The effects of familiarity on escape responses in the Trinidadian guppy (*Poecilia reticulata*) (#17311)

First submission

Please read the **Important notes** below, the **Review guidance** on page 2 and our **Standout reviewing tips** on page 3. When ready **submit online**. The manuscript starts on page 4.

Important notes

Editor and deadline

Lydia Hopper / 3 May 2017

Files	4 Figure file(s) 2 Table file(s) 1 Video file(s) 1 Raw data file(s) Please visit the overview page to **download and review** the files not included in this review PDF.
Declarations	**Involves vertebrate animals.**

Review guidelines

Please read in full before you begin

How to review

When ready **submit your review online**. The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

To finish, enter your editorial recommendation (accept, revise or reject) and submit.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to **PeerJ standards**, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see **PeerJ policy**).

EXPERIMENTAL DESIGN

- Original primary research within **Scope of the journal**.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.
- Speculation is welcome, but should be identified as such.

The above is the editorial criteria summary. To view in full visit **https://peerj.com/about/editorial-criteria/**

7 Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that your international audience can clearly understand your text. I suggest that you have a native English speaking colleague review your manuscript. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Give specific suggestions on how to improve the manuscript	*Line 56: Note that experimental data on sprawling animals needs to be updated. Line 66: Please consider exchanging "modern" with "cursorial".*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

The effects of familiarity on escape responses in the Trinidadian guppy (*Poecilia reticulata*)

Hayley L Wolcott [1] , **Miguel Barbosa** [Corresp., 1] , **Alfredo F Ojanguren** [1]

[1] Centre for Biological Diversity, School of Biology, University of St. Andrews, St Andrews, Fife, United Kingdom

Corresponding Author: Miguel Barbosa
Email address: mb334@st-andrews.ac.uk

Predation is the main driver of mortality during early life stages. The ability to avoid and evade potential threats is, therefore, favoured to evolve during the early stages of life. It is also during these early stages that the process of familiarization occurs. It has long been recognized that associating with familiar individuals confers anti predator benefits. Less, however, is known about how predator evasion is affected by social experience during early stages. In this study we test the hypothesis that familiarization acquired during early life stages improves anti predator escape responses. Using the Trinidadian guppy we examine the effect of different early social conditions in the three main components of predator evasion. Using high-speed motion analysis we compared the responsiveness, reactive distance and magnitude of the response (maximum speed, maximum acceleration and distance) of the response to a visual stimulus in groups composed either of familiar or non-familiar individuals. Surprisingly, groups composed by familiar individuals were less responsive than groups of unfamiliar individuals. It is plausible that familiarity equips individuals with better skills to accurately assess the threat avoiding false alarms. Reactive distance and magnitude of response were more dependent on individual size than on familiarity. Larger individuals reached higher maximum speeds and total distances in their escape response. Our approach allowed us to tease apart which aspects of an escape response are more likely to be influenced by early social conditions.

The effects of familiarity on escape responses in the Trinidadian guppy (*Poecilia reticulata*)

Hayley L Wolcott[1], Miguel Barbosa [1*] & Alfredo F Ojanguren[1]

[1]Centre for Biological Diversity
Sir Harold Mitchell Building
University of St Andrews
St Andrews
Fife
KY16 9TF
UK

Corresponding Author:
Miguel Barbosa [1]
Email address: mb334@st-andrews.ac.uk

ABSTRACT

Predation is the main driver of mortality during early life stages. The ability to avoid and evade potential threats is, therefore, favoured to evolve during the early stages of life. It is also during these early stages that the process of familiarization occurs. It has long been recognized that associating with familiar individuals confers antipredator benefits. Less, however, is known about how predator evasion is affected by social experience during early stages. In this study we test the hypothesis that familiarization acquired during early life stages improves antipredator escape responses. Using the Trinidadian guppy we examine the effect of different early social conditions in the three main components of predator evasion. Using high-speed motion analysis we compared the responsiveness, reactive distance and magnitude of the response (maximum speed, maximum acceleration and distance) of the response to a visual stimulus in groups composed either of familiar or non-familiar individuals. Surprisingly, groups composed by familiar individuals were less responsive than groups of unfamiliar individuals. It is plausible that familiarity equips individuals with better skills to accurately assess the threat avoiding false alarms. Reactive distance and magnitude of response were more dependent on individual size than on familiarity. Larger individuals reached higher maximum speeds and total distances in their escape response. Our approach allowed us to tease apart which aspects of an escape response are more likely to be influenced by early social conditions.

INTRODUCTION

Predation is a powerful agent of mortality, particularly during early life stages when organisms are at heightened risk due to their smaller size (Cushing 1974). Natural selection is therefore expected to favour the development of antipredator behaviours early in life (Braithwaite & Salvanes 2005; Vilhunen & Hirvonen 2003). Antipredator behaviours are generally divided into two major types; 1) avoidance and 2) evasion (Fuiman & Magurran 1994; Weihs & Webb 1984). Avoidance includes any pre-emptive behaviours in which the individual reduces the likelihood of encountering a predator and consequently of its attack (Fuiman & Magurran 1994). Evasion, on the other hand, occurs once the predator initiates the attack. As predator avoidance is not always possible, successful predator evasion tactics are essential for survival. The behaviour and frequency at which each evasion tactic is employed is context-dependent; individuals adopt behaviours that improve their evasive response and, thus, enhance survival (Domenici 2010).

One way in which organisms may reduce the risk of predation is by associating with others, either by schooling or just by joining a group (Ruxton & Johnsen 2016; Ward & Webster 2016). Though groups might be more conspicuous for a predator, each individual within the group has a smaller probability of being predated than if alone. Among the group antipredator benefits of enhanced vigilance, dilution of risk, predator confusion and coordinated antipredator maneuverers (Krause & Ruxton 2002; Ward & Webster 2016), there is strong evidence showing that familiarity within individuals in a group enhances antipredator behaviours (Griffiths et al. 2004). Familiarity can be broadly defined as the ability to discriminate between individuals based on previous interactions (Griffiths 2003). The process of familiarization is based on visual, auditory and olfactory cues (Coffin et al. 2011; Reby et al. 2001; Zajitschek & Brooks 2008). Repeated interactions and resource-sharing leads to the familiarization. Joining a group

composed of familiar conspecifics brings greater fitness benefits than joining a group composed of unfamiliar individuals (Barber & Wright 2001; Griffiths & Magurran 1997b).

The benefits in associating with familiar individuals for the development and acquisition of successful antipredator behaviours and responses are acknowledged (Ward & Hart 2003). There is evidence that groups composed by familiar individuals are more cohesive and have reduced neighbour distance (Chivers et al. 1995; Höjesjö et al. 1998), characteristics which enhance predator confusion and dilute individual risk. Further, familiar groups ~~have been found to~~ have reduced within-group aggression and evolve more stable social hierarchies (Griffiths et al. 2004; Höjesjö et al. 1998; Johnsson 1997; Tanner & Keller 2012). Reduced aggression within familiar groups allows more time for predator vigilance, which may improve escape latency (Griffiths et al. 2004; Strodl & Schausberger 2012). Additionally, individuals are more likely to perform cooperative antipredator behaviours when in familiar groups, as they will have an idea of whether the others have behaved cooperatively in the past (Dugatkin & Alfieri 1991). For example, individuals in familiar groups may be more likely to perform more risky antipredator manoeuvres (Chivers et al. 1995), join predator mobbing (Grabowska-Zhang et al. 2012), or perform predator inspection (Dugatkin & Godin 1992). Such antipredator behaviours put individuals at higher risk, but improve group antipredator response.

