PERSONALITY AND PREDATION IN A CHANGING WORLD

by

Chelsea A. Blake

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Committee Members:

Caitlin Gabor, Chair

Andrea Asbury

Chris Nice

Alison Bell

Brian Langerhans

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DEDICATION

I dedicate this work to my loving partner, Augustyn Rox Blake.

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ABSTRACT

The interaction between predators and prey is one of the driving forces that shape not only animal behavior, but also the evolution and ecology of organisms. However, predator-prey interactions are now taking place in an unprecedented and rapidly changing world, as humans introduce new species and alter habitat conditions. Thus examining the anthropogenic introduction of novel predators is key to the contemporary study of behavioral ecology. Further, not all individual animals behave the same way within the same species or population, thus it is important to also assess behavior at the level of the individual. Individual behavioral types, or "personalities" of animals can have far-reaching implications for their ecology. Here I have explored predator-prey interactions in the context of changing environments from the perspective of individual-level variation to provide novel insights into species interactions. I have found that the personality of prey can affect how they fare with predators, but that the effect depends on which predator species they face. Additionally, I have shown that although behavioral type is important in predator interactions, it does not affect whether prey are able to recognize a novel predator. I have also explored how physical antipredator characteristics of individuals might relate to their behavioral type. I have found that although physical traits are not necessarily inherently correlated with behavioral traits, altering the physical condition of an individual can affect their behavioral traits. Ultimately, my work contributes to the understanding of how prey personality could interact with introduced predators to either aid or hinder the survival of native species.

CHAPTER I

FOUNDATIONS FOR THE STUDY OF PERSONALITY AND PREDATION IN A CHANGING ENVIRONMENT

Introduction

Our current world is one characterized by human impacts that have so thoroughly reached into the most intricate and the most vast ecosystem processes, that many scientists have begun referring to this altered world as the Anthropocene (Waters et al. 2016). In light of the far-reaching implications of anthropogenic impacts, the behavior and ecology of organisms must be considered within this new and dynamic context. At the same time, remaining rooted in an evolutionary perspective is necessary to understand the origins of current traits and the potential for these traits to change in response to changing environmental conditions through selection or plasticity.

These unprecedented and rapid changes are relevant in many areas of ecology, including predator-prey interactions. The interaction between predators and prey is one of the driving forces that shapes animal behavior and is arguably one of the most important relationships shaping the evolutionary ecology of organisms. Predators can directly affect the fitness of prey, and thus exert strong selection pressure on prey species (Vermeij 1994; Abrams 2000). Additionally, predators shape the morphology, behavior, and life history traits of individuals over the course of their lives (Lima and Dill 1990; Lima and Bednekoff 1999; Relyea 2001; Werner and Peacor 2003). Prey may respond to predators by altering their behavior in many contexts, from reproductive behavior and foraging activity, to habitat selection (Magnhagen 1991; Werner and Anholt 1993; Martin 1995;

Lima 1998). For example, non-consumptive effects of predators can include a decrease in opportunities to perform other behaviors such as mating and foraging (Ferrari et al. 2007; Sih et al. 2010), and/or physiological effects of heightened stress levels (Cockrem and Silverin 2002; Fraker et al. 2009; Davis and Gabor 2015). Predators can thus decrease prey fitness through indirect non-consumptive effects as well as direct consumption. In order to remain relevant in today's rapidly-changing environment, predator-prey research must recognize the central importance of anthropogenic impacts on these relationships.

Predator introductions and invasion

Predator introductions are a striking example of how human activity can rapidly change ecosystems. Predator introduction can refer to several different ecological scenarios, but here I will concentrate on anthropogenic predator introductions that involve a predator species establishing contact with prey species with which they share no recent sympatry in their evolutionary history. Anthropogenic predator introductions can occur through many means, including range expansion due to climate change, accidental transport or exotic species, purposeful introductions for management or recreation, and unintentional escape of captive animals (Mills et al. 1993). The rate of species introductions increases as trade increases worldwide, for example the rate of marine introductions has increased exponentially in the last 200 years (Ruiz et al. 2000).

The introduction of non-native predators into an ecosystem can have damaging effects on native prey populations. Theory predicts that native prey should suffer greater consumptive effects from introduced predators than from native predators due to the lack of adaptation to non-native predators (Sih et al. 2010). A meta-analysis incorporating many empirical studies of native and nonnative predators suggested the harmful effects of alien predators was double that of native predators (Salo et al. 2007). Prime examples of these effects include the decline in native fish populations due to introduced brown trout and salmon in the Great Lakes, and a widespread pattern of native species declines due to mammal introduction on islands (Mills et al. 1993; Courchamp et al. 2003).

Novel predators and predator recognition in fish

Predator species newly introduced into an environment may experience certain advantages that can actually facilitate invasion. In predator-prey relationships between native sympatric species, prey may experience high non-consumptive costs of defending themselves, but antipredator defenses may reduce predation rates and result in low consumptive effects from native predators (Sih et al. 2010). However, introduced predators can have a novelty advantage as a result of prey naïveté (Sih et al. 2010). In other words, native prey species may not even recognize introduced species as threatening (Salo et al. 2007; Anson and Dickman 2013). In this case, prey species are likely to experience high consumptive effects from novel predators that they either do not recognize or do not have effective antipredator behavior to defend themselves (Sih et al. 2010). Prey species that do not exhibit effective antipredator behavior in response to novel predators may suffer high predation rates that endanger the persistence of these native species (Rehage et al. 2005; Nannini and Belk 2006; Banks and Dickman 2007). It is important to explore the response of native prey to introductions to understand the impacts and provide management implications.

However, not all native prey species will respond to introduced predators in the same way. The likelihood of a native prey species responding with effective defenses to introduced predators depends on evolutionary history, phenotypic and behavioral plasticity, and learning ability (Kelley and Magurran 2003b; Nannini and Belk 2006; Ferrari et al. 2007). If prey have a history of sympatry with predators similar to the introduced predator, they may be able to generalize from the native predator to recognize and show antipredator behavior to morphologically similar or closely-related non-native predators (Ferrari et al. 2007; Mitchell et al. 2011; Davis et al. 2012). However, when an introduced predator is morphologically, phylogenetically, or behaviorally dissimilar to native predators, prey may not perceive the predator as a threat and may not respond adaptively. In comparison to terrestrial prey species that have historically large contiguous ranges with many predators, aquatic species in less connected freshwater systems may be less likely to share an evolutionary history with an introduced predator (Cox and Lima 2006).

