Early interactions with adults mediate the development of predator defenses in guppies

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Antipredator defenses in many species have been shown to exhibit phenotypic plasticity in response to variable predation risk. Some evidence suggests that in certain species adults act as proxy predators, triggering the development of adaptive defenses in juveniles where interaction with a predator is unlikely to occur. However, almost nothing is known about how adult/juvenile interactions mediate plasticity. Here, we examine the nature of the antipredator defenses that develop in Trinidadian guppies as a function of early social experience and investigate the importance of different types of cue (physical, visual, and olfactory) by rearing juveniles under 3 different social conditions. In the first, only juveniles are present; in the second, only visual and olfactory interaction occurs between adults and juveniles; and in the third, adults physically interact with juveniles. Our analyses show that juveniles reared in the physical presence of adults spend significantly less time shoaling with adults than fish from other treatments in an adult versus juvenile shoal-choice trial. Further, we show that juveniles with experience of adult aggression have a decreased response latency to a simulated avian predation attempt and travel a greater distance in the first 5 frames of movement after the simulated strike. Finally, juveniles reared with physical experience of adults developed relatively deeper bodies and were significantly shorter in standard length than guppies reared without physical experience of adults.

Keywords: development, inducible defenses, phenotypic plasticity, rearing experience, social environment.

Prediction is a powerful selective force, contributing significantly to mortality in many ecological systems. This means that the development of effective antipredator defenses can be of huge importance to individual fitness. Antipredator defenses have been shown to have a strong genetic basis across a wide range of taxa (damselﬁshes: Stoks et al. 2003; ﬁsh: Huntingford and Wright 1993; birds: Veen et al. 2000; amphibians: Gallie et al. 2001). However, the development of defenses can be costly (Tollrian and Harvell 1999), and so when predation risk varies temporally or spatially it may be adaptive for prey organisms to phenotypically respond to changes in the environment to avoid incurring such costs where unnecessary. This is known as phenotypic plasticity (Pigliucci 2001). Phenotypic plasticity can take a variety of forms, including morphological (Olsson et al. 2007), life historical (Evans et al. 2007), and behavioral (e.g., learning: Svanch and Bolnick 2007).

Learning is an important component in the acquisition of antipredator defenses. Exposing coho salmon fry Oncorhynchus kisutch to a predator early in life led to a 75% survival rate in the subsequent encounter, compared with a 46% survival rate for naïve fry (Patten 1977), highlighting the importance of early experience in successfully evading predators. More recent work supports the importance of learning across a range of taxa (e.g., birds: Maloney and Maclean 1995, fish: Kelley and Magurran 2003, mammals: Mineka et al. 1984).

Inducible morphological defenses have been documented across a variety of both vertebrate and invertebrate taxa (Tollrian and Harvell 1999). For example, experience of predator cues induces morphological defenses in Daphnia (Laforsch et al. 2004) and crucian carp Carassius carassius (Bronmark and Miner 1992). Carp exposed to piscivorous pike Esox lucius develop deeper bodies, which lead to increased handling times for pike and a higher probability of escape for prey. Such individuals also constitute a less desirable prey phenotype than more fusiform carp (Nilsson et al. 1995).

Early experience is therefore profoundly important in the development of antipredator defenses, but the mechanism for this is unclear as when faced with highly effective predators, naïve juveniles may have little opportunity to hone their responses to predator attacks. Some evidence suggests that adult/juvenile interactions may constitute a proximate mechanism by which juveniles can develop antipredator defenses prior to direct experience with a predator (Goodey and Liley 1986; Tulley and Huntingford 1987). In sticklebacks Gasterosteus aculeatus, fry from high-predation populations raised with parental care showed a significant improvement in their antipredator responses compared with fry raised with no care (Tulley and Huntingford 1987).

The Trinidadian guppy has no such system of parental care, and neonates respond to conspeciﬁc adults by displaying a variety of antipredator behaviors (Magurran and Seghers 1990). Adults behave agonistically to juveniles and have been shown to prey on fry (Magurran 2005), suggesting that adults may behave as proxy predators in this system and provide juveniles with their ﬁrst opportunity to develop antipredator skills.

