Social Context Modulates Predator Evasion Strategy In Guppies

Eva K. Fischer*, Adina J. Schwartz†, Kim L. Hoke* & Daphne Soares‡

* Department of Biology, Colorado State University, Fort Collins, CO, USA
† Biology Department, University of Maryland, College Park, MD, USA
‡ Department of Biological Science, New Jersey Institute of Technology, University Heights, Newark, NJ, USA

Abstract

Social context is a powerful mediator of behavioral decisions across animal taxa, as the presence of conspecifics comes with both costs and benefits. In risky situations, the safety conferred by the presence of conspecifics can outweigh the costs of competition for resources. How the costs and benefits of grouping influence decisions among alternative antipredator behaviors remains largely unexplored. We took advantage of the Trinidadian guppy (Poecilia reticulata) to examine the influence of social context on alternative behavioral responses to threats. We compared the frequency of active (startle) versus passive (freeze) responses to sudden acoustic stimuli in the presence and absence of conspecifics. We found that fish were relatively less likely to startle and more likely to freeze when in a group than when alone, indicating that immediate social context modulates predator evasion strategy in guppies. We suggest that these social context-dependent changes reflect trade-offs between survival and energy expenditure. To our knowledge, an effect of immediate social environment on startle probability has not been previously demonstrated in a teleost.

Introduction

Social context is a powerful mediator of behavioral decisions across animal taxa, as the presence of conspecifics influences the relative value of diverse behaviors. The mere presence of conspecifics alters behavior when conspecifics represent competition for resources, such as food (e.g., Pitcher & Parish 1993) and mates (e.g., Farr & Herrkind 1974; Doutrelant 2001; Sadowski et al. 2002). But competitors can turn into allies under certain environmental conditions. In risky situations, the safety afforded by the presence of conspecifics can outweigh costs, such as intraspecific competition or increased conspicuousness to predators (Abrahams & Dill 1989; Lima & Dill 1990; Krause & Ruxton 2002). Being in a group confers safety by a number of mechanisms, including risk dilution, predator confusion, and joint predator inspection (e.g., Magurran & Pitcher 1987; Abrahams & Dill 1989; Lima & Dill 1990; Milinski 1993). In addition, being in a group conserves energy by increasing vigilance at the group level, such that individuals can be less vigilant and spend more time engaging in profitable behaviors such as foraging and courting (reviewed in Krause & Ruxton 2002). Despite many examples of social context altering behavior and of the antipredator benefits of grouping, few studies have examined how social context modulates predator evasion tactics.

Animals have various tactics for predator evasion, ranging from active (fleeing) to passive (freezing, hiding) responses. The relative efficacy of these alternative tactics may depend on social context. Nearly all animal taxa display a stereotyped startle response following sudden, unexpected stimuli (Eaton & Hackett 1984; Eaton et al. 1991; Koch 1999). Active escape responses are effective but costly because they (1) typically involve fast, sudden movements that are energetically expensive and inefficient (e.g., Webb 1984; Jayne & Lauder 1993; Hughes & Kelly 1996), (2) carry the indirect cost of interrupting and/or interfering with profitable behaviors (e.g., Ydenberg & Dill 1986; Milinski 1993), and (3) may be undesirable if predators have not yet detected prey animals, as movement...
— in particular fast movement — can make prey more conspicuous (e.g. Krause & Godin 1995). Although passive tactics, such as freezing and/or hiding, may also interrupt profitable behaviors they have the advantage of allowing animals to remain undetected, as well as conserve energy. However, passive tactics do not carry the animal away from the threat and are thus less effective if prey animals are detected (Staudinger et al. 2011). In sum, there are trade-offs between active and passive predator avoidance tactics, and we expected behavioral decisions among tactics to balance these trade-offs in a context-dependent manner.