In the absence of any innate and/or evolved antipredator behaviors that may help native prey species cope, the introduction of a novel predator can create a situation where prey must learn to recognize novel cues and/or adopt new escape strategies to respond successful. Fish can learn to respond to novel predators via associating chemical alarm and disturbance cues of conspecifics, or diet cues from a predator (Ferrari et al. 2010). Further, prey are sometimes able to identify unfamiliar predators from kairomones, or chemical signals of the predator alone (Brown 2003; Kelley and Magurran 2003b; Wisenden 2003). When native species are able recognize the threat of introduced predators they may be able to perform antipredator behaviors that can mitigate the impact of the invasive predator.

Antipredator behavior in fish

Like any prey species, fish prey can employ general antipredator strategies such as avoiding predators or avoiding habitats frequented by predators, as well as more specific antipredator adaptations. A general response to predation risk can include a reduction in activity, such as decreased foraging behavior, more time spent hiding in refuge areas, and freezing behavior (Brown and Smith 1998). For some species inactivity is also reflected in their position in the water column, as inactive prey fish usually spend time near the substrate (Wisenden et al. 1999).

Other antipredator behaviors identified in fish include, shoaling or schooling, predator inspections, and C-start escapes (Pitcher et al. 1986; Dugatkin and Godin 1992b; Domenici and Blake 1997; Brown and Smith 1998). Shoaling is a behavior defined as fish grouping together for social reasons (Pitcher 1986). Shoaling fish may or may not exhibit schooling behavior, a more narrowly defined behavior in which fish synchronize their swimming and all face in the same direction (Pitcher 1986). Aggregation into schools or shoals serves many of the same purposes for fish that it does for herds of terrestrial animals, including confusion effects on the predator, increased vigilance of the shoal, and improved predator recognition and risk assessment through increased communication with conspecifics within the shoal (Magurran 1990; Hoare and Krause 2003).

Predator inspections are another important component of antipredator behavior in fish. Predator inspections at first appear counterintuitive, because the behavior is defined by a prey fish approaching a predator and lingering near it for several seconds before retreating (Pitcher et al. 1986). However, research has shown that predator inspections can allow prey to gain information about the diet and current riskiness of the predator through chemical cues, as well as potentially deter predator attacks by communicating to the predator the prey's own level of alertness and physical condition (Dugatkin and Godin 1992a; Brown 2003; Kelley and Magurran 2003a).

If a predator attacks, prey fish may employ specialized escape movements, termed fast-start or C-starts. The initiation of a C-start escape is characterized by the body of the fish forming into a C-shaped (or sometimes S-shaped) curve before the fish darts quickly away (Webb 1976; Domenici and Blake 1997). The behavior usually lasts less than one second, and when successful, propels the prey individual out of danger of the rapidly approaching predator (Domenici and Blake 1997). Some fish also display a related behavior in which they thrust upwards and actually jump out of the water to move themselves away from the predator (Christensen 1996). The velocity and ability of the prey individual to adjust their direction during the fast-start movement predict its ability to escape and survive a predator attack (Walker et al. 2005).

Behavioral syndromes

In addition to emerging research in the area of anthropogenic impacts and introduced predators, research in recent years has revealed that assessing behavior at the level of the individual can be quite revealing. Not all individual animals behave the same way, even within the same species and population. Individual behavioral types, or "personalities" of animals can have far-reaching implications for their ecology, especially in human-altered environments (Sih et al. 2011; Sih 2013).

Individual variation in behavior among conspecifics is considered a behavioral syndromes if behavior is consistent within individuals across ontogeny, environmental situations, or life history contexts. Behavioral syndromes need not preclude plasticity completely, because an individuals' rank ordered behavioral tendencies within the population can be maintained even in instances where they exhibit behavioral plasticity (Sih et al. 2004a). Behavioral syndromes often encompass correlations among multiple correlated traits, such that an individual may display a behavioral type that is more bold, active, and exploratory than its conspecifics (Sih et al. 2004b; Cote et al. 2010; Cote et al. 2011). Behavioral syndromes can sometimes act as constraints on behavioral responses, and cause behaviors that are advantageous in one situation to carry over into situations in which they are maladaptive (Sih et al. 2003a; Sih et al. 2004b; Johnson and Sih 2005). Despite these occasional suboptimal behaviors, there is also evidence that behavioral correlations themselves may be selected for, especially by predation pressure (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007).

Behavioral syndromes not only affect the behavioral ecology of single species, but can also impact the ecology of entire communities. These impacts include contributing to enhanced invasion success by pest species (Cote et al. 2010; Cote et al. 2011), modifying species interactions (Pruitt and Riechert 2011; Pruitt et al. 2012), and limiting the abundance of species in different habitats (Sih et al. 2012). Behavioral syndromes can also affect an animal's ability to respond rapidly to the novel selection pressures of human induced rapid environmental change (HIREC) (Robertson et al. 2013; Sih 2013).

Experimental Approach

To explore the importance of behavioral syndromes and anthropogenic predator introductions on the behavioral ecology of native species, I conducted five studies asking several related questions. In chapter two of this dissertation, I examine responses of native prey to both native and novel predators, and explore correlations between personality traits of prey and their predator recognition. In chapter three, I examine how personality of native prey individuals influences their behavior and survival of several different predator species, including native, novel, and invasive predators. In chapter four, I present a study exploring the possibility of selective foraging by two different predators on prey of different behavioral characteristics. The final two experiments in chapters five and six focus on the relationship of behavioral traits to physical body traits associated with predator escape ability, and plasticity of behavior in response to morphological changes. The experiments presented add to our understanding of how the behavior and personality traits of native species interplay with anthropogenic changes to shape outcomes for native species in shifting ecological landscapes.

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CHAPTER II

EXPLORATION AND PREDATOR RECOGNITION: BEHAVIORAL CORRELATIONS ACROSS CONTEXT

It was hypothesized that the exploratory behaviour of an individual measured in a novel environment could predict its behaviour in response to a novel predator. The present study examined novel predator recognition in the Western mosquitofish *Gambusia affinis*, a species with individual differences in risk-taking, activity, and exploration in novel environments. Prey responded with characteristic shoaling and avoidance in response to native predators, but did not show characteristic antipredator behaviour towards novel predators. Further, *G. affinis* exhibited individual-level behavioural correlations across contexts, but only when prey were tested with native predators. This could be the result of native predatory selection on behavioural correlations in the prey species.

Key words: Antipredator behaviour; behavioural syndromes; boldness; introduced predators; invasive species.