While the effect and importance of familiarity on predator avoidance is well recognised, how familiarity shapes predator evasion, particularly the escape response, remains fairly unexplored. Furthermore, studies to date only focus on the effect of familiarity on the latency of the escape response (Griffiths et al. 2004; Strodl & Schausberger 2012). Successful escape responses depend on various components, such as latency, velocity and distance travelled in the response (Domenici & Blake 1997). For instance, latency considered as the time between the

onset of the predator attack and the start if the response is crucial for the outcome of the interaction (Fuiman et al. 2006). Also, an effective response requires moving away from the attack trajectory fast enough so the predator can not adjust it (Fuiman & Cowan 2003). Given the context-dependent nature of escape responses, it is possible that familiarity may aid predator escape by improving certain aspects of the escape response. The aim of this study was to address the role of familiarity acquired during early life stages in shaping the different components of the antipredator escape responses in the Trinidadian guppy (*Poecilia reticulata*).

Guppies shoal immediately after birth, and it is during these early stages that, by interacting with other individuals within the group, that the ability to discriminate between familiar and unfamiliar starts (Laland et al. 2003; Magurran et al. 1994). The importance of early conditions for the establishment and reinforcement of individual discrimination in guppies has been extensively studied (Barbosa et al. 2016; Barbosa et al. 2013; Chapman et al. 2008a; Chapman et al. 2008b). Guppies respond to a predator attack by performing a "fast-start" escape response, characteristic to most fish species (Dial et al. 2015). This evasion tactic consists of an unambiguous quick and sudden burst of swimming activity usually of only tenths of a second that propels the fish away from an oncoming predator (Domenici & Blake 1997; Webb 1978; Weihs 1973). Fast-start escape responses integrate a combination of behavioural and kinematic components (Marras et al. 2011), both of which were examined in this study.

In view of the evidence of the antipredator benefits of familiarity, we predicted that juvenile guppies are also more responsive and perform more successful escape responses when in groups of familiar conspecifics. To test this hypothesis, we exposed familiar and unfamiliar groups of juvenile guppies to a digital display of a looming object and quantified the difference in responsiveness (number of fish responding), reactive distance (based on the size of the

stimulus when the response started) and magnitude of the escape response (maximum speed and acceleration achieved during the response, and distance covered by the escaping fish). This approach allows us to identify the role of familiarity in a behaviour closely related to survival during early life stages and to pinpoint which components of an escape response are more likely to be affected by social experience.

METHODS

All guppies used were descendants of individuals collected from the Lower sections of the Tacarigua River in Trinidad. Several species of fish predators have been reported in this locality including the pike cichlid (*Crenicichla alta*), the blue acara (*Aequidens pulcher*) and the wolf fish (*Hoplias malabaricus*), which also prey intensively on juvenile guppies (Magurran & Seghers 1994). Experimental fish were housed, and all observations recorded, at the aquarium facility at the Sir Harold Mitchell Building, University of St Andrews, UK. The aquarium has an air temperature control system, which kept the tank temperatures at a mean (±SD) temperature of 24.5°C (± 0.3 °C). All stock tanks contained similar numbers of males, females and juveniles. Lighting conditions followed a 12-hour light/dark cycle. All fish were fed daily with TetraMin® flake food.

Test Fish Collection and Rearing

Prior to the experiment, we collected three juveniles from three different stock tanks that contained a mix of males, females and juveniles using a dip net. This ensured that the test groups were composed neither of familiar conspecifics nor of close kin. Juveniles were allocated to a 20

x 22 x 30 cm holding tank to create a test group. A total of 42 holding tanks were used. Black plastic sheets were placed between each tank to ensure each test group was visually isolated from adjacent groups. Fish were of similar size and randomly distributed between holding tanks (mean (±SD) 10.8 (± 1.7) mm). Nevertheless, in order to be able to identify each individual during tracking, test groups were carefully constituted of different sized individuals. This size disparity, however, was not different to the one observed in groups of juvenile fish shoaling in Trinidadian rivers (personal observation). Each test group remained in its holding tank for two weeks to ensure the establishment of familiarity between tank mates (Griffiths & Magurran 1997a).

Escape Response Trials

Six groups were tested each day, split into three 'familiar' and three 'unfamiliar' groups. In familiar groups, individuals were tested with those they shared the holding tank with for two weeks. Unfamiliar groups were treated as a control. For unfamiliar groups, we took one fish, each from a different holding tank, and put them together in the observation chamber for testing (Figure 1).

All tests occurred between 9:00 and 11:00 am and at least an hour after being fed. These measures were taken to avoid differences in satiation rate and time of day that might affect the behaviour of the individuals. The experimental setup used to assess escape response was based on an established protocol (Fuiman et al. 2010), but modified for this experiment (Figure 2). Each trial involved presenting a digital display of a looming object to a test group. The digital display consists 1.8-second sequence showing black oval in the middle of a white background that increases its size to simulate an approaching object (Supplementary Information). The same stimulus has been shown to elicit a startle response in larval fish of similar size (Fuiman et al. 2006; Ojanguren & Fuiman 2010). The video was presented using a LCD screen (Braun 1210)

153 located 0.23 cm from a 10x10x10 cm glass test chamber. Water depth within the observation
154 chamber was kept at 225 ml to minimise vertical movement in escape responses. This depth was
155 within the range which juveniles would be likely to experience in the wild (Magurran 2005). For
156 each trial, a test group was transported to the observation chamber and given at least 10 minutes
157 of acclimatisation to their new surroundings before testing began.

158 Individual response to the visual stimulus was recorded at 240 frames s^{-1} using a high-
159 speed video camera (Casio EX-FH25 EXILM) through a 45º-angled mirror to obtain an overhead
160 view of the observation chamber. The observation chamber sat on top of a black surface and was
161 illuminated by lamps positioned left and right of the chamber so that the response could be
162 clearly observed. All individuals tested were gently transferred to a small petri dish with a small
163 amount of water (so no anaesthesia was required) and photographed from above. Individual
164 sstandard length was measured to nearest millimetre using ImageJ analysis software (Abràmofff
165 et al. 2004). All tested individuals resumed normal routine swimming activity immediately after
166 the scape responses. No fish died during the tests and after the picture was taken. After the
167 terminus of the study, all individuals returned to stock tanks.