Active escape responses are particularly amenable for study in fish, as they are characterized by highly stereotyped c-startle escape responses present in nearly all fish species (Roberts 1992). This behavior typically involves two stages: In the first stage, the body bends away from the stimulus, creating the arched posture that gives the behavior its name. In the second phase, the body straightens, creating a propulsive force that moves the animal away from the threatening stimulus (Eaton et al. 1977, 2001). Startle responses are mediated primarily by two large neurons in the brainstem, the Mauthner cells. A startle response occurs only when an action potential is fired in one of the Mauthner cells (Korn & Faber 2005). The Mauthner cells receive inputs from the auditory, visual, and lateral line systems, and startle responses can be elicited by a single stimulus type (Eaton et al. 1977, 1991; Zottoli 1977), or by several, simultaneous stimulus types, as is probably the case during actual predator encounters (Eaton & Hackett 1984). Visual, mechanical, and auditory stimuli are commonly used to elicit startle escape responses. Auditory stimuli are advantageous as they are easily controlled and manipulated (Preuss 2006; Kastelein et al. 2008; Neumeister et al. 2008) and because auditory inputs to the Mauthner cells come directly from the 8th nerve (auditory nerve) (e.g., Eaton & Popper 1995; Zottoli & Faber 2000), making this stimulus modality independent of the acoustic details of the stimulus (Preuss 2006; Kastelein et al. 2008; Neumeister et al. 2008). Although the threshold for startle initiation can be modulated, once a startle response is initiated the behavior is almost always completed (Eaton & Hackett 1984). While the startle response is highly stereotyped overall, inter- and intra-specific variation exists in various response parameters, including response latency, acceleration, maximum velocity, body angle, and response distance (Eaton et al. 1977; Ghalambor & Reznick 2004; Neumeister et al. 2010). Grouping behavior has been shown to influence response distance and response latency in a number of fish species (e.g., Eaton et al. 1977; Seghers 1981; Abrahams 1995; Domenici & Batty 1997; Semeniuk & Dill 2004), but to our knowledge, the effects of immediate social context on startle probability have not been explored.

We examined the influence of immediate social context on alternative predator evasion tactics in Trinidadian guppies (Poecilia reticulata). Trinidadian guppies are small, freshwater teleosts, native to the island nation of Trinidad and Tobago as well as the adjacent South American mainland. The Trinidadian guppy is a well-established model system in ecology, evolution, and ethology due to its ability to rapidly adapt to environmental challenges. Guppies are known to alter courtship (e.g. Farr 1975; Rodd & Sokolowski 1995), foraging (e.g. Zandonà et al. 2011; Elvidge et al. 2014), and antipredator (e.g., Seghers 1974; Breden et al. 1987; Botham et al. 2008; Torres-Dowdall et al. 2012) behaviors in an adaptive manner in response to changing predation pressure on evolutionary and developmental timescales, as well as in response to immediate changes in predation threat (e.g., Seghers 1974; Abrahams & Dill 1989; Houde 1997; Huizinga et al. 2009). In response to a threat, guppies may mount an active escape response, freeze/hide, or increase shoaling (general affiliative) behavior (reviewed in Magurran 2005), but it is not clear what cues guppies use to decide among strategies and whether social context plays a role.

In this study, we examined the influence of immediate social context on responses to acoustic stimuli in guppies. We compared the probability of startle versus freeze responses of individual guppies in the presence and absence of conspecifics. To control for potential confounding effects of behavioral differences between social contexts, we examined the effect of an individuals’ behavior prior to stimulus presentation and the behavior of other group members following stimulus presentation on response strategy. In addition, we examined responses over a range of stimulus intensities to determine whether changes in response probability across social contexts were consistent among amplitudes. Given that the presence of conspecifics has consequences for both energetics and conspicuousness, we predicted that social context could shift the balance between active versus passive evasion tactics.

Methods

Animals

The adult male fish in this study were bred in our fish facility at Colorado State University before transportation.
to the Marine Biological Laboratory in Woods Hole, Massachusetts, where we conducted all behavioral experiments. We established laboratory populations at Colorado State University from wild-caught, gravid females collected from the Guanapo high-predation locality in the Northern Range Mountains of Trinidad in 2009. To establish family lines, we separated first-generation laboratory-born fish by sex and kept them in isolated tanks under identical conditions. Once mature, we uniquely crossed first-generation fish to generate the second generation of laboratory-born fish for this study. We housed adult fish in single-sex, 5 l group tanks in a recirculating water system containing conditioned water with a pH, hardness, temperature, and chemistry similar to natural streams. We kept fish on a 12:12 h light cycle and fed them a measured diet twice daily, once in the morning (Tetramin™ tropical fish flake paste) and once in the afternoon (hatched Artemia cysts).

We transported fish used in the experiment to the Woods Hole Marine Biological Laboratory, where we housed fish in 3.2 l group tanks containing conditioned water. We kept fish on a 12:12 h light cycle and fed them twice daily (Tetramin™ tropical fish flake paste). We photographed all fish prior to the experiment and used unique male color patterns to track individuals. All male fish were mature at the time of the experiment. All animal husbandry protocols were approved by the Colorado State University Animal Care and Use Committee (protocol #12-3818A), and all behavioral methods were approved by the Marine Biological Laboratory Animal Care and Use Committee (protocol # 11-44).