INTRODUCTION

As human activities lead to the introduction of predator species into new habitats, the ability of prey to recognize and respond to novel predators becomes increasingly important for the survival of prey species. Native species can suffer indirect damage from invasive species that alter habitats and ecosystem processes, or direct effects from interactions with introduced predators (Cox & Lima, 2006; Sih *et al.*, 2010; Strayer, 2010). Prey species that do not respond with effective antipredator behaviour towards novel predators may suffer lethal effects that can put native species at risk (Nannini & Belk, 2006; Banks & Dickman, 2007; Salo *et al.*, 2007).

The effects of a novel predator can be mitigated if the prey species can recognize it as a threat, through innate and/or learned responses. For aquatic organisms, chemical cues, or kairmones, given off by predators are often important for prey to identify and respond to potential predator threats (Chivers & Smith, 1998; Kelley & Magurran, 2003). Fishes can sometimes use associative learning to respond to novel predator cues that are paired with alarm cues of attacked or consumed conspecifics (Kelley & Magurran, 2003; Wisenden, 2003; Aizaki & Yusa, 2010; Mitchell *et al.*, 2011). In contrast, innate recognition can allow an immediate response to a novel predator that does not require multiple experiences. Innate recognition of non-native visual or chemical predator cues without prior exposure is possible when prey species are able to generalize from a native predator to recognize and perform antipredator behaviour towards a similar, non-native predator (Ferrari *et al.*, 2007; Davis *et al.*, 2012; Mitchell *et al.*, 2013). However, generalization is less likely in situations where the introduced predator is phylogenetically distant and dissimilar from native predators in morphology and behaviour (Blake *et al.*, 2014).

In addition to understanding how prey species react to introduced predators, it is important to consider how prey may differ in their response to novel predators at the individual level. Individual variation in behavioural traits within a species, termed behavioural syndromes or personalities, has been shown to affect many aspects of ecology (Sih *et al.*, 2012), and appears to be particularly important in predator-prey relationships (Biro *et al.*, 2004; Bell, 2005; Bell & Sih, 2007; Dingemanse *et al.*, 2007). In addition to observed population-level effects, prey personalities can affect direct behavioural interactions among predator and prey individuals (Dugatkin & Alfieri, 2003; Smith & Blumstein, 2010; Pruitt *et al.*, 2012; Blake & Gabor, 2014).

Research on behavioural syndromes in animals has often examined risk-taking behaviour, for example latency to remerge after a simulated predator attack (Webster *et al.*, 2007; Burns, 2008; Carter *et al.*, 2013). Studies on behavioural syndromes also often test response to novelty, such as willingness to enter a novel environment or approach a novel object (Brown *et al.*, 2007; Dingemanse *et al.*, 2007; Carter *et al.*, 2013). Additional research has shown that some individuals respond with less caution to novelty, but may also be slower in responding to changes in their environment (de Lourdes Ruiz-Gomez *et al.*, 2011). As facing an introduced predator combines elements of both risk-taking and novelty, individual prey may differ in their reaction, and these individual differences could have important consequences for their recognition and response to novel predators, and ultimately their survival when faced with novel predators. Further, there may be correlations between risk-taking and reaction to novelty in the context of novel predator recognition to these same behavioural characteristics measured in other contexts, such as emerging from an enclosed structure into a novel environment. For example, individual prey that are more likely to take risks such as emerging quickly from shelter into an unfamiliar environment may be less likely to recognize a novel predator as threatening.

The present study investigated recognition of a novel predator, and how other behavioural characteristics of prey individuals are connected to responses to predators. In this experiment, responses of western mosquitofish *Gambusia affinis* (Baird & Girard 1853) were examined to native predatory, novel predatory, and novel non-predatory stimuli fishes. The first prediction was that on average prey would not respond as strongly to the novel predators as native predators because the novel species was dissimilar from native predators phylogenetically, morphologically, and behaviourally. The novel predator in this study, the cyprinid Gulf killifish *Fundulus grandis* (Baird & Girard 1853), is contrasted with a native predator stimulus, the centrarchid Green sunfish *Lepomis cyanellus* (Rafinesque 1819). In addition to being taxonomically distant, these two predators contrast in their hunting styles, with *F. grandis* being more active in searching for prey. *Fundulus grandis* has been introduced into several freshwater drainages sympatric with *G. affinis* in central Texas; thus a lack of recognition of this predator implies detrimental impacts of this introduction for wild *G. affinis* populations.

Secondly, this experiment explored whether the behaviour of an individual towards native predatory, novel predatory, and novel non-predatory stimuli correlated with behaviour of these same prey individuals during novel environment emergence tests. Previous studies have found that bold or risk-taking behaviours occur with consistent individual differences in several species of mosquitofishes (Cote *et al.*, 2010; Cote *et al.*, 2011; Blake & Gabor, 2014). It was hypothesized that individual risk-taking behaviour in a novel environment could predict the behaviour of these same prey individuals in response to a novel predator. If some behavioural types were better able to recognize or respond to novel predators, this could modify the effects of introduction on the prey species, and could also lead to new selection pressures on behavioural syndromes in the prey population.

MATERIALS AND METHODS

STUDY SPECIES

Gambusia affinis is a widespread species, its native range stretching from Alabama to some parts of New Mexico and as far north as Illinois, and has also become invasive in other introduced habitats. *Gambusia affinis* is common in fresh water throughout Texas, and is small (<65 mm), mostly insectivorous, and live-bearing. *Lepomis cyanellus* is a common native piscivorous predator in central Texas, and thus is currently and historically sympatric with *G. affinis* (Hubbs *et al.*, 1991). *Fundulus grandis* is native to fresh and brackish waters along the coasts of northeastern Florida and the Gulf of Mexico, and consumes an omnivorous diet including invertebrates and small fishes (Rozas & Lasalle, 1990; Hubbs *et al.*, 1991). *Fundulus grandis* can tolerate a range of salinity and have been introduced into many freshwater environments in Texas and New Mexico through bait-bucket releases (Hillis *et al.*, 1980). In its current distribution *F. grandis* co-occur with *G. affinis* in some areas of Texas, but are novel to the *G. affinis* population used in this study (Hillis *et al.*, 1980; Thomas *et al.*, 2007; Perkin & Bonner, 2014). Guppies *Poecilia reticulata* (Wilhelm Peters 1859) from a laboratory stock population roughly equivalent in size to the focal *G. affinis*, were used as an allopatric, non-predatory control. For the predator treatments juvenile individuals [60-100 mm standard length (L_S)] were used to allow for ease of maintenance in laboratory tanks, but all individuals were large enough to potentially consume focal *G. affinis* individuals (20-35 mm L_S). Mean L_S was similar between the two predator species (*F. grandis*: 82mm, *L. cyanellus* 86 mm).