In support of this idea, Goodey and Liley (1986) demonstrated that juveniles with early experience of encounters with adults had a signiﬁcantly improved escape ability later in life. How this enhanced escape ability develops is unknown. Here, we investigate the nature of the defenses induced by adults in fry by rearing guppies in the presence and absence of adults. We predict that juveniles with early experience of adults will modify their shoaling behavior (a common antipredatory strategy in ﬁsh; Krause and Ruxton 2002) and respond more quickly to a fright stimulus. We also examine the impact of early experience on morphological development. Previous work has shown that olfactory cues are sufﬁcient to trigger the development of morphological defenses in a number of species (Tollrian and Harvell 1999). We investigate the type of cues that “activate” plasticity in this system by rearing fry in...
3 different treatments: with physical experience of adults, with visual and olfactory adult cues but no physical mixing, and in the absence of adults.

**METHODS**

**Study population and maintenance**

All fish used in this experiment were the descendants of wild-caught guppies sourced from the Tacarigua River in the northern mountain range of Trinidad in 2005 (Trinidad national grid reference: PS 787 804; coordinates: 10°40.736’N 061°19.168’W). This site is characterized as a high-predation locale (Croft et al. 2006) due to the presence of a variety of aquatic piscivore predators, particularly the pike cichlid *Crenicichla alta*.

Stockfish were reared at the University of Leeds in a 100 × 100 × 25 cm tank with gravel substrate and clumps of moss to provide refuge for fry. Controlled temperature (28 °C) and lighting conditions were maintained (18 W overhead fluorescent strips set on a 12-h light/dark cycle). Water depth was kept constant at 13 cm. All fish were fed daily ad libitum with dried flake food.

**Rearing conditions**

We captured juveniles of ≤10-mm size (mean ± standard deviation [SD]: 8.09 ± 0.09) using a dip net and assigned them randomly into one of 3 different rearing conditions. This meant that fry were unlikely to be from the same brood, ruling out the importance of any maternal effects. In the first treatment, 4 of these juveniles were reared in the presence of 2 adult males. In this “mixed” treatment, adults and juveniles were free to interact physically. Previous work has shown that agonistic and competitive encounters between adults and juveniles when allowed to interact physically were commonplace (Goodey and Liley 1986); our observations confirmed this. In the second treatment (to be referred to as “v and o”), we were interested in isolating the impact of visual and olfactory interactions and reared 4 neonate fry separated from 2 adult males by a transparent perforated screen. To ensure the transmission of olfactory cues, water was pumped from the adult compartment to the juvenile compartment. This occurred in all treatments to control for flow patterns. In the third, control treatment, 4 neonate fry were reared in the absence of adults (to be referred to as the “all juvenile” treatment). Although density differed among treatments, the size of the tanks rendered any variation negligible.

Each rearing tank was of the same design: tanks were 50 × 20 × 20 cm, had a gravel substrate, and had opaque sides to minimize disturbance and to mimic natural conditions. Water depth was kept constant at 12 cm deep. Tanks were divided into 2 compartments by a transparent, perforated plastic screen (see Figure 1). In each tank, the larger compartment (30 × 20 × 20 cm) contained a free-floating circular plastic feeding ring (diameter = 8 cm) with 2 square polystyrene floats (2 × 2 × 1 cm) opposite to one another, which floated on the surface of the water. In addition, a strip of green plastic mesh (3 × 25 cm) was tied loosely into a knot and placed on the substrate to provide a refuge for the juvenile guppies. The larger compartment contained 4 fry in each rearing treatment and 2 additional adult males in the mixed treatment. In the mixed and all juvenile treatments, the smaller compartment was unoccupied; in the “v and o” treatment the smaller compartment contained 2 adult males. A foam filter was fixed to the side of the smaller compartment with silicon sealant. A length of hosing (34 cm) was attached to the outlet pipe of the filter with silicon sealant and placed so that the open end was below the water surface in the larger compartment. This pumped water from the small compartment to the larger one and ensured that olfactory cues were transmitted from the adults to the juveniles in the “v and o” treatment.