168

169 *Data Analysis*

170 Video recordings were analysed frame by frame to determine responsiveness (the number
171 of fish that responded to the stimulus in each test group) and the reactive distance (the virtual
172 distance between the looming object and the first individual that responded, calculated from the
173 size of the oval on the screen at the moment of the start of the response and the distance of the
174 fish from the screen) (see (Fuiman et al. 2010) for details). This method allowed us to know the
175 exact position of the fish and determine its speed and therefore calculate maximum speed,

maximum acceleration and total distance covered during the escape response (magnitude of the response).

Videos of the individual responses were imported to ImageJ and analysed frame-by-frame to determine the reactive distance and track the fish position during the response. Reactive distance was calculated by combining perceived distance of the looming object (displayed in the top left screen of digital display) at the frame of the start of the response with the distance of the head of the individual from the screen. The position of the fish in 2-dimensional coordinates for the overhead view was obtained using the manual tracking plugin in ImageJ (Cordelières 2005), this allowed us to calculate maximum speed, maximum acceleration and total distance covered in the response (see (Fuiman et al. 2010; Fuiman et al. 2006)).

The responsiveness of each test group was ranked according to the number of individuals within the group that responded (either 0, 1, 2 or 3). We considered that the response was over when the distance travelled between three consecutive frames (12.5 milliseconds) was 1 mm or less. Reactive distance, maximum speed, maximum acceleration and distance travelled during a response were measured on the first fish that responded. On the only trial that two fish responded in the same frame, the fish that had the larger reactive distance was considered the first responder. The first author performed sampling and motion analysis. Blind data collection was, therefore, not possible. Nevertheless, the strict criterion for defining escape characteristics minimizes any observation bias.

196 *Statistical Analysis*

197 Differences in responsiveness between familiar and unfamiliar groups were tested with a
198 Wilcoxon rank sum test to account for the fact that responsiveness was a discrete variable. In
199 order to investigate the effect of familiarity on reactive distance and in the magnitude of the
200 response (maximum speed, maximum acceleration and distance covered in a response) we used
201 General Linear Models (GLMs). Each full model included familiarity as main effect treatment
202 and standard length as a covariate, as well as their interaction. Diagnostic plots revealed
203 significant departures from normality of the residuals for both responses variables reactive
204 distance and total distance. Normal distribution of residuals was achieved by log-transformation.
205 We tested if all factors were needed in the minimal adequate model using Akaike's Information
206 Criterion (Burnham & Anderson 2002). Specifically, we calculated ΔAIC, the difference
207 between the AIC of each model and that of the estimated best model (the model with the lowest
208 AIC) (Supplementary Information). We also calculated Akaike weights, which are estimates of
209 the probability that each model is the best in the model set, to assess uncertainty about which
210 model is best (reflected in multiple models having similar Akaike weights). All analyses were
211 performed in using R (Team 2015).

212

213 # RESULTS

214

215 *Responsiveness*

216 A total of 42 groups composed by three different sized individuals were tested. Of the 30
217 groups in which one or more individuals responded, 17 groups were familiar and 13 groups were

unfamiliar. There was a significant effect of familiarity on responsiveness (Wilcoxon rank sum: W=1197, $p < 0.005$) (Figure 3), where responsiveness was higher in unfamiliar groups. In the majority of familiar groups only one individual in the group responded, whereas the unfamiliar groups showed more instances where two or more individuals reacted to the stimulus.

Reactive distance

The best explanatory model for the effect of familiarity on reactive distance did include the main effects and interaction between standard length and treatment (Table 1, Figure 4A, Supplementary Information). We failed to detect an effect of familiarity on reactive distance ($F_{1,28} = 0.194$, $p = 0.663$) (Figure 4A).

Magnitude of the response

The best selected GLM for explaining the effect of familiarity on maximum speed, maximum acceleration and distance did not include the interaction between standard length and treatment, but length was important as a covariate (Table 1, Figure 4B, C, Supplementary Information). There was no significant effect of familiarity on maximum speed ($F_{1,27} = 2.53$, $p = 0.123$), maximum acceleration ($F_{1,27} = 3.47$, $p = 0.07$) or total distance ($F_{1,27} = 2.34$, $p = 0.138$). Individual length, however, had a significant effect on maximum speed ($F_{1,27} = 15.59$, $p = 0.004$), maximum acceleration ($F_{1,27} = 6.42$, $p = 0.017$), and total distance ($F_{1,27} = 12.17$, $p = 0.001$) (Table 1, Figure 4, Supplementary Information).

239 DISCUSSION

240 A novel contribution of this study is that it examines the consequences of familiarity
241 during early stages in the performance of escape responses separating the multiple aspects of the
242 response to determine which parts depend on the social environment. Through high-speed
243 analysis of the escape responses in familiar and unfamiliar groups of guppies, we were able to
244 unambiguously demonstrate that familiarity plays a significant role in shaping how groups of
245 fish respond to a stimulus. Unfamiliar groups had more individuals perform an escape response
246 than those in familiar groups. A plausible explanation is that familiarity ~~could allow individuals~~
247 ~~to be better able perceiving a lower~~ threat from the stimulus. Rather unexpectedly, other
248 components of the escape response, namely latency and magnitude, were not affected by
249 familiarity. Furthermore, the speed and distance covered in the response were correlated with
250 individual size rather than with level of familiarity within the group. In combination, our study
251 suggests that, while familiarity affects how groups respond to a visual stimulus, it plays a less
252 meaningful role in determining the quality of the escape response.

253 Our results are clear in demonstrating that familiarity affects group responsiveness. There
254 were a greater number of individuals responding within each group among unfamiliar groups
255 than among familiar groups. While most fish species rely on the escape response to avoid a
256 potential predator (Domenici 2010; Fuiman & Magurran 1994), escaping may not always be the
257 best strategy (Lima & Dill 1990; Ward & Webster 2016; Ydenberg & Dill 1986). If there is
258 enough information to accurately predict the level of threat in a given environment, then it is
259 advantageous for a prey to only flee when it is necessary for survival avoiding false alarms that
260 could in turn attract the attention of nearby predators (Ward et al. 2011). For example, minnows
261 performed antipredator behaviours in response to a realistic pike model, whereas an unrealistic

stimulus elicited no response (Magurran & Girling 1986). The lower responsiveness in familiar groups may be a result of improved vigilance. According to the theory of limited attention, performance is reduced when attention must be divided among different tasks (Dukas 2002). Therefore, if individuals are not spending time inspecting or acting aggressively toward group mates, as is often found among unfamiliar individuals (Griffiths et al. 2004; Johnsson 1997; Tanner & Keller 2012), then they are likely to have more time to dedicate to other tasks, such as predator vigilance (Strodl & Schausberger 2012; Strodl & Schausberger 2013; Zach et al. 2012). Guppies from familiar groups may have been able to accurately assess the non-threatening nature of the stimulus. Contrastingly, unfamiliar groups may have been more skittish and, thus more likely to be startled by the stimulus. Interacting with unfamiliar individuals can be stressful (Choleris et al. 1998), particularly if such interactions are associated with increased aggression (Galef et al. 1984). Individuals may perceive higher risk when shoaling with unfamiliar conspecifics, as was found in fathead minnows who had a higher production of epidermal alarm substance cells when in unfamiliar shoals than familiar shoals (Wisenden & Smith 1998). Furthermore, escape responses from the digital display may be misinterpreted as an attack by the other group mates. Aggression is common among guppies, in both natural as well as laboratory conditions (Magurran 2005; Thibault 1974). Therefore, it is plausible that an individual guppy would flee from an unfamiliar group mate that is performing a fast-start response, as this could be misinterpreted as an attack.