COLLECTION AND LABORATORY MAINTENANCE

Wild-caught *G. affinis* were collected from the Comal River, Comal County, TX (N 29°42'15", W 98°7'49") in February 2014. *Poecilia reticulata* were selected haphazardly from a stock laboratory population of several hundred fish. Before and between trials *G. affinis* and *P. reticulata* resided in 38l aquaria (50 x 25 x 30 cm) on a 14:10 h light cycle at 25-27°C and ate flake food (Ocean Star International, http://www.oceanstarinternational.com) *ad libitum* once a day at 1630 hours. *Lepomis cyanellus* were collected from Spring Lake, Hays County, TX (N 29°53'40", W 97°55'49"), and *F. grandis* were collected from the Brazos River, Hill County, TX (N 31º52'23" N, W 97º21'53"). *Lepomis cyanellus* and *F. grandis* were maintained in single-species 150l aquaria (91 x 46 x 41 cm) on a 14:10 h light cycle at 25-27°C, and fed them pellet food (Purina Aqua Max 200, http://www.purinamills.com) *ad libitum* once a day at 1630 hours.

NOVEL ENVIRONMENT TRIALS

Several weeks before testing, focal *G. affinis* were injected immediately anterior to the pectoral fin with an individualized tag, using up to two colours of elastomer (Northwest Marine Technology, WA, USA, http://www.nmt.us/). Mortality from elastomer was less than 1.0% and generally occurred within the first 24 h after injections. Novel environment trials were conducted in a shallow, opaque plastic tank (52x35cm) with video cameras mounted above the tank to record trials. Tanks contained 8 cm of water, so that movement was primarily horizontal, and water temperature ranged from 25-27°C. *G. affinis* were placed individually into in an opaque container (9 x9 x 18 cm) and allowed to acclimate for 5 min before opening the door (5 x 5 cm) of the container remotely using a pull string. Latency to emerge was calculated as the log of the maximum time allowed to exit (10 min) minus the log of the time to until the *G. affinis* exited the container into the novel tank environment. Thus, a higher value for latency to emerge indicates that the *G. affinis* entered the novel tank environment sooner. Time spent moving was calculated as the proportion of the 5 min observation period the individual spent moving, and area used as the proportion of the tank area explored, each calculated from videos of the trials using Image (http://imagej.nih.gov/ij/; Blake & Gabor, 2014). During the novel environment assays, most *G. affinis* exited the chamber within the time allotted, and only these *G. affinis* with complete novel environment data were included in analysis of behavioural correlations across contexts (*n* = 42 per treatment). These trials were performed in between 0800-1300 hours March - April 2014.

PREDATOR RECOGNITION TRIALS

Predator recognition trials were conducted using the same marked individuals, 4-6 weeks following the novel environment trials. Each *G. affinis* was exposed to all three treatments in random order: (1) native *L. cyanellus* predator (2) novel *F. grandis* predator (3) novel non-predatory *P. reticulata*. Before predator recognition trials *G. affinis* were also photographed for another study, measured for standard length (L_S) from tip of the snout to the end of the last vertebra, and weighed for body mass. Predator recognition trials used 76 l testing tanks (76 x 30 x 30 cm) filled with 15 cm of dechlorinated tap water. Tanks were covered on three sides with opaque barriers and window tinting was put on the front of the tank to reduce visual disturbance. The tank was divided into two unequal sections by a clear, water-permeable plastic barrier, one 56 cm section containing the focal individual and shoalmates, and the other 20 cm section for the stimulus fish. The focal fish section was marked with lines on the outside of the glass to indicate three (5 cm) vertical zones and fourteen (4 cm) horizontal zones. Thus a *G. affinis* in horizontal zone one was within 4 cm of the barrier to the stimulus fish section, whereas a *G. affinis* in zone fourteen was 52-56 cm from the stimulus fish area.

To initiate the predator recognition trial, a marked focal individual was placed into the testing tank with two unmarked conspecifics collected at the same site and location as the focal fish. These conspecific shoalmates were haphazardly selected for each trial from another tank in the lab. After a 5 min acclimation period, vertical position of the focal *G. affinis*, horizontal position, and shoaling behaviour were recorded every 30 s for 5 min. The focal *G. affinis* was considered to be exhibiting shoaling behaviour if they were within two body lengths of a conspecific by visual estimation of the observer. After the 5 min pre-stimulus observation period, the stimulus fish was introduced with a net into the separated section of the experimental tank. Multiple individuals of each stimulus species were used and rotated between trials so that no one stimulus fish became too stressed (*F. grandis* = 9, *L. cyanellus* = 5, *P. reticulata* = 15). Following the introduction of the stimulus fish, vertical and horizontal position and shoaling of the focal *G. affinis* were recorded every 30 s for 5 min. After the trial all fishes were moved back to their home tanks. This process was repeated on subsequent days (24 h later) for all three treatments, using a repeated measures design so that each focal *G. affinis* received each treatment (*n* = 51 per treatment).

Behavioural responses of *G. affinis* were calculated by subtracting the focal *G. affinis'* average zone during the pre-stimulus from its average zone during post-stimulus observation. Thus a positive value for change is distance from the stimulus represented a move away from the stimulus, while a positive value for change in water column use indicated a higher position in the water column post-stimulus. Similarly, shoaling response was calculated as the number of times the focal individual was observed shoaling in the pre-stimulus period subtracted from their shoaling in the post-stimulus period. Increased shoaling, moving upwards in the water column, and moving away from predator cues were considered indications of antipredator behaviour (Magurran, 1990; Christensen, 1996; Kelley & Magurran, 2003; Zheng *et al.*, 2005). Thus positive values for all three behavioural responses were associated with an increase in antipredator behaviour. Predator recognition trials were performed between 0800-1300 hours at 25-27°C in May 2014. The experimental methods described above were approved under IACUC protocol 0515_0612_13, and follow guidelines for ethical use of experimental animals, and fish specifically (Metcalfe & Craig, 2011; ASB/ABS, 2012).

ANALYSES

Preliminary analyses showed testing order and L_S of the focal individual did not have significant effects and these factors were not included in the final analyses. Latency to emerge was calculated on a log scale to improve normality. Following this, Shapiro-Wilks tests and diagnostic plots showed that all variables were normal or near-normal in distribution. To assess effects of treatment on behavioural responses to the stimuli fish, a repeated measures ANOVA was used, followed by Tukey's post-hoc test to compare means of behavioural responses among treatment groups. Pearson correlations were used to examine relationships among behaviours across contexts. Analyses were conducted in R 2.15.0 (www.r-project.org).