We reared 14 replicates of the mixed and all juvenile treatments and 11 replicates of the visual and olfactory treatment for a total of 47 days each between April and December 2006. Replicates had staggered start dates, with one replicate of each of the 3 treatments beginning at a given time to control for any effect of start date. On the start date of a set of replicates, 12 fry and 4 adult males were captured from the source population and randomly assigned to rearing treatments. Individuals’ body lengths were measured and recorded. Adult males only were used: in high-predation populations, guppies segregate by sex, with more males tending to occupy the shallower waters. Juveniles are hence more likely to have a higher frequency of encounter with males than with females (Croft et al. 2006). In each rearing regime, fish were fed daily ad libitum with dried flake food. Food was sprinkled into the feeding ring located on the water surface in the larger compartment in all treatments. This aggregated the food resource spatially, encouraging competitive and agonistic interactions to occur between adults and juveniles in the mixed treatment. After 47 days of rearing, we recorded the final tank density.

**Experimental assays**

After 42 days, we carried out 2 behavioral assays. The first was a binary shoal-choice test that took place on day 42; the second examined individual escape responses to a simulated avian predation attempt (day 47). Finally, individuals’ body lengths were measured and recorded. A subset of the fish were photographed for morphometric analysis (see below), and all were returned to a stock tank.

**Shoaling tendency**

To investigate shoaling behavior, focal fish were allowed to swim freely in a tank containing 2 choice chambers; one contained 2 adult males and the other 2 juvenile stimulus fish. All stimulus fish were taken from the original stock...
population. The choice arena was a rectangular tank of dimensions 22 × 20 × 20 cm, of 7-cm water depth with gravel substrate. A transparent perforated cylindrical plastic choice chamber (diameter = 8 cm) was placed 2 cm from the side of the tank at each side along the midline, with 12 cm separating the 2 choice chambers. A shoaling zone of 2 body lengths width (approximately 3 cm) surrounded each chamber, with a neutral zone in the center of the tank (of approximately 6 cm at the narrowest point). To minimize disturbance, the sides of the tank were opaque; focal individuals were observed with the use of a mirror angled at 45° above the tank. In the trial, 2 adult male stimulus fish were placed in one of the choice chambers and 2 juvenile fish placed in the other chamber to investigate individual shoaling preferences.

At the beginning of each trial, a focal fish was placed in a transparent plastic cylinder in the neutral zone. Once all stimulus fish had resumed movement, this holding chamber was removed. To ensure the fish had recovered from any disturbance this may have caused, the trial began once the focal was removed. To ensure the fish had resumed movement, this holding chamber was a transparent plastic cylinder in the neutral zone. Once all chambers were opaque; focal individuals were observed with the use of a mirror angled at 45° above the tank. In the trial, 2 adult male stimulus fish were placed in one of the choice chambers and 2 juvenile fish placed in the other chamber to investigate individual shoaling preferences.

Response to a simulated threat

Escape response trials took place in a 22 × 20 × 20 cm tank with opaque sides. Water depth was kept at 2 cm to minimize vertical movement in escape responses, a depth within the range of which juveniles would likely to experience in the wild. Focal individuals were introduced into the arena and allowed 5 min to acclimatize. After this, a plastic stick (diameter = 0.8 cm, length = 50 cm with Bluetak attached to the tip) was dropped vertically from 18 cm above the water surface within 5 cm of the focal fish to mimic an avian predation attempt and elicit an escape response (Ward et al. 2004). This was repeated for each of the 4 fish in each of the rearing treatments. Trials were recorded at 25 frames/s using a Sony DSR-PD100AP video camera suspended 32 cm above the top of the tank. Variables measured were an individual’s latency to respond (frames), the distance traveled in the first 5 frames of movement (millimeters), and the maximum speed achieved in the first 10 frames (millimeters per frame). Video was transferred to .avi using vDub freeware (http://www.virtualdub.org/) and analyzed frame by frame using ImageJ (also available free at http://rsb.info.nih.gov/ij/). Each fish was subjected to this stimulus only once.

