6.36	**0.027**
Sex	4.55	**0.036**	0.01	0.918	0.52	0.473
Predation × river	0.28	0.610	3.70	0.078	0.69	0.422
Predation × sex	0.05	0.823	1.47	0.230	12.20	**0.001**
River × sex	<0.01	0.974	0.74	0.392	2.45	0.122
Predation × river × sex	0.01	0.905	0.03	0.861	0.01	0.917

Degrees of freedom are 1,76 for effects that include sex, and 1,12 for all other effects. Significant effects are shown in bold.
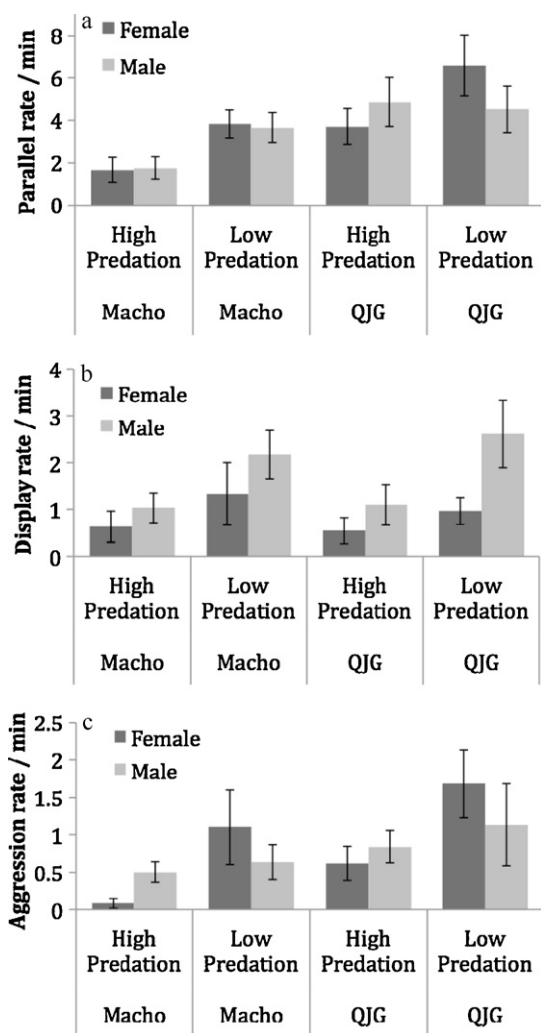
Fig. 1. The effect of predation regime, river of origin and sex on behaviours performed by individual fish in front of the mirror in Experiment two. (a) Number of parallel behaviours per minute, (b) number of display behaviours per minute, (c) number of aggressive behaviours per minute. Data shown are untransformed means ± SE.

but the predation × sex interaction was significant. Fig. 1c shows that females from low predation sites had higher rates of aggression than those from high predation sites, while males had intermediate rates under both predation regimes.

Correlations, split between the sexes for each tank (all $N = 16$), showed that display rate was not correlated with parallels rate (Pearson's rank correlation: females $r_p = 0.06$, $P = 0.816$, males $r_p = 0.39$, $P = 0.131$). Male display rate and aggression rates were highly correlated ($r_p = 0.79$, $P < 0.001$), but this was not the case for females ($r_p = 0.05$, $P = 0.842$). In contrast, female parallels rate was highly correlated with aggression rate ($r_p = 0.79$, $P < 0.001$). Male parallels rate was also correlated with aggression ($r_p = 0.52$, $P = 0.041$), but this relationship is not significant after sequential Bonferroni correction for multiple tests ($\alpha = 0.004$ for each sex). For both sexes, none of the MIS behaviours were correlated with movement rate in any of the three phases of the trials (Table 2).

The rate at which fish moved between the six areas of the test compartment did not differ between predation regimes, rivers or sexes. However, movement rate between the areas differed significantly between the three phases of each trial (Table 3). Pair-wise comparisons found that fish moved between the areas more in control phases than when the mirror was present (phase 1 vs. 2 = 9.77, $P < 0.001$; phase 2 vs. 3, −7.77, $P < 0.001$). The difference between

Table 2
Correlations between the rate of behaviours performed in front of the mirror and rate of movement (MR) in the three phases of trials, where MR2 is the test phase with the mirror present, and MR1 and MR3 are control phases with no mirror. Behaviours are swimming parallel to the mirror, displaying to the mirror, and an aggregate variable for aggression, that is the sum of biting and swiping.