We failed to detect an effect of familiarity on the reactive distance of an escape response. Comparable studies have found that familiarity reduces the latency of an escape response. For example group-living mites *Phytoseiulus persimilis* reacted more quickly to an attack of a predator when they were in a familiar pair (Strodl & Schausberger 2012). Similarly, familiar

juvenile brown trout responded 14% faster than unfamiliar ones when exposed to a simulated predator attack (Griffiths et al. 2004). In both studies reduction in reaction time has been attributed to the associated benefits of the theory of limited attention. Our results therefore indicate that familiarity is more important in antipredator behaviours earlier in a predator sequence. A predator must successfully encounter, attack and capture a prey, where a prey's strategy is to interrupt this sequence. It has been suggested that avoiding the encounter and attack are a prey's best strategy (Fuiman & Magurran 1994). Previous experiments included an entire predator interaction, such as a model heron swinging forward and plunging its beak into the water (Griffiths et al. 2004) or a live predator (Strodl & Schausberger 2012), and could, therefore elicit such behaviours. In contrast, our experiment only elicited behaviours seen in the last few milliseconds of the attack.

Familiarity has been found to enhance avoidance tactics. For example, predator confusion was enhanced in shoals of familiar fathead minnows that had reduced neighbour distance and more shoal cohesion in response to predator stimuli compared to unfamiliar shoals (Chivers et al. 1995). Tighter shoal cohesion reduces the probability of being captured by a predator (Mathis & Smith 1993). In addition, familiar shoals exhibited a greater number of predator inspections with more inspectors per inspection when faced with a model pike (Chivers et al. 1995). Predator inspection, where an individual or small group of individuals approach a predator, pause and swim away (Pitcher 1992), enables prey to gain valuable information on the threat of a predator. This behaviour, though risky to inspectors, is associated with improved avoidance of a predator attack (Godin & Davis 1995; Magurran 1990; Magurran & Pitcher 1987). Therefore, it is likely that familiarity is more crucial in antipredator behaviour associated with predator avoidance than predator evasion.

The effect of familiarity on the magnitude of the response was not significant. It is recognized that there is a crucial need to implement an integrative approach that accounts for all aspects of an escape response in order to obtain a clear understanding of the mechanisms of response to a predator (Domenici 2010). While other behavioural variables may affect the magnitude of an escape response, our study provides strong evidence that familiarity is not one of them. Our results showed that size had a far greater effect in the magnitude of the response than familiarity. This result is consistent with previous studies that have shown that the magnitude of the fast-start response in young fish increases with body length (Dial et al. 2015). While behavioural effects on the locomotive performance cannot be ruled out (Domenici 2010), our study and others (Gibb et al. 2006; Ojanguren & Braña 2003) lend strong support that the magnitude of a fast-start response is largely determined by morphology, rather than by social conditions.

In this study we provided a strong test for the relative effect of familiarity in modulating predator avoidance behaviour measuring several aspects of the escape responses using high speed video analysis. The familiarity effect on group responsiveness may be an adaptive response in which familiar groups have improved antipredator performance, as individuals conserve energy and are less conspicuous by not fleeing in a non-threatening situation. Nevertheless, further studies are necessary to elucidate this. Our results also suggest that the effects of familiarity on the response are perhaps unlikely to play a role on escape performance in the last few milliseconds of a predator attack. Instead, we believe that familiarity is more likely to affect behaviour earlier in a predator-prey interaction, which then affects the quality of the response. Taken together our study further contributes to previous ones, by distinguishing which components of an escape response are modulated by familiarity.

ACKNOWLEDGMENTS

We are grateful to Maria Dornelas, Anne Magurran and Mike Webster for providing helpful comments on early drafts. We are also thankful to the Biodiversity and Behaviour Group for the rewarding discussions and comments throughout this research.

REFERENCES

Abràmofff MD, Magalhães PJ, and Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* 11:36-43.

Barber I, and Wright HA. 2001. How strong are familiarity preferences in shoaling fish? *Animal Behaviour* 61:975-979. 10.1006/anbe.2000.1665

Barbosa M, Camacho-Cervantes M, and Ojanguren AF. 2016. Phenotype matching and early social conditions affect shoaling and exploration decisions *Ethology* 122:171-179. 10.1111/eth.12455

Barbosa M, Ojanguren AF, and Magurran AE. 2013. Courtship display persists despite early social deprivation. *Ethology* 16:496-502. 10.1111/eth.12087

Braithwaite VA, and Salvanes AGV. 2005. Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. *Proceedings of the Royal Society B-Biological Sciences* 272:1107-1113. 10.1098/rspb.2005.3062

Burnham KP, and Anderson DR. 2002. *Model selection and multi-model inference: A practical information- Theoretic approach*. New York: Springer-Verlag.

Chapman BB, Morrell LJ, Benton TG, and Krause J. 2008a. Early interactions with adults mediate the development of predator defenses in guppies. *Behavioral Ecology* 19:87-93. 10.1093/beheco/arm111

Chapman BB, Ward AJW, and Krause J. 2008b. Schooling and learning: early social environment predicts social learning ability in the guppy, *Poecilia reticulata*. *Animal Behaviour* 76:923-929. 10.1016/j.anbehav.2008.03.022

Chivers DP, Brown GE, and Smith RJF. 1995. Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Canadian Journal of Zoology* 73:955-960. 10.1139/z95-111

Choleris E, Valsecchi P, Wang Y, Ferrari P, Kavaliers M, and Mainardi M. 1998. Social Learning of a Food Preference in Male and Female Mongolian Gerbils is Facilitated by the Anxiolytic, Chlordiazepoxide. *Pharmacology Biochemistry and Behavior* 60:575-584. 10.1016/S0091-3057(98)00005-7