RESULTS

Changes in distance of the focal *G. affinis* from the stimulus differed among treatments (ANOVA: $F_{2,100}$=6.21, P=0.001). Focal mosquitofish also varied in their shoaling response to the stimulus among the different treatments (ANOVA: $F_{2,100}$=6.36, P=0.001), and in their water column use (ANOVA: $F_{2,100}$=3.12, P=0.05). Focal *G. affinis* responded to native predatory *L. cyanellus* by moving away from the stimulus and shoaling more closely (Figs. 1 and 2). In contrast, focal *G. affinis* moved towards novel predatory *F. grandis* and did not change their shoaling behaviour following stimulus introduction (Figs. 1 and 2). Focal *G. affinis* also responded to *F. grandis* by moving downwards in the water column, but did not change their water column usage following introduction of native *L. cyanellus* (Fig. 3). Responses to *P. reticulata* stimuli were near zero for all three behaviours, indicating little change in focal *G. affinis* behaviour following stimulus introduction.

Behaviour in the novel environment assays and in the predator recognition trials showed several significant relationships among the behaviours measured in these two contexts. However, there were only significant correlations across contexts when focal *G. affinis* were tested with the native *L. cyanellus* treatment (Table I). The amount of time an individual spent moving in the novel environment was positively correlated with their change in distance from *L. cyanellus* stimulus (*i.e.*, more active individuals moved further from *L. cyanellus* than less active individuals). The area used by an individual in the novel environment assay was negatively correlated with their change in shoaling tendency, and change in water column use in response to the *L. cyanellus* stimulus. The most exploratory individuals showed a decrease in shoaling and moved lower in the water column in response to the *L. cyanellus*. There were no significant correlations across the two testing contexts when individuals were exposed to the *P. reticulata* or *F. grandis* treatments (Table I).

DISCUSSION

The results indicate that *G. affinis* used in this study did not recognize and respond to a novel predator stimulus. *Gambusia affinis* behaviour towards native predatory *L. cyanellus* was characterized by increased shoaling and moving away from the stimulus, with no significant change in water column use. Increased shoaling and moving away coincide with previous descriptions of antipredator behaviour in *G. affinis* (Magurran, 1990; Kelley & Magurran, 2003). In contrast, behavioural responses to novel *F. grandis* stimuli were characterized by moving towards the bottom and towards the stimulus fish, without significantly changing shoaling behaviour. Overall, behavioural responses to the *F. grandis* were more similar to responses toward non-predatory *P. reticulata* than to native predator responses. These results contrast with other research showing that native species can sometimes respond in a threat-sensitive way to novel predators (Brown & Morgan, 2015). However, our results coincide with a previous study on the closely-related Largespring mosquitofish *Gambusia geiseri* (Hubbs & Hubbs

1957), which also showed a lack of antipredator response to the *F. grandis* (Blake *et al.*, 2014).

Changes in water column use can be important in antipredator responses, especially for fishes that use jumping behaviour as an escape mechanism. Moving upwards in the water column is a response to acute predation risk, as this allows fishes to perform antipredator behaviour by jumping out of the water (Christensen, 1996; Blake & Gabor, 2014). However, previous research suggests that the vertical movement of prey can also depend on water column use and hunting strategy of the particular predator species (Staudinger *et al.*, 2013). It is possible that prey *G. affinis* in the present study did not change water column use in response to native *L. cyanellus* because they were responding to the way predator individuals were moving in the water column. Similarly, moving towards the bottom in response to novel *F. grandis* could be related to the water column usage of the predator fish, especially as the observations made indicated that *F. grandis* spent a large amount of time near the surface compared to native sunfish predators. However, predator behaviour was not recorded during the trials so any behavioural differences there may have been between predators cannot be quantified. In addition, future studies could further explore the effect of individual predator characteristics such as hunger-level and body size on the strength of antipredator responses towards novel predators.

Increasing the average distance from the stimulus is consistent with an adaptive antipredator response to the native *L. cyanellus*. However, focal *G. affinis* moving closer to the novel *F. grandis* predator could have several explanations. Moving towards *F. grandis* after the stimulus was introduced could be the result of predator inspection behaviour, in which prey move closer to a potential threat to gain more information about the size and/or hunger level of the predator (Dugatkin & Godin, 1992). Inspection behaviour in response to the novel predator could indicate perception of a possible threat that requires more information gathering rather than immediate recognition of a predation threat. It is also possible that prey *G. affinis* simply did not recognize the novel *F. grandis* predator as threatening at all, and so were not deterred from going closer. However, the response to novel *F. grandis* was distinct from the response to non-predatory *P. reticulata*, indicating that the focal *G. affinis* did distinguish between these two types of stimuli. Furthermore, in a previous study *F. grandis* actually consumed *Gambusia geiseri* more quickly than native *L. cyanellus* in one-on-one predation trials (Blake & Gabor, 2014). Thus in a direct interaction, the lack of an immediate moving away response with novel *F. grandis* predators would likely be detrimental to prey survival. The present findings suggest that naïve *G. affinis* may not exhibit optimal behaviour when exposed to *F. grandis* in the wild, and could suffer high consumptive effects from these introduced predators.

Contrary to predictions, exploratory behaviour in a novel environment predicted antipredator behaviour when *G. affinis* were tested with native predators, but not novel predators. Behavioural correlations between the novel environment and predator recognition contexts were not significant for either novel predatory *F. grandis* or novel non-predatory *P. reticulata* stimuli. This finding was contrary to the prediction that individual responses to novel environment and emergence tests would predict predator recognition responses. This finding is also contrary to previous studies in which proactive (bold) vs. reactive (shy) individuals show unique responses to novel stimuli (de Lourdes Ruiz-Gomez *et al.*, 2011).

Instead, there were significant behavioural correlations across contexts when the focal *G. affinis* were exposed to a native *L. cyanellus* predator (Table I). Although the mean level responses to native *L. cyanellus* predators were characteristic of antipredator behaviour, the expression of these responses differed greatly among different prey individuals. Individuals that were more active in the novel environment moved further away than their less active conspecifics, possibly because they were simply moving around more. Exploratory individuals showed less characteristic antipredator behaviour in shoaling and water column use. The most exploratory individuals actually decreased their shoaling behaviour in response to *L. cyanellus*. This correlation could indicate a maladaptive behavioural carry-over in which individuals with a high ability to explore and exploit novel environments continue highly exploratory behaviour in the context of a predation threat, when it is no longer advantageous (Johnson & Sih, 2005). Alternately, it is possible that there is more than one effective antipredator strategy, and individuals may employ distinct antipredator coping styles based on their individual characteristics. In other words, the negative correlation between exploration in a novel context and shoaling in response to predators could be adaptive (Dall *et al.*, 2004).