Behavior

Visual, mechanical, and acoustic stimuli are commonly used to elicit startle escape responses in guppies and other fish (e.g. Ghahambor & Reznick 2004; Preuss 2006; Dadda et al. 2010). Acoustic stimuli are easily controlled and manipulated, and stimulus intensity is directly correlated with Mauthner cell excitability and startle response probability (Preuss 2006; Kastelein et al. 2008; Neumeister et al. 2008). Moreover, auditory inputs to the Mauthner cells come directly from the 8th nerve (e.g. Eaton & Popper 1995; Zottoli & Faber 2000), and thus these inputs are not sensitive to detailed stimulus characteristics. We chose acoustic stimuli for this study because this allowed us to modify stimulus intensity and ensure intermediate response probabilities, allowing us to detect subtle shifts in response probability among groups. Group differences cannot be accurately quantified when stimulus intensity is so high that response probability in all groups is close to one, or conversely, when stimulus intensity is so low that response probability in all groups is close to zero (e.g. Neumeister et al. 2010).

All behavioral trials were conducted in a circular, 12-cm diameter arena filled with conditioned water. The arena was lit from below, and we used a filter paper screen to evenly diffuse the light and increase visual contrast. We recorded behavioral responses using a high-speed camera (X-PRI model, Del Imagineering, Cheshire, CT, USA) positioned above the tank and connected to a computer running AOS Imaging studio light software (AOS technology, Baden Daettwil, Switzerland). We delivered acoustic stimuli from a speaker (RadioShack, Fort Worth, TX, USA) positioned 5 cm from the side of the tank. Acoustic stimuli were made in Matlab (Mathworks, Natick, MA, USA) and were 2 ms 200 Hz sine wave tones at one of four amplitudes, representing increasing stimulus intensity (0.2, 0.4, 0.6, 0.8 volts). We conducted pre-trials at a range of stimulus amplitudes to ensure that startle probabilities were intermediate at the amplitudes we chose (>20% and <80%).

Individual fish were tested in 3–6 acoustic startle trials, with one trial (either social or isolated condition) conducted every 1–2 d. Each trial consisted of a train of 20 stimuli with a unique combination of interstimulus intervals and stimulus amplitudes. We generated four unique stimulus trains. In each stimulus train, the number of stimuli at each amplitude was equal (i.e., five stimuli at each of four amplitudes), but we randomized amplitude order to control for order effects. Similarly, we randomized interstimulus intervals between 2 and 8 min to prevent the fish from anticipating or habituating to stimulus presentation. Because individuals experienced a different stimulus train during each trial, this allowed us to minimize habituation to stimulus presentation. The order in which fish experienced the different trial versions and social conditions was randomized to control for order effects.

Fish were placed in the arena either individually (isolate condition) or in a group of three (social condition) and allowed to acclimate for 1 h, after which the trial began. Social groups were selected randomly and altered among trials to control for potential group level effects (i.e., changes in one individuals behavior due to the behavior of other group members). We identified fish prior to the trial using unique color markings and tracked individuals visually during the trial. We recorded behavioral responses at 500 frames/second and subsequently analyzed all videos.
using Proanalyst software (Xcitex, Woburn, MA, USA). We scored responses as startle, freeze, or no response. To be categorized as a startle, fish had to exhibit the characteristic c-bend following stimulus presentation. In addition, we recorded fish behavior just prior to stimulus presentation as either moving or stationary. We could not accurately distinguish freeze responses from no response when fish were already stationary prior to stimulus presentation, so trials in which fish were stationary and did not startle were scored as no response. Fish were typically moving rather than stationary prior to stimulus presentation, and the time stationary did not differ based on social condition; fish were moving at stimulus presentation in 77% of all social trials and 80% of all individual trials. Context-dependent differences in the proportion of freezing thus were not an artifact of differences in prior behavior, as fish in each experimental condition were equally likely to be stationary when the stimulus sounded. Given the difficulty in detecting a freezing response in stationary fish, we did not assess the influence of prior behavior on probability of freezing and only report the effect of prior behavior on startle probability.