366 Coffin HR, Watters JV, and Mateo JM. 2011. Odor-Based Recognition of Familiar and Related
367 Conspecifics: A First Test Conducted on Captive Humboldt Penguins (*Spheniscus*
368 *humboldti*). *PLoS One* 6:e25002. 10.1371/journal.pone.0025002
369 Cordelières FP. 2005. Manual Tracking. http://rsbweb.nih.gov/ij/plugins/track/Manual
370 Tracking plugin.pdf.
371 Cushing DH. 1974. The possible density-dependence of larval mortality and adult mortality
372 in fishes. In: Blaxter JHS, editor. The Early Life History of Fish. New York: Springer-
373 Verlag. p 103-111.
374 Dial TR, Reznick DN, and Brainerd EL. 2015. Effects of neonatal size on maturity and escape
375 performance in the Trinidadian guppy. *Functional Ecology* 30:943-952.
376 10.1111/1365-2435.12565
377 Domenici P. 2010. Context-dependent variability in the components of fish escape
378 response: integrating locomotor performance and behavior. *Journal of Experimental*
379 *Biology* 313:59-79.
380 Domenici P, and Blake RW. 1997. The kinematics and performance of fish fast-start
381 swimming. *The Journal of Experimental Biology* 200:1165–1178.
382 Dugatkin LA, and Alfieri M. 1991. Guppies and the TIT FOR TAT Strategy: Preference Based
383 on Past Interaction. *Behavioral Ecology and Sociobiology* 28:243-246.
384 Dugatkin LA, and Godin J-GJ. 1992. Prey approaching predators: a cost-benefit perspective.
385 *Annales Zoologici Fennici* 29:233-252.
386 Dukas R. 2002. Behavioural and ecological consequences of limited attention. *Philosophical*
387 *Transactions of the Royal Society B: Biological Sciences* 357:1539-1547.
388 10.1098/rstb.2002.1063
389 Fuiman LA, and Cowan JH. 2003. Behaviour and Recruitment Success in Fish Larvae:
390 Repearability and Covariation of Survival Skills. *Ecology* 84:53-67. 10.1890/0012-
391 9658(2003)084[0053:BARSIF]2.0.CO;2
392 Fuiman LA, and Magurran AE. 1994. Development of predator defences in fishes. *Reviews in*
393 *Fish Biology and Fisheries* 4:145-183. 10.1007/bf00044127
394 Fuiman LA, Meekan MG, and McCormick MI. 2010. Maladaptive behavior reinforces a
395 recruitment bottleneck in newly settled fishes. *Oecologia* 164:99-108.
396 10.1007/s00442-010-1712-3
397 Fuiman LA, Rose KA, Cowan Jr JH, and Smith EP. 2006. Survival skills required for predator
398 evasion by fish larvae and their relation to laboratory measures of performance.
399 *Animal Behaviour* 71:1389-1399. 10.1016/j.anbehav.2005.11.013
400 Galef BG, Kennett DJ, and Wigmore SW. 1984. Transfer of information concerning distant
401 foods in rats: A robust phenomenon. *Animal Learning & Behavior* 12:292-296.
402 10.3758/bf03199970
403 Gibb A, C. , Swanson B, O. , Wesp H, Landels C, and Liu C. 2006. Development of the Escape
404 Response in Teleost Fishes: Do Ontogenetic Changes Enable Improved
405 Performance? *Physiological and Biochemical Zoology* 79:7-19. doi:10.1086/498192
406 Godin J-GJ, and Davis SA. 1995. Who Dares, Benefits: Predator Approach Behaviour in the
407 Guppy (Poecilia reticulata) Deters Predator Pursuit. *Proceedings of the Royal Society*
408 *of London B: Biological Sciences* 259:193-200. 10.1098/rspb.1995.0028
409 Grabowska-Zhang AM, Sheldon BC, and Hinde CA. 2012. Long-term familiarity promotes
410 joining in neighbour nest defence. *Biology Letters* 8:544-546.
411 10.1098/rsbl.2012.0183

412 Griffiths SW. 2003. Learned recognition of conspecifics by fishes. *Fish and Fisheries* 4:256-
413 268. 10.1046/j.1467-2979.2003.00129.x
414 Griffiths SW, Brockmark S, Höjesjö J, and Johnsson JI. 2004. Coping with divided attention:
415 the advantage of familiarity. *Proceedings of the Royal Society of London B: Biological*
416 *Sciences* 271:695-699. 10.1098/rspb.2003.2648
417 Griffiths SW, and Magurran AE. 1997a. Familiarity in schooling fish: how long does it take
418 to acquire? *Animal Behaviour* 53:945-949. 10.1006/anbe.1996.0315
419 Griffiths SW, and Magurran AE. 1997b. Schooling Preferences for Familiar Fish Vary with
420 Group Size in a Wild Guppy Population. *Proceedings: Biological Sciences* 264:547-
421 551.
422 Höjesjö J, Johnsson JI, Petersson E, and Järvi T. 1998. The importance of being familiar:
423 individual recognition and social behavior in sea trout (Salmo trutta). *Behavioral*
424 *Ecology* 9:445-451. 10.1093/beheco/9.5.445
425 Johnsson JI. 1997. Individual Recognition Affects Aggression and Dominance Relations in
426 Rainbow Trout, Oncorhynchus mykiss. *Ethology* 103:267-282. 10.1111/j.1439-
427 0310.1997.tb00017.x
428 Krause J, and Ruxton GD. 2002. Living in groups. In: Evolution OSiEa, editor: New York:
429 Oxford University Press. p 228.
430 Laland KN, Brown C, and Krause J. 2003. Learning in fishes: from three-second memory to
431 culture. *Fish and Fisheries* 4:199-202. 10.1046/j.1467-2979.2003.00124.x
432 Lima SL, and Dill LM. 1990. Behavioural decisions made under the risk of predation: a
433 review and prospectus. *Canadian Journal of Zoology* 68:619-640.
434 Magurran AE. 1990. The adaptive significance of schooling as an anti-predator defence in
435 fish. *Annales Zoologici Fennici* 27:51-66.
436 Magurran AE. 2005. *Evolutionary Ecology: The Trinidadian Guppy*: Oxford University Press,
437 New York.
438 Magurran AE, and Girling SL. 1986. Predator model recognition and response habituation
439 in shoaling minnows. *Animal Behaviour* 34:510-518. 10.1016/S0003-
440 3472(86)80119-1
441 Magurran AE, and Pitcher TJ. 1987. Provenance, Shoal Size and the Sociobiology of
442 Predator-Evasion Behaviour in Minnow Shoals. *Proceedings of the Royal Society of*
443 *London Series B Biological Sciences* 229:439-465. 10.1098/rspb.1987.0004
444 Magurran AE, and Seghers BH. 1994. Predator Inspection Behaviour Covaries with
445 Schooling Tendency Amongst Wild Guppy, Poecilia reticulata, Populations in
446 Trinidad. *Behaviour* 128:121-134.
447 Magurran AE, Seghers BH, Shaw PW, and Carvalho GR. 1994. Schooling preferences for
448 familiar fish in the guppy, Poecilia reticulata. *Journal of Fish Biology* 45:401-406.
449 10.1111/j.1095-8649.1994.tb01322.x
450 Marras S, Killen SS, Claireaux G, Domenici P, and McKenzie DJ. 2011. Behavioural and
451 kinematic components of the fast-start escape response in fish: individual variation
452 and temporal repeatability. *The Journal of Experimental Biology* 214:3102-3110.
453 10.1242/jeb.056648
454 Mathis A, and Smith RJF. 1993. Chemical alarm signals increase the survival time of fathead
455 minnows (Pimephales promelas) during encounters with northern pike (Esox
456 Lucius). *Behavioral Ecology* 4:260-265. 10.1093/beheco/4.3.260