Morphological measurements

After the behavioral assays, we measured the body length of the fish and placed it in an individual container for morphometric analysis. To investigate whether early experience of adults triggered the development of differential morphologies in juvenile guppies, we calculated the relative body depth of test fish after 47 days.

An image of each fish was captured using a JVC TK1280E color video camera using the program Imagedok 2.0 (Kinetic Imaging Ltd, Liverpool, United Kingdom). The relative caudal base depth of each fish was calculated by dividing caudal base depth by standard length (Figure 2). The standard length is the distance between the fish’s snout and the point at which the spine meets the base of the caudal fin. We defined “depth” as the distance from the anus to a point on the dorsal edge that could be located by drawing a line perpendicular to the line of standard length through the anus and the dorsal edge. Distances were calculated using tpsDIG (available free at http://life.bio.sunysb.edu/morph/).

Statistical analysis

All data were checked for deviations from normality and homoscedasticity. Only individual response latency data failed to conform to parametric model assumptions; when tank means were calculated, the necessary assumptions were met. In this case, we used a general linear model to investigate the effects of final tank density, mean body length, and treatment on mean response latency. All other statistical analysis utilized mixed-effects models with “tank” as a random factor to avoid pseudoreplication of the data and to control for any tank effect, with details of specific fixed effects below. In multiple comparisons of parametric data, the false discovery rate post hoc test was carried out to investigate pairwise differences (Verhoven et al. 2005). All statistical analysis was carried out using R (freeware available at www.r-project.org/) and SPSS 11.0. Where mortality had occurred during the 47-day rearing period, this was either controlled for statistically by including tank as a random factor where data fulfilled parametric assumptions or by including density as a fixed factor where tank means were used. Nonsignificant interactions were dropped, and then the analysis was rerun. Differences in sample sizes between experiments are indicative of technical issues related to video and image capture or exclusion from trials based on the specific criteria detailed.
RESULTS

Mortality

Levels of mortality did not differ between rearing treatments (Kruskal–Wallis: $\chi^2 = 0.196$, degrees of freedom = 2, $P = 0.907$; 4 out of 56 fry died over the 70-day rearing period in the mixed and all juvenile treatments and 4 out of 44 fry in the visual and olfactory treatment.

Shoaling behavior

We found no evidence to suggest that experience of adult/juvenile interactions affected overall shoaling tendency (i.e., total time spend shoaling with adults or juveniles [seconds]; restricted maximum likelihood [REML]: $F_{2,36} = 0.478$, $P = 0.62$). However, when we examined how individuals with differing early social environments spent their time, differences became clear. Rearing treatment influenced the time adult fish spent shoaling with juveniles (REML: $F_{2,36} = 12.7041$, $P < 0.001$; Figure 3). Juveniles reared with physical experience of adults spent significantly more time shoaling with juveniles than fry reared in other treatments.

Response to a simulated avian predation attempt

Our analysis of variables relating to escape performance in juvenile guppies uncovered evidence of differences related to both early social environment and body length. Juveniles with experience of adults reacted significantly faster than juveniles lacking physical experience of adults (general linear model (LM): $F_{2,36} = 12.58$, $P < 0.001$; Figure 4a). Mean individual body length and final tank density had no significant effect on mean response latency (LM: $F_{1,36} = 0.18$, $P = 0.67$; $F_{1,26} = 2.65$, $P = 0.12$, respectively). For this analysis, data from the single treatment with a density of 2 were excluded as all other treatments had a final density of 3 or 4 (mean ± SD = 3.76 ± 0.43) and tank density was explicitly included in the model as a fixed factor. Individuals that initially froze before taking evasive action (fish with latencies of ≥10 frames, $n = 7$ out of 132 individuals) were also excluded from this analysis. Juveniles with experience of adults reacted significantly faster than juveniles lacking physical experience of adults. Both individual body length and early social environment influenced the distance traveled in the first 5 frames of movement after a fright stimulus, with larger fish traveling farther (REML: $F_{1,54} = 7.04$, $P = 0.01$; treatment $F_{2,54} = 3.81$, $P = 0.035$; Figure 4b). No relationship between an individual’s relative caudal depth and the distance traveled in 5 frames was found ($F_{1,54} = 2.30$, $P > 0.05$). Fish with physical experience of adults traveled farther in the first 5 frames of movement than fish lacking actual physical experience of adults.