	Displays		Parallels		'Aggression'	
	R_p	p	R_p	p	R_p	p
(a) Females						
MR1	−0.19	0.479	0.09	0.735	0.17	0.521
MR2	−0.30	0.259	0.06	0.816	0.08	0.760
MR3	−0.28	0.297	0.25	0.360	0.49	0.053
(b) Males						
MR1	−0.40	0.124	−0.18	0.506	−0.23	0.402
MR2	−0.31	0.243	−0.18	0.512	−0.29	0.279
MR3	−0.30	0.252	0.15	0.590	−0.08	0.779

Data are split by sex, and aggregated per tank, giving $N = 16$ for each test (with $\alpha = 0.004$ for each sex).

the two control phases was not significant (phase 1 vs. 3 = 2.00, $P = 0.133$).

The proportion of time fish spent in the mirror zone (the two areas, top and bottom, that were at end of the tank) also varied (Table 3). Pair-wise comparisons show that fish spent more time in the mirror zone when the mirror was present than in either of the control phases of the trial (phase 1 vs. 2 = −0.27, $P < 0.001$; phase 2 vs. 3 = 0.21, $P < 0.001$), with no difference between the control phases (phase 1 vs. 3 = −0.06, $P = 0.169$). Additionally, fish from the Rio QJG spent more time in the mirror zone compared to Rio Macho fish (Table 3; Fig. 2a). There was a significant interaction between phase and river: time in the mirror zone was approximately equal for fish from both rivers in the first control period, but fish from the Rio Macho spent less time in the mirror zone compared with QJG fish during the mirror phase, and this difference continued into the second control phase.

Low predation fish spent more time in the mirror zone than did fish from high predation sites (Table 2; Fig. 2b). There was a non-significant trend ($P = 0.054$) for the phase by predation interaction. Fig. 2b shows that, as for the phase by river interaction, time in the mirror zone was similar for fish from high and low predation sites in the first control period, but low predation fish spent more time in the mirror zone in the mirror phase, and possibly the second control phase. These interactions suggest carry-over effects of the mirror that vary by predation regime and river of origin. Time in the mirror zone did not differ between sexes, and no other interaction terms were significant (Table 3).

Table 3
Effect of predation regime, river of origin, sex and phase of trial on the rate of movement between the areas of the test compartment, and the proportion of time spent in the mirror zone (arcsine square-root transformed prior to analysis).

Effect	Rate of movement			Time in mirror zone		
	F	df	P	F	df	P
Predation	0.08	1,12	0.783	4.68	1,16.36	**0.046**
River	0.31	1,12	0.587	9.56	1,16.36	**0.007**
Sex	0.64	1,76	0.427	0.11	1,91.25	0.745
Phase	53.95	2,176	**<0.001**	29.66	2,97.97	**<0.001**
Predation × river	0.02	1,12	0.899	1.52	1,16.36	0.236
Predation × sex	0.02	1,76	0.879	0.93	1,91.25	0.338
Predation × phase	1.00	2,176	0.370	3.00	2,97.07	0.054
River × sex	0.38	1,76	0.542	2.40	1,91.25	0.125
River × phase	1.93	2,176	0.149	5.03	2,97.07	**0.008**
Sex × phase	0.45	2,176	0.636	1.35	2,97.07	0.265