457 Ojanguren AF, and Braña F. 2003. Effects of size and morphology on swimming
458 performance in juvenile brown trout (Salmo trutta L.). *Ecology of Freshwater Fish*
459 12:241-246. 10.1046/j.1600-0633.2003.00016.x
460 Ojanguren AF, and Fuiman LA. 2010. Seasonal variability in antipredator performance of
461 red drum larvae. *Marine Ecology Progress Series* 413:117-123. 10.3354/meps08697
462 Pitcher TE. 1992. Who dares, wins: the function and evolution of predator inspection
463 behaviour in shoaling fish. *Netherlands Journal of Zoology* 42:371-391.
464 Reby D, Hewison M, Izquierdo M, and Dominique P. 2001. Red deer (Cervus elaphus) hinds
465 discriminate between the roars of their current harem-holder stag and those of
466 neighbouring stags. *Ethology* 107:951-959. 10.1046/j.1439-0310.2001.00732.x
467 Ruxton GD, and Johnsen S. 2016. The effect of aggregation on visibility in open water.
468 *Proceedings of the Royal Society B: Biological Sciences* 283. 10.1098/rspb.2016.1463
469 Strodl MA, and Schausberger P. 2012. Social Familiarity Reduces Reaction Times and
470 Enhances Survival of Group-Living Predatory Mites under the Risk of Predation.
471 *PLoS One* 7:e43590. 10.1371/journal.pone.0043590
472 Strodl MA, and Schausberger P. 2013. Social familiarity relaxes the constraints of limited
473 attention and enhances reproduction of group-living predatory mites. *Oikos*
474 122:1217-1226. 10.1111/j.1600-0706.2012.20833.x
475 Tanner CJ, and Keller L. 2012. Nest distribution varies with dispersal method and
476 familiarity-mediated aggression for two sympatric ants. *Animal Behaviour* 84:1151-
477 1158. 10.1016/j.anbehav.2012.08.018
478 Team RC. 2015. R: A Language and Environment for Statistical Computing. R Foundation
479 for Statistical Computing. R 2.15.3 ed. Vienna, Austria: R Foundation for Statistical
480 Computing.
481 Thibault RE. 1974. Genetics of cannibalism in a viviparous fish and its relationship to
482 population density. *Nature* 251:138 - 140.
483 Vilhunen S, and Hirvonen H. 2003. Innate antipredator responses of Arctic charr
484 (Salvelinus alpinus) depend on predator species and their diet. *Behavioral Ecology*
485 *and Sociobiology* 55:1-10. 10.1007/s00265-003-0670-8
486 Ward A, and Webster M. 2016. *Sociality: The Behaviour of Group-Living Animals*: Springer
487 International Publishing.
488 Ward AJW, and Hart PJB. 2003. The effects of kin and familiarity on interactions between
489 fish. *Fish and Fisheries* 4:348-358. 10.1046/j.1467-2979.2003.00135.x
490 Ward AJW, Herbert-Read JE, Sumpter DJT, and Krause J. 2011. Fast and accurate decisions
491 through collective vigilance in fish shoals. *Proceedings of the National Academy of*
492 *Sciences* 108:2312-2315. 10.1073/pnas.1007102108
493 Webb PW. 1978. Fast-start Performance and Body Form in Seven Species of Teleost Fish.
494 *The Journal of Experimental Biology* 74:211-226.
495 Weihs D. 1973. The mechanism of rapid starting of slender fish. *Biorheology* 10:343-350.
496 Weihs D, and Webb PW. 1984. Optimal avoidance and evasion tactics in predator-prey
497 interactions. *Journal of Theoretical Biology* 106:189-206.
498 Wisenden BD, and Smith RJF. 1998. A re-evaluation of the effect of shoalmate familiarity on
499 the proliferation of alarm substance cells in ostariophysan fishes. *Journal of Fish*
500 *Biology* 53:841-846.

Ydenberg RC, and Dill LM. 1986. The Economics of Fleeing from Predators. In: Jay S. Rosenblatt CBM-CB, and Peter JBS, eds. *Advances in the Study of Behavior*: Academic Press, 229-249.

Zach GJ, Peneder S, Strodl MA, and Schausberger P. 2012. Social familiarity governs prey patch-exploitation, - leaving and inter-patch distribution of the group-living predatory mite *Phytoseiulus persimilis*. *PLoS One* 7:e42889. 10.1371/journal.pone.0042889

Zajitschek Susanne RK, and Brooks Robert C. 2008. Distinguishing the Effects of Familiarity, Relatedness, and Color Pattern Rarity on Attractiveness and Measuring Their Effects on Sexual Selection in Guppies (Poecilia reticulata). *The American Naturalist* 172:843-854. 10.1086/593001

Figure 1(on next page)

Figure 1

Figure 1 - Diagram of the two experimental treatments (familiar and unfamiliar). Individuals were allocated to a holding tank with two other conspecifics for two weeks. Each testing day, three groups were tested where fish remained with those they had been sharing a tank with (familiar treatment). The other three groups had the individuals swapped so that none of the fish had encountered each other previously (unfamiliar treatment). Forty-two groups were tested in total, 21 of each treatment.

Figure 1

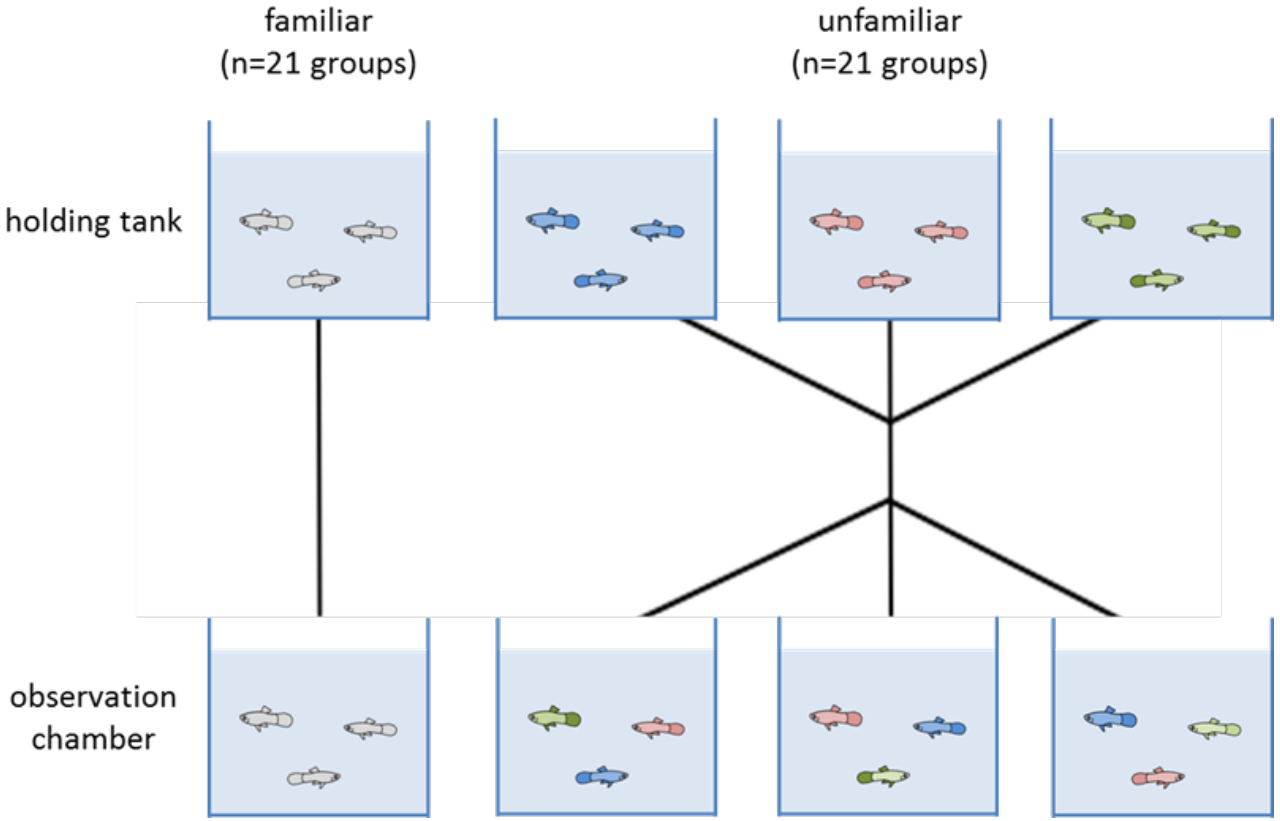
familiar
(n=21 groups)
unfamiliar
(n=21 groups)
holding tank
observation
chamber