Our analysis of the greatest distance a juvenile traveled in a single frame (in the first 10 frames), that is, the maximum speed achieved (millimeters per frame) suggested that only body length influenced maximum speed achieved (REML: body length $F_{1,54} = 5.16$, $P = 0.011$; Figure 5a). Fish reared in the physical presence of adults were, on average, significantly shorter than fish reared without experience of such interactions with adults.

Finally, our analyses revealed a treatment effect on relative body depth of fish (REML: $F_{2,34} = 7.914$, $P < 0.001$, Figure 5b). Fish with experience of adults had relatively deeper bodies than fish reared in the absence of any form of interaction with adults.

DISCUSSION

Our principal findings clearly show for the first time that early experience of adult/juvenile interactions impact on the
behavioral and morphological development of Trinidadian guppies. We found that this experience affected shoaling behavior, response to a simulated avian predation attempt, and morphological development. Furthermore, we investigated the importance of different cues, finding that physical experience of adults was of primary importance in the acquisition of heightened evasive responses, morphological development, and mediating shoal-choice behavior. How might this behavioral and morphological plasticity translate into actual fitness benefits in the wild? We argue that the plasticity evident in these traits may confer enhanced defenses in a predator situation in real life.

Grouping behavior

We found evidence of differences in shoal-choice behavior as a function of early social environment. Juveniles reared with physical experience of adults spent significantly more time shoaling with other juveniles than either guppies reared with only the visual and olfactory cues of adults or guppies reared in the absence of adults. This means that juveniles from the mixed treatment spent significantly more time in phenotypically homogenous (i.e., size matched) shoals. Both the dilution effect and the confusion effect rely for their maximum effect on shoals being homogenous (Krause and Ruxton 2002); should individuals join shoals with phenotypically different fish, their probability of being attacked will be greater than if they had joined shoals containing similar individuals. This is known as the oddity effect (Landeau and Terborgh 1986) and has been experimentally shown to be important in increasing predator preference and attack success for “odd” prey (Krause and Ruxton 2002). Hence, juveniles reared without experience of physical interactions with aggressive adults may suffer greater predation risk due to their more readily forming mixed size shoals. Two hypotheses may explain this pattern: firstly, experience of adult aggression and competition may lead to avoidance and the passive formation of homogenous shoals. Secondly, the process may be active—adult/juvenile interactions may adaptively mediate the phenotypic structure of shoals through modifying the shoal-choice preferences of juveniles.

Evasive response

Juveniles with experience of adults responded faster to a fright stimulus and also traveled farther in the first 5 frames of movement after the fright stimulus. Contrary to our initial prediction, the maximum speed achieved did not differ between juveniles reared in the different treatments. Although it seems plausible that an increase in response latency and the propensity to travel farther after a fright stimulus could increase survival probabilities in a predator encounter in the wild, empirical tests of which components of the escape response are important in an actual predator attack are surprisingly rare. Fuiman et al. (2006) describe how “responsiveness” to a predator accounted for 86% of the variation in escape potential in red drum larvae Sciaenops ocellatus, and Webb (1981) found that 24–30% of anchovy larvae responded too late to attack by clown fish. Both these studies implicate response latency as critical in surviving a predator attack, supporting its adaptive value. Furthermore, Webb (1981) suggested that maximum speed achieved was not an important characteristic in the evasive response as the confusion caused by the rapid initial movement of the prey was adequate to increase the likelihood of survival, which may explain why we found no differences in maximum speed achieved between treatments.