Only the results of main effects and two-way interactions are shown. All three- and four-way interactions were non-significant at $P > 0.193$. Significant effects are shown in bold.
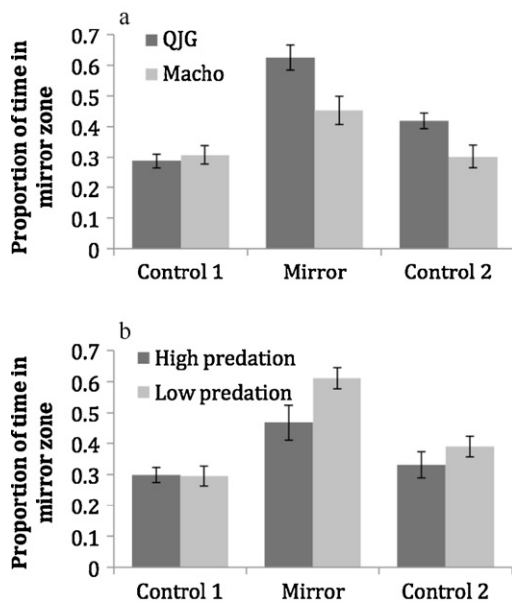
Fig. 2. Mean proportion of time spent by individual fish in the mirror zone in Experiment two. Data are split by phase of trial (control 1, mirror, control 2), and by (a) river of origin, or (b) predation regime. Data shown are untransformed means ± SE.

4. Discussion

B. episcopi responded to mirror images with direct approaches, parallel inspections, displaying and aggressive physical contacts with the mirror (biting and tail swiping). These were similar to the aggressive interactions observed between individual *B. episcopi* as the fish interact or compete for food in their laboratory tanks, indicating that they treat the mirror image as a conspecific. We observed behavioural differences between populations and between the sexes. In Experiment one, fish approached a mirror stimulus more often than a novel object stimulus. Similar effects have been seen in other fish species (e.g. cichlids, Ros et al., 2006). *B. episcopi* from low predation sites approached both stimuli more frequently. Previous studies, on different individuals, have found that *B. episcopi* from high predation populations are bolder than those from low predation populations; they emerged more quickly from a shelter (e.g. Brown et al., 2005, 2007). In general, animals from high predation populations are considered to be bolder because they have to trade-off the risk of predation with other activities such as foraging and mating (Lima and Bednekoff, 1999). If approach to a novel object is considered a measure of boldness, and approach to an image of an unfamiliar conspecific (often accompanied with physical attacks) is considered a measure of aggression, then the first experiment found that both boldness and aggression were greater in low predation populations.

Boldness, however, is a broad term with multiple definitions, that has been used to describe behavioural variation in a variety of situations. As such, boldness may be a composite measure that includes elements of several behavioural axes, e.g. responses to both new and known situations and objects, responses to risk, and activity levels. To combat this problem, Réale et al. (2007) suggest that temperament traits should be defined by ecological situation. Under this classification, boldness is an individual's response to risky, but not novel, situations. Responses to novelty are considered exploration/avoidance, and activity is also a separate trait, that can affect both boldness and exploration. These distinctions may account for differences between the current and earlier studies on *B. episcopi*: our results indicate aggression is correlated across populations with willingness to explore, not boldness per se.

In Experiment two, individual fish responded to the mirror by moving between zones of the test area less frequently, and by spending more time in the mirror zone in phases when the mirror was in the tank. As in Experiment one, low predation fish spent more time in front of the mirror, and performed more parallel inspections of their image, compared to fish from high predation populations. There were no effects of predation on display or aggression rates, although there was a tendency for higher levels of aggression in low predation populations (Fig. 1, $P = 0.083$). The increased effect of the mirror on fish from low predation populations is further demonstrated by the fact that after the mirror test fish from low predation populations maintained an increased presence in the mirror zone, whereas fish from high predation populations did not. This difference was not caused by freezing behaviour–the fish actively moved when they were in the mirror zone.

Performing parallels may have the dual function of gaining information about a potential rival, and allow them to gain information about you. In the wild and the laboratory, female *B. episcopi* frequently approach one another, after which one female swims away. These approaches may be equivalent to the parallels seen in the MIS, and so function as aggressive signals that test and reinforce dominance hierarchies, without need for physical interactions.