Figure 2(on next page)

Figure 2

Figure 2 - Illustration of the experimental setup. A camera was placed 1 m away from a glass tank (10 x 10 x 10 cm) positioned before the LDC screen that showed the digital display of a looming object. The front of the tank and the overhead view of the tank were recorded in high-speed video for each trial. The distance in centimetres of the digital looming object was displayed on the top left of the screen.

Figure 2

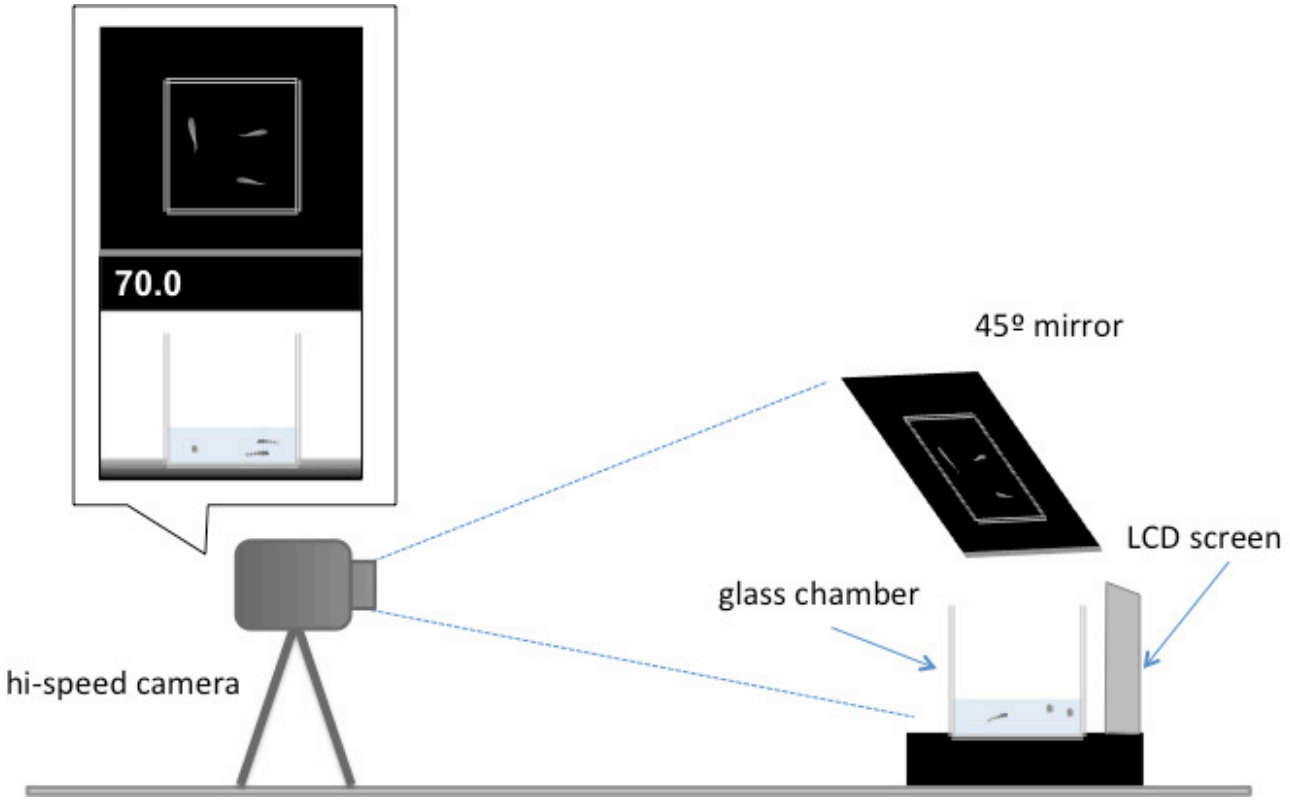

Figure 3(on next page)

Figure 3

Figure 3 - Responsiveness for familiar and unfamiliar groups in terms of how many individuals in a group of three responded to the stimulus. The numbers within the bubbles give the number of groups.

Figure 3

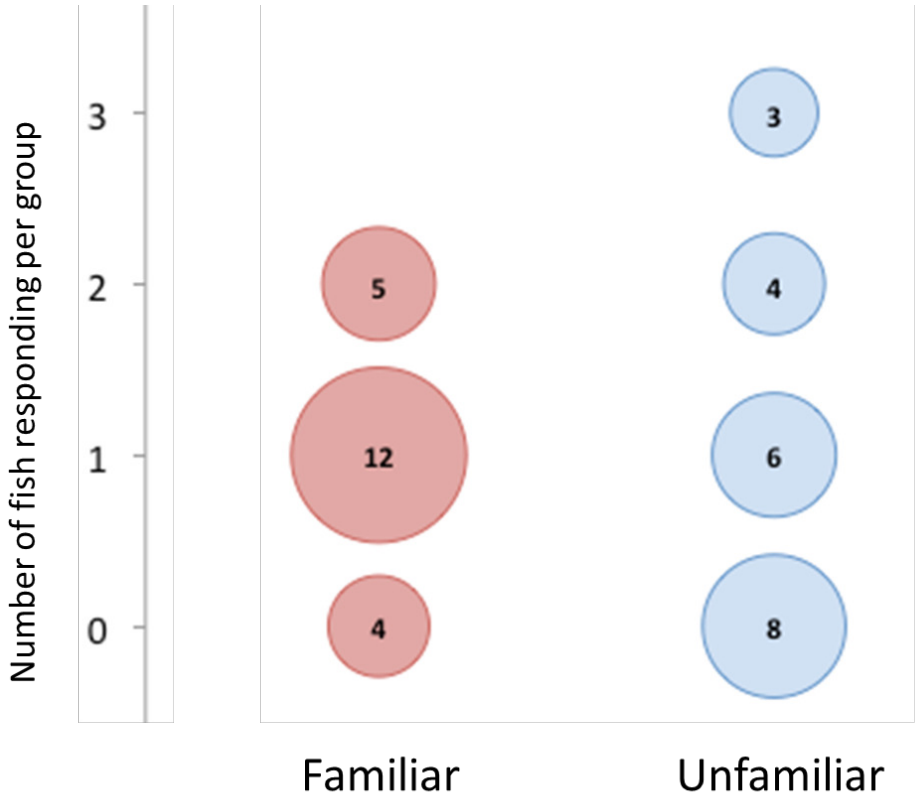
Number of fish responding per group
3
2
1
0
5
12
4
3
4
6
8
Familiar
Unfamiliar