Statistical Analysis

We tested the effects of social condition and stimulus amplitude on startle and freeze probabilities using logistic regression analyses. We modeled startle and freeze probabilities separately as binary responses with a logit link function. Social condition, stimulus amplitude, and their interaction were included in the model as fixed effects. To test for the influence of other group members’ behavior, we performed an additional analysis of startle probability that included the behavior of other group members (whether one or more group members startled versus no group members startled) as a predictor of startle probability in the social condition. We included stimulus number within trial as a covariate in all analyses to control for changes in response probability over time. Behavior prior to stimulus presentation (moving or stationary) was included as a covariate for startle but not freeze responses, as described above. We included fish identity as a random effect to control for individual differences in overall responsivity. All post hoc comparisons were Tukey’s adjusted to control for multiple hypothesis testing.

Results

Fish responded (startled or froze) on average 64.5% of the time, with individual response probabilities ranging from 53.3% to 77.7%. Independent of social context, fish were more likely to startle (71.4% of all responses) than freeze (28.6% of all responses). Over the course of the trial, startle probability decreased by an average of 18% ($F_{19,1302} = 2.20, p = 0.0021$) and freeze probability increased by an average of 7% ($F_{19,1302} = 2.01, p = 0.0060$). Although startle probability decreased, it remained on average high (>50%) indicating that fish did not completely habituate to stimulus presentation. We included stimulus number in all statistical analyses to control for differences in response probability over the course of the trial. We also included fish identity as a random effect to control for differences in individual response probability (covariance parameter estimates: startle: 0.7767 ± 0.366, freeze: 0.5952 ± 0.361). Raw counts and percentages for behavioral responses by social condition and stimulus amplitude are shown in Table 1.

Social condition strongly influenced response type. Fish were relatively more likely to startle ($F_{1,1302} = 54.88, p < 0.0001$) and less likely to freeze.

| Table 1: Raw counts and percentages for behavioral responses by social condition are shown for each stimulus amplitude and averaged across stimulus amplitudes |
|----------------------------------|---|---|---|---|---|---|---|---|
| **Stimulus amplitude (volts)**   |   |   |   |   |   |   |   |
|                                 | Iso | Social | Iso | Social | Iso | Social | Iso | Social |
| Startle                         | 129 | 54 | 161 | 56 | 184 | 67 | 192 | 63 |
|                                | 44.0% | 28.7% | 54.9% | 37.3% | 62.2% | 38.0% | 64.9% | 35.3% |
| Freeze                          | 73 | 50 | 54 | 43 | 39 | 30 | 41 | 23 |
|                                | 24.9% | 33.3% | 18.4% | 28.7% | 13.3% | 20.0% | 13.9% | 15.3% |
| Neither                         | 91 | 57 | 78 | 51 | 71 | 63 | 63 | 74 |
|                                | 31.1% | 38.0% | 26.6% | 34.0% | 24.1% | 42.0% | 21.3% | 49.3% |

Iso, isolate condition, social, social condition.
Startle probability was not affected by the behavior of other individuals in the social condition ($F_{1,575} = 2.76$, $p = 0.0969$). In fact, fish were slightly less likely to startle (45.51% of responses) than not startle (54.49% of responses) when other group members startled.

Startle probability increased with increasing stimulus amplitude (Fig. 2; $F_{3,1302} = 7.57$, $p < 0.0001$), while freeze probability decreased with increasing stimulus amplitude (Fig. 2; $F_{3,1303} = 5.86$, $p = 0.0006$), and this effect did not differ by social condition (social condition *amplitude, startle: $F_{3,1302} = 1.97$, $p = 0.1163$; freeze: $F_{3,1302} = 0.13$, $p = 0.9446$). Post hoc tests revealed that startle probability increased from 0.2 to 0.4 amplitude and then plateaued at an apparent maximum. Conversely, freeze probability decreased from 0.2 to 0.6 amplitude and then reached a minimum (Fig. 2). Also independent of social condition, fish were more likely to startle if they were moving, rather than stationary, prior to stimulus onset (Fig. 3; $F_{1,1302} = 69.12$, $p < 0.0001$).

**Discussion**

Social context changed threat responsiveness and modulated predator evasion strategy in guppies. Guppies were less likely to startle and more likely to freeze in response to a sudden acoustic stimulus when they were in a group as compared to when they were alone. As expected, startle probability increased and freezing probability decreased with increasing stimulus amplitude, but this effect was independent of social condition. Similarly, the influence of prior behavior on startle probability was independent of social condition, and fish in both social contexts were more likely to startle when moving prior to the stimulus. The behavior of other group members did not significantly affect startle probability. To our knowledge, an effect of immediate social environment on startle probability has not been previously demonstrated in a teleost. We suggest these patterns result from trade-offs between predator evasion and energy expenditure.