This raises the question, why do parallel rates differ between populations with different predation regimes? There are a number of possible explanations. First, increased risk of predation in downstream habitats may increase the need to school with conspecifics such that it outweighs any benefits of maintaining clear dominance hierarchies or territories. Unlike many other poeciliid species, however, *B. episcopi* do not frequently school, even when threatened (pers. obs.). Second, downstream, high predation populations are larger than those upstream above the waterfalls. This means that high predation fish may simply be unable to remember the identity of all members of their population. Griffiths and Magurran (1997) found that the preference for wild females guppies, *Poecilia reticulata*, to school with familiar individuals declines as group size increases, suggesting that females remember individual conspecifics, but this effect is weaker in larger groups. Recently, it has been shown that fish can identify unfamiliar conspecifics on the basis of olfactory cues originating from habitat or diet alone (Ward et al., 2005). However, most sections of *B. episcopi* habitat are well connected, with similar physical and biotic characteristics, which makes self-referent matching processes unlikely to work in this situation. Thirdly, the encounter rate of unfamiliar conspecifics may be so great in high predation populations that constant challenges may be energetically unviable. This effect may be heightened by increased downstream movement of individuals, as is the case for guppies (van Oosterhout et al., 2007).

There were clear sex differences in mirror responses, and these interacted with the effects of predation regime. As might be expected for a poeciliid species, where mating systems are often based on female choice, males displayed more often than did females. Indeed, perhaps the fact that females displayed at all is more noteworthy. Males obviously display to solicit matings, whereas females have not been observed displaying to conspecifics of either sex in the wild, and only very rarely in the laboratory. The fact that both sexes display to their own image suggests that displays form part of the aggressive response to unknown same-sex conspecifics, and thus in males have more than one function. It also means that 'testing' of unfamiliar individuals may start with parallel inspections and then escalate, at rates that vary between individuals, through displaying, to physical aggression. Certainly, care has to be taken when introducing new individuals of either sex into tanks: if lone *B. episcopi* are introduced, they are chased repeatedly by the existing tank mates, and are nearly always attacked,

sometimes leading to death (V. Braithwaite, pers. obs.). Increased aggression towards unfamiliar individuals compared to familiar ones is well-documented in fish (e.g. in trout, *Salmo trutta*, Höjesjö et al., 1998; and references therein).

We found no sex differences in overall levels of aggression. Female aggression rates, however, were lower than those for males in high predation populations, but higher than male rates in low predation populations. Thus, the relative cost of aggression varies for females across predation regime. In males, intra-sexual aggression is likely to arise over access to females, whereas for females it seems more likely that aggression will be linked with access to food resources. Females are larger than males, invest more in reproduction, and hence require more energy for maintenance. Female fecundity in livebearing poecilids is determined by size (e.g., in guppies, Herdman et al., 2004), and hence food availability. *B. episcopi* are omnivorous but, unlike many other poecilids, much of their diet consists of invertebrates (Angermeier and Karr, 1983) rather than plant material. Abundance of prey is both much lower and more patchy upstream (Angermeier and Karr, 1983). Intra-specific competition for food in upstream habitats is also much higher, because *B. episcopi* dominate the fish fauna here (Brown and Braithwaite, 2004). In contrast, downstream habitats have both greater food abundance and more conspecifics, making female aggression and the maintenance of dominance hierarchies both unnecessary and untenable. Fish living downstream must also invest time in predator avoidance. Together, these factors may underlie the differences in female aggression seen between populations.

Fish from the two rivers also behaved differently. QJG fish spent more time in the mirror zone than those from the Macho populations, although they were not more active overall. QJG fish performed more parallel inspections, and were more aggressive. On both rivers, *B. episcopi* are replaced downstream by the congener *B. cascajalensis*, before they flow separately into the Panama Canal. Populations from different rivers are therefore clearly separated, and are likely to be more genetically distinct than those from different predator regimes within rivers, which are connected by low rates of upstream migration and higher rates of downstream migration (van Oosterhout et al., 2007).

In summary, *B. episcopi* from high predation populations are bolder, whereas low predation populations are more explorative, with more aggressive females. Recent studies on three-spined sticklebacks have shown that a relationship between aggressiveness, activity and exploration only exists in high predation populations (Bell, 2005; Dingemanse et al., 2007). The results from these studies provide support for an adaptationist explanation of the relationships between temperament traits, rather than the hypothesis that genetic constraints determine behavioural relationships (Sih et al., 2004; Dingemanse and Réale, 2005). Our explanation of why females, but not males, are more aggressive in low predation populations is inherently adaptationist, and highlights the fact that more than one environmental variable (i.e. not just predation) may be a driving natural selection in this species.

Acknowledgements

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