If the behavioural correlations across contexts are the result of adaptation, it would be unsurprising that behaviour correlated across contexts only when prey were exposed to native predators. Native predation pressure can effect prey behaviour over evolutionary time, and previous evidence suggests that predators may select for behavioural correlations across contexts (Bell, 2005; Bell & Sih, 2007). If this is the case for the prey species here, it would be less likely for behavioural correlations to occur across contexts when the situation includes a novel predator like the *F. grandis* or a novel non-predator like a *P. reticulata* that has not evolved in sympatry with the native prey. The lack of correlation between novel environment emergence tests and reactions to a novel predator found here are contrary to previous studies that have found correlations among proactive or bold behaviour and responses to novel stimuli (Dugatkin & Alfieri, 2003; de Lourdes Ruiz-Gomez *et al.*, 2011). However, others have found that some behaviours that have been associated with "boldness", such as responses to novel food, may not correlate to other measures associated with "boldness", like emergences tests (Carter *et al.*, 2013). Future studies could explore how individuals may differ upon repeated exposure to novel predators, because there may still be differences in speed of learned predator recognition among individuals with different coping styles (Dugatkin & Alfieri, 2003).

In summary, *G. affinis* did not show antipredator behaviour to a novel *F. grandis* predator. The introduction of *F. grandis* into Central Texas could cause problems for native prey species unfamiliar with this type of predator. Further, behavioural correlations across contexts were only significant when prey were exposed to a native predator stimulus. This suggests that individual behavioural traits cannot be generalized to all contexts, especially when environmental conditions deriving from anthropogenic effects are unprecedented in the evolutionary history of a species.

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TABLE II.1

Correlations among behaviours measured in *Gambusia affinis* in a novel environment emergence test and in response to stimuli fishes. Significant Spearman correlation (ρ) in bold ($n = 42$).

	Latency to emerge	Time spent moving	Area used
Native *Lepomis cyanellus*			
Δ distance from stimulus	0.03, $P > 0.05$	**0.38, $P = 0.01$**	0.01, $P > 0.05$
Δ water column use	0.08, $P > 0.05$	-0.07, $P > 0.05$	**-0.35, $P = 0.01$**
Δ shoaling tendency	-0.17, $P > 0.05$	-0.03, $P > 0.05$	**-0.36, $P = 0.01$**
Novel *Fundulus grandis*			
Δ distance from stimulus	0.18, $P > 0.05$	0.26, $P > 0.05$	0.26, $P > 0.05$
Δ water column use	0.002, $P > 0.05$	0.09, $P > 0.05$	-0.10, $P > 0.05$
Δ shoaling tendency	0.06, $P > 0.05$	0.30, $P > 0.05$	0.17, $P > 0.05$
Novel non-predatory *Poecilia reticulata*			
Δ distance from stimulus	-0.01, $P > 0.05$	-0.05, $P > 0.05$	0.04, $P > 0.05$
Δ water column use	-0.06, $P > 0.05$	0.06, $P > 0.05$	0.02, $P > 0.05$
Δ shoaling tendency	0.09, $P > 0.05$	0.02, $P > 0.05$	-0.18, $P > 0.05$

FIGURES

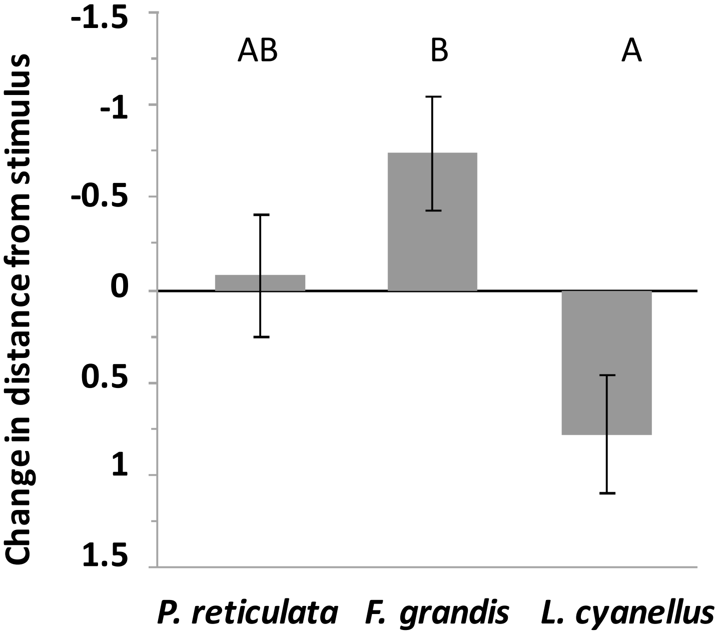

FIG. II.1. Mean change in distance from stimulus fish by treatment for *G. affinis*, calculated as post-stimulus minus pre-stimulus zone such that a positive value indicates moving further from the stimulus. One zone represents 4 cm of the tank. *F. grandis* is a novel predator, *L. cyanellus* is a native predator of *G. affinis*, and *P. reticulata* is a novel non-predator. Responses varied by treatment and letters distinguish significant difference from Tukey's post-hoc test.

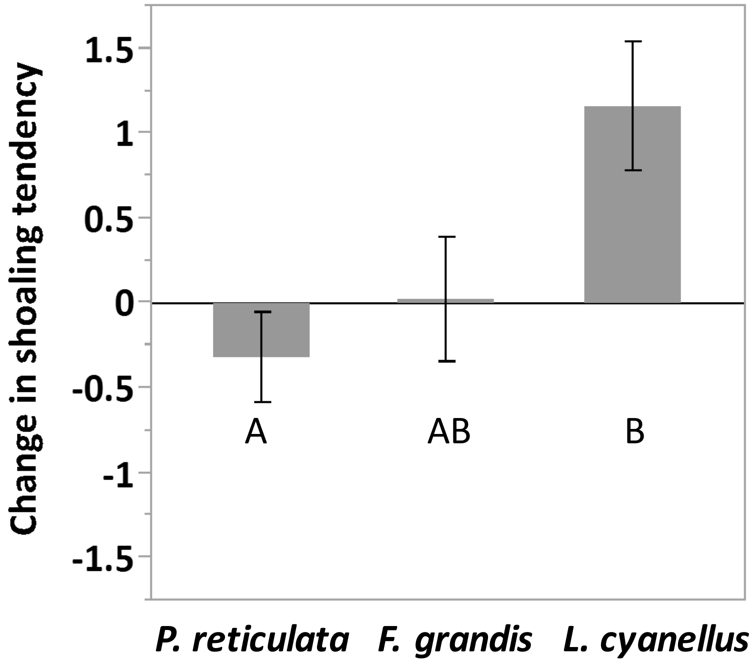

FIG. II.2. Mean change in shoaling tendency (post-stimulus minus pre-stimulus) by treatment for *G. affinis*. Units are number of times shoaling out of 10 observations over 5 min. *F. grandis* is a novel predator, *L. cyanellus* is a native predator of *G. affinis*, and *P. reticulata* is a novel non-predator. Responses varied by treatment and letters distinguish significant difference from Tukey's post-hoc test.