Grouping behavior is a common predator evasion tactic that can also conserve energy. The presence of conspecifics confers safety by creating a dilution effect, such that the probability of attack is less for any given individual, and by increasing vigilance at the group level. Grouping is energetically favorable, as it reduces the need for costly active escape responses and enables individuals to spend more time engaging in other profitable behaviors (reviewed in Lima & Dill 1990). We found that guppies were relatively less likely to startle (i.e., mount an active, high-energy escape response) when they were in a group than...
when isolated. A decrease in startle responses in concert with a relative increase in freezing behavior in the social condition could conserve energy and maintain shoal cohesion, both of which are beneficial if grouping does indeed increase survival and decrease energetic costs in the presence of predators.

One limitation of our experimental design is that we cannot distinguish whether decreased startle behavior in the social condition is a response to the proximity of conspecifics or is a more generalized response to any nearby object. Decreased startling and increased freezing in the presence of conspecifics and/or objects could reflect a sit-and-hide strategy in response to a threat (Templeton 2004), which would conserve energy and reduce detection probability, as discussed above. Alternatively, decreased startle probability could serve to reduce the likelihood of collision with neighboring fish and/or objects. Proximity to other individuals and/or the side of the tank does not appear to prevent startle responses in guppies (E. K. Fischer, personal observation), but further work is necessary to explicitly test this idea. Our data support the hypothesis that guppies may be utilizing alternative behavioral strategies in the presence of conspecifics, although we acknowledge that this behavioral switch could apply more generally in any structurally complex environment that provides shelter and reduces fish conspicuousness.

While social context influenced response tactics, stimulus intensity modulated response probability independently of social condition. As stimulus intensity increased, startle probability increased and freeze probability decreased. Similar increases in startle probability at higher stimulus intensities have been observed in other teleosts (e.g., Neumeister et al. 2010), but the mechanistic basis for this behavioral pattern remains unclear. Given that the intensity of behavioral responses should scale with the level of danger (Ydenberg & Dill 1986; Helfman 1989; Lima & Bednekoff 1999), increased startle probability is often considered indicative of an increase in the perceived danger of a threat (reviewed in Domenici 2010). Alternatively, increased startle probability could have a purely physiological basis, for example, if increased stimulus intensity increases sensory neuron input to brainstem escape circuitry and Mauthner cell excitability (Neumeister et al. 2008; Mirjany & Faber 2011). Whichever the mechanism by which stimulus amplitude influences startle probability, we emphasize that the reduction in startle probability associated with the presence of conspecifics was consistent across stimulus intensities, suggesting a global shift in startle response threshold in the presence of conspecifics.

The influence of behavior prior to stimulus presentation on response probability was also independent of social condition. Fish that were moving prior to the stimulus were more likely to startle in response to a stimulus. Animals engaging in behaviors that distract them and/or make them more conspicuous may be more likely to mount an active escape response because they are more vulnerable to attack (Lima & Dill 1990; Milinski 1993). Future studies could explicitly test whether the increase in startle probability we observe in moving fish does indeed have an adaptive value, for example, by examining the relationship between alternative behavioral tactics and survival probability in staged predator encounters.

Conclusions

We found that immediate social context influenced predator evasion strategy in guppies. The presence of conspecifics shifted antipredator tactic from active toward passive responses, demonstrating that immediate social context can shift the balance between alternative evasion tactics. Stimulus intensity and behavior prior to stimulus presentation altered behavioral responses independently of social context. We suggest that these behavioral changes balance
trade-offs between predator evasion and energy expenditure. Given that natural populations of guppies adapted to different predation regimes differ in shoaling propensity and startle probability, these trade-offs may also influence the evolution of behavioral differences among populations in the wild.

Acknowledgements

We thank A. Streets for help with data collection, A. Scott-Johnston, and L. Rose for help with behavior quantification, G. Haspel for assistance with equipment construction, the members of the Guppy Lab for fish care, and two anonymous reviewers for helpful comments on a previous version of the manuscript. We gratefully acknowledge support from the Marine Biological Laboratory Associates Endowed Fellowship and Colwin Endowed Summer Research Award (to K.L.H. & D.S.) and NSF DEB-0846175 (to C.K. Ghalambor).

Literature Cited