Figure 4(on next page)

Figure 4

Figure 4 - Variation in reactive distance (A), maximum speed (B), maximum acceleration (C) and total distance (D), in familiar (open circles) and unfamiliar (closed circles) groups. Lines were fitted using the coefficients of linear models.

Figure 4

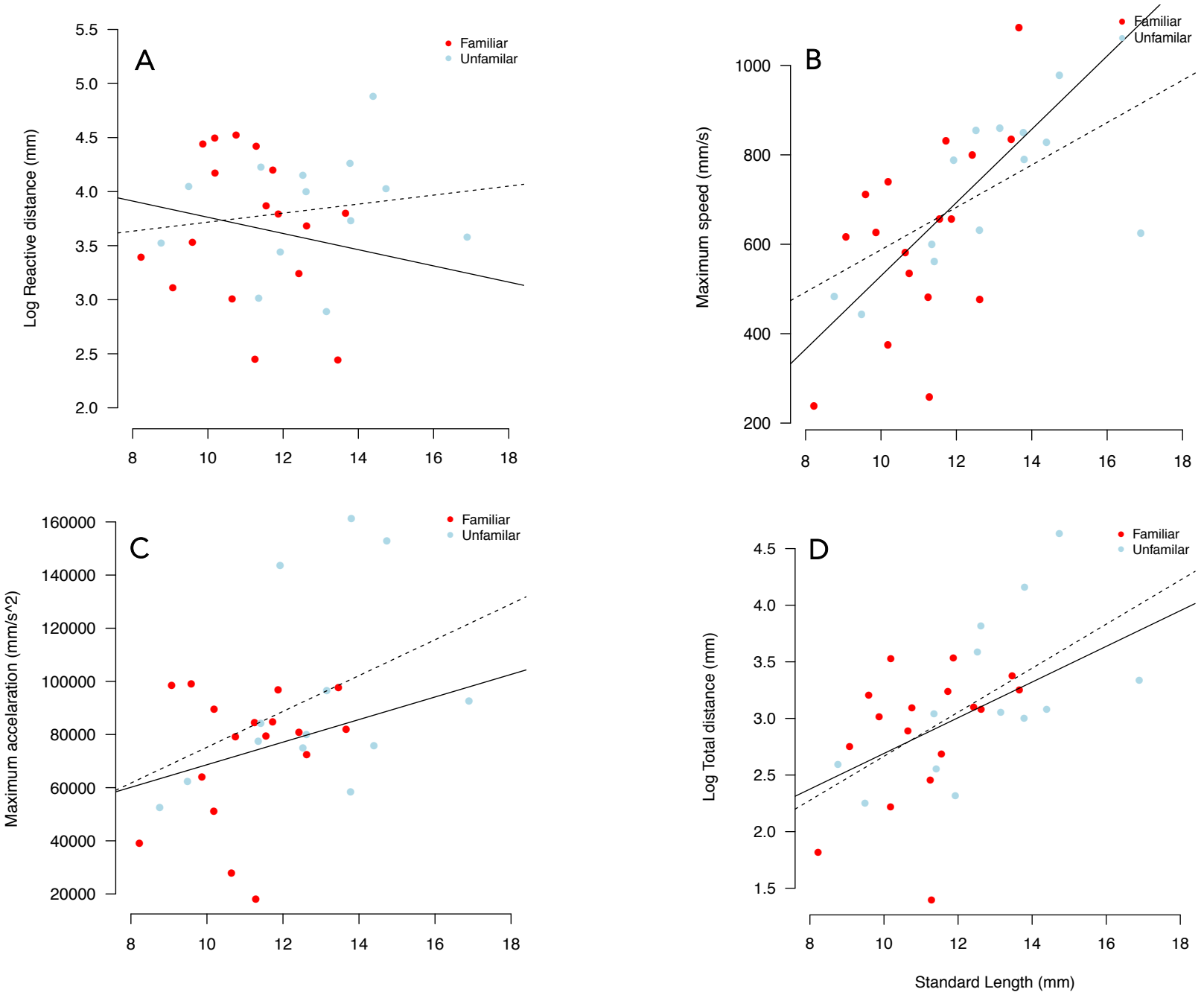
A
Familiar
Unfamilar
Log Reactive distance (mm)
B
Maximum speed (mm/s)
C
Maximum accelaration (mm/s^2)
D
Log Total distance (mm)
Standard Length (mm)

Table 1(on next page)

Table 1

Table 1 - Generalized linear models for testing the effect of familiarity on different qualitative measures of response. Model selection was performed using Akaike's information criterion (AIC). Both maximal and minimal adequate models are shown. The model with the lowest AIC was selected as being the minimum adequate model.

1
2
3 Table 1

4

Response variable	Explanatory variable	df	Sum Sq	F value	p-value
Reactive distance AIC - 63.4	Treatment	1	163	0.194	0.663
	Length	1	32	0.038	0.847
	Treatment + length	1	713	0.850	0.365
Reactive distance AIC - 58.7	Intercept	29	10.88		
Maximum speed AIC – 397.34	Treatment	1	6913	2.527	0.123
	Length	1	3414	12.48	0.001
	Treatment + length	1	2623	0.959	0.336
Maximum speed AIC – 394.43	Length	1	4106	15.59	0.004
Maximum acceleration AIC – 708.15	Treatment	1	2.96e+09	3.427	0.075
	Length	1	3.10e+09	3.591	0.069
	Treatment + length	1	1.36e+08	0.158	0.694
Maximum acceleration AIC – 705.26	Length	1	5.34e+09	6.425	0.017
Total distance AIC – 57.91	Treatment	1	0.781	2.342	0.138
	Length	1	3.015	9.037	0.005
	Treatment + length	1	0.030	0.089	0.767
Total distance AIC – 54.04	Length	1	3.788	12.17	0.001