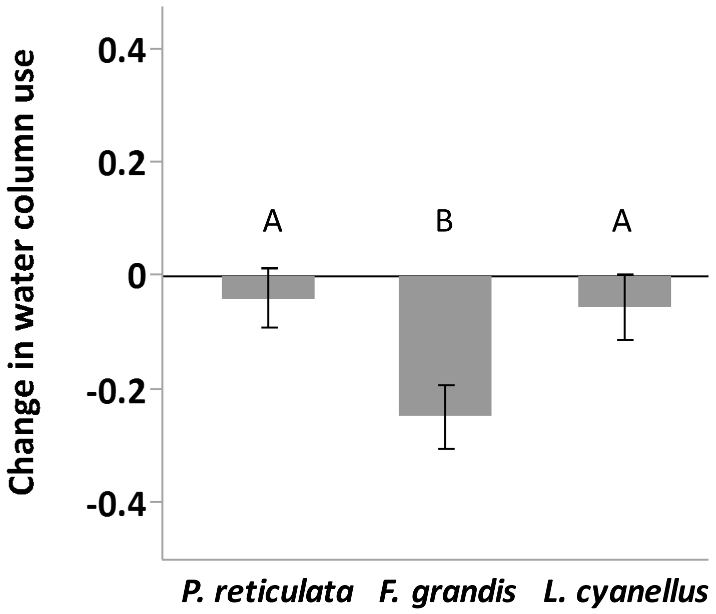

FIG. II.3. Mean change in water column zone use (post-stimulus minus pre-stimulus) by treatment for *G. affinis*. One zone represents 4cm of the tank. *F. grandis* is a novel predator, *L. cyanellus* is a native predator of *G. affinis*, and *P. reticulata* is a novel non-predator. Responses varied by treatment and letters distinguish significant difference from Tukey's post-hoc test.

CHAPTER III

EFFECT OF PREY PERSONALITY DEPENDS ON PREDATOR SPECIES

The ability of prey to respond to predators is especially important in human-altered habitats, where prey are faced with changing predator regimes. A growing body of research has shown the important ecological impacts of intraspecific, individual differences in behavior, but few studies have shown direct fitness consequences of behavioral types in a predation context. We examined behavioral syndromes in the endemic Largespring mosquitofish (*Gambusia geiseri)*, and how the behavioral type of individual *G. geiseri* affected their behavior and survival when exposed to three different species of predators. We measured latency to emerge from a container, time spent moving, and tank area used for individual *G. geiseri* in the presence and absence of predators. We then measured behavior and survival of these same individuals in one-on-one predation trials. We found that behavioral types and correlations between latency to emerge, time spent moving, and area used were consistent regardless of predator presence. Behavioral type did not predict survival of the predation trial. However, higher behavioral scores correlated with more escapes from *Fundulus grandis* predators. We argue this result indicates that active/exploratory fish have a greater ability than their conspecifics to escape this species, which is a novel predator. Our results illustrate the potential importance of considering individual differences in behavior in studying the impacts of introduced predator species.

INTRODUCTION

Individual differences in behavior among conspecifics can have far-reaching ecological impacts. These impacts include contributing to enhanced invasion success by pest species (Cote et al. 2010; 2011), modifying the magnitude and nature of species interactions (Pruitt et al. 2011; 2012a), and limiting the presence and abundance of species in different habitats (reviewed in Sih et al. 2012). Behavioral syndromes are individual differences in behavior that are consistent within an individual across environmental situations or life history contexts (Sih et al. 2004a). Behavioral syndromes often encompass multiple correlated traits, such that an individual may display a behavioral type that is more bold, active, and exploratory relative to its conspecifics (Cote et al. 2010; 2011). Behavioral syndromes can act as a constraint on behavioral responses, and cause behaviors that are advantageous in one situation to carry over into situations in which they are maladaptive (Sih et al. 2003, 2004a; Johnson and Sih 2005). However, behavioral syndromes need not preclude plasticity completely. For example, individuals' rank ordered behavioral tendencies could be maintained even in instances where they exhibit a high degree of behavioral plasticity (Sih et al. 2004 a, b,). Indeed, behavioral plasticity itself can be an important trait that varies among individuals (Nussey et al. 2007; Dingemanse 2010; Ensminger and Westneat 2012)

Ecological consequences of behavioral syndromes are easily characterized in predator-prey interactions. For example, when shoals of guppies, *Poecilia reticulata,* are exposed to a predator, individual fish with an active/bold/exploratory behavioral type survive longer than their shy shoal mates (Smith and Blumstein 2010). Further, prey from high predation environments often exhibit stronger correlations between behavioral traits (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007), and in some instances they display more bold, active, exploratory behavior than prey from low predation environments (Reale and Festa-Bianchet 2003; Brown et al. 2005; Archard and Braithwaite 2011). In contrast, in other systems, high predation environments are associated with reduced boldness, activity, and exploration (Riechert and Hedrick 1990; Hedrick and Riechert 1989; Biro et al. 2004). Although population level differences in behavioral syndromes between environments with different predator regimes imply predator-induced selection on behavioral types, experimental demonstrations that predators differentially consume particular behavioral types are comparatively rare (Bell and Sih 2007, Smith and Blumstein 2010; Pruitt et al. 2012b).

Individual variation in behavior is especially relevant in habitats that have been invaded by novel predators. Prey are severely impacted by invasion when they are not able to respond effectively to a novel predator (Courtenay and Moyle 1992; Rehage et al. 2005; 2009; Sih et al. 2010). The study of human induced rapid environmental change (HIREC) must begin to consider behavioral syndromes, because of the potential for behavioral syndromes to affect an animal's ability to respond rapidly to novel selection pressures (Sih 2013). The introduction of a novel predator can create a situation where prey must learn to recognize novel cues and/or adopt new escape strategies to respond successfully to the predator (Kelley and Magurran 2003; Rehage et al. 2009; Sih et al. 2012). Previous research suggests that bold fish may fare better in these circumstances, because they are more likely to perform predator inspections, allowing them to gather information about predation risk (Brosnan et al. 2003; Dugatkin and Alfieri 2003; Pellegrini et al. 2012).