Size and shape

Guppies with physical experience of agonistic and competitive encounters with conspecific adults were shorter in standard length but had deeper caudal peduncles than individuals reared in the absence of adults. This variation in body shape matches variation between high- and low-predation sites in the wild, with guppies from high-predation rivers (and the mixed treatment) being smaller (Reznick and Endler 1982) and having deeper caudal peduncles (males only—Hendry et al. 2006) than fish from low-predation rivers (and the all juvenile treatment). Furthermore, Langerhans and Dewitt (2004) found consistent patterns of morphological divergence in a cross-population comparison of 3 species of livebearing fish. They reported that individuals from high-predation rivers had relatively larger caudal peduncle regions than fish from low-predation rivers, suggesting that these morphological features may have adaptive antipredatory significance. In support of this, Langerhans et al. (2004) showed that caudal size was positively related to enhanced locomotor performance in mosquito fish Gambusia affinis.

Conversely, relative caudal depth was not statistically related to any of the components of escape response measured in our study. It is possible that at this stage in the guppy’s ontogeny differences in escape response are related to differences in risk perception (potentially via experience of adult aggression) rather than physiological differences (i.e., differences in caudal size). It would be interesting to examine the impact of adult/juvenile interactions in the juvenile stage on the morphology and escape responses of adult guppies to further investigate whether this early morphological divergence has implications for escape response later in life.

We have proposed that adults behaving as proxy predators toward juveniles drive the development of the defenses acquired by juveniles in the mixed treatment. However, an alternative hypothesis might be that this plasticity may be a product of competition—antagonism between adults and juveniles. Juveniles with experience of competitive interactions may devote more time to vigilance to avoid potentially costly competitive interactions with adults, which could have consequences for their response latency. Competitive exclusion from access to food may also impact on the morphological
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The development of fry. Hence, enhanced antipredator skills may be a beneficial side effect of juveniles being wary to avoid aggressive competition for food, rather than a direct consequence of experience evading predatory specifics. Furthermore, it is possible that these defenses have developed in response to higher levels of density (and a higher frequency of interactions per se) in the mixed treatment. We assert that although this is possible, there is no strong a priori evidential or theoretical basis for this hypothesis. Conversely, previous empirical work supports the hypothesis that specifically adult/juvenile interactions drive the development of these defenses (Goodey and Liley 1986).

Conclusions

Previous work has clearly elucidated the importance of natural selection on the evolution of antipredator defenses in guppies (Magurran et al. 1992; O’Steen et al. 2002). In this study, we provide evidence that a number of these antipredator traits exhibit a high degree of phenotypic plasticity. For this plasticity to be adaptive, juveniles from high-predation rivers would have to encounter adults at a greater frequency than in low-predation rivers in order for adult/juvenile interactions to act as accurate cues of predation risk. This “adaptive interaction” hypothesis could be tested with relative ease through field observations, comparing the spatial distribution of adults and juveniles between environments of varying predation risk. Also central to the notion of adaptive plasticity is that plastic traits are costly to produce and maintain. It is likely that the production and maintenance of deeper caudal peduncle regions are energetically expensive; individuals may trade off burst-speed against endurance (Reidy et al. 2000). Burst swimming speed is likely to be of benefit in evading predator attacks, whereas sustained swimming may be beneficial in foraging and mate acquisition (Domenici 2003). The trade-offs involved in preferentially shoaling with juveniles over adults are unclear.

In summary, we report that adult/juvenile interactions mediate the development of antipredator responses in the Trinidadian guppy. Furthermore, we elucidate the importance of different cues, finding that physical cues—experience of agonistic/competitive interactions—are primarily important in the acquisition of behavioral and morphological defenses. The defenses we describe may act to enhance individual survival probability at a number of stages in the predator sequence (Fuiman and Magurran 1994). Testing whether fish reared in differential social environments survive more effectively in the wild would provide an interesting basis for future work in the field.

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REFERENCES

Patten BG. 1977. Body size and learned avoidance as factors affecting predation on coho salmon fry, Oncorhynchus kisutch, by torrent sculpin Cottus rhodent. Fish Bull. 75:457–459.