Thus, there may be differences in the ability of prey of different behavioral types to gather information and develop behaviors to cope with a novel predator.

Here we examine behavioral syndromes in a prey species, the Largespring Mosquitofish (*Gambusia geiseri*), and how exposure to three different species of predators affect prey behavior and survival. We examine individuals' behavior in a novel tank environment, including latency to emerge, time spent moving, and area of the tank used, because these behaviors are relevant to a predation context, and because these behaviors have been used to examine correlations among boldness, activity, and exploration in many fish species (Bell et al. 2009; Wilson and Godin 2009; Smith and Blumstein 2010; Cote et al. 2010; 2011). Additionally, as mentioned above, risk-taking behavior and general activity have been linked with prey survivorship in a variety of predator-prey systems (Smith and Blumstein 2010; Pruitt et al. 2012b). Specifically, we test the following hypotheses: (1) *G. geiseri* exhibit correlated behaviors, (2) Behavioral syndromes in *G. geiseri* are consistent regardless of predator presence, (3) The behavioral type of an individual can predict survival during direct exposure to predators. We can then assess whether these different predator species could increase the prevalence of some prey behavioral types, either through plastic changes in individual behavior, or through differential consumption of certain behavioral types. Further, understanding the effects of novel predator species on prey within the context of intraspecific behavioral variation is increasingly important as human-altered habitats present new predation pressures that threaten the persistence of native prey species.

MATERIALS AND METHODS

Study species

Gambusia geiseri is endemic to the headwaters of the San Marcos and Comal Rivers in Central Texas, and is exposed to variety of predators in these habitats. The Green Sunfish (*Lepomis cyanellus*) is a native piscivorous predator in Central Texas (Hubbs et al. 1991), and thus shares an evolutionary history with *G. geiseri*. The Rio Grande Cichlid (*Herichthys cyanoguttatus*) is an invasive predator found in the San Marcos Spring. Originally native to the Rio Grande River and Northeastern Mexico, *H. cyanoguttatus* has spread through deliberate introductions by the U.S. Fish and Wildlife Service and accidental aquarium releases throughout Texas and the southern United States since 1928 (Hubbs et al. 1978). Invasive cichlids have been present in San Marcos Spring for several decades, and it is possible that rapid evolution of the prey species may already have occurred in response to this predator (Strauss et al. 2009). Therefore, we also included a novel, allopatric predator in this study, the Gulf killifish (*Fundulus grandis*). *Fundulus grandis* is native to fresh and brackish waters along the coasts of Northeastern Florida and the Gulf of Mexico. *Fundulus grandis* can tolerate a range of salinity and has been introduced into many freshwater environments in Texas and New Mexico through bait-bucket releases (Hubbs et al. 1991). In its current distribution *F. grandis* co-occurs with the Western mosquitofish (*Gambusia affinis*), but is novel to our focal species, *G. geiseri* (Hillis et al. 1980; Thomas et al. 2007).

Collection and laboratory maintenance

We used adult, wild-caught *G. geiseri* (17-31mm) collected with dip and seine nets from Spring Lake Marcos River, Hays County, TX (29-53'41" N, 097-55'49" W) and Comal Springs, Comal County, TX (29-42'37" N, 098-07'49" W) in September 2011, January 2012, and January 2013. We transported fish back to the laboratory in insulated five gallon buckets. We used only female fish for this study because their larger body size makes them easier to inject with elastomer tags, and because we found no difference in male and female behavior in *G. geiseri* in a similar study (Blake unpublished data). We began testing fish after they had acclimated to the lab for between 5 and 12 weeks in isolated female tanks. Once we began testing, we maintained mosquitofish in groups of four in 19L tanks on a 14:10h light cycle at 25-27C and fed flake food (Ocean Star International) *ad libitum* once a day. We collected sympatric *H. cyanoguttatus* and *L. cyanellus* from Spring Lake, and collected allopatric *F. grandis* from Galveston Bay, Galveston County, TX (29-12'42" N, 94-57'06" W). We used juveniles (5-8 cm SL) of *L. cyanellus* and *H. cyanoguttatus* because adults of these two species grow to be much larger than *F. grandis*, and we wanted to ensure that we used similar-sized predators for all treatments. We maintained three predators of each species in individual tanks on a 14:10h light cycle, and fed them pellet food (Purina Aqua Max 200) *ad libitum* once a day.

Experimental protocol

We tested the same fish throughout all three experiments, although control group fish were not a part of the final predation trials. We tested a total of 60 treatment group fish, and 70 control group fish. Some fish had to be eliminated from certain analyses due to missing data from video recording errors. At least one week before testing, we injected individuals with one of four colors of elastomer (Northwest Marine Technology, WA, USA). Mortality from elastomer tagging was less than 10% and generally occurred within the first 48 hours after injections. We housed fish in tanks with three other individuals of different elastomer colors to provide a natural shoaling environment, while still being able to distinguish individuals throughout the duration of all three experiments. We conducted initial experiments between February and June 2012, and tested additional control fish (50 of the total 70) in April-May 2013.

Experiment one: Behavioral correlations in *G. geiseri*

We conducted behavioral trials in a novel tank environment. The terminology and methodology of testing boldness, activity, and exploration varies throughout the literature on behavioral syndromes, and behavior in a novel environment is sometimes included in a wider definition of an exploratory syndrome (Reale et al. 2007). Here we follow the methods of Cote et al. (2010), but will use terminology specific to the variables measured in our behavioral assay rather than general trait terms. We tested fish in a shallow, white plastic tank (52x35cm). We filled the tank with 8cm of water, so that movement was primarily horizontal. Each of these tanks also contained a clear plastic enclosure (9x9x18cm) that remained empty for experiment one, but was placed in the tank to maintain a consistent set up between the first and second experiment. Water temperature ranged from 25-27C. We placed a mosquitofish in an opaque container and allowed the fish to acclimate for 5 minutes before we opened the door of the container. We calculated *latency to emerge* as the log of the maximum time allowed to exit (10 minutes) minus the log of the time to until the fish exited the container into the novel tank environment (Cote et al. 2010). Thus, a higher value for latency to emerge indicates that the fish entered the novel tank environment sooner. We calculated *time spent moving* as the proportion of the 5-minute observation period the individual spent moving, and *area used* as the proportion of the tank area explored (detailed below).

We recorded behavioral assays in EvoCam at 1 frame per second, using Dynex 1.3 megapixel webcams mounted above the experiment tanks. We processed videos as virtual stacks in Image J to obtain (*x,y*) coordinates of the individuals' position in the tank during each second of the observation period. We defined "movement" as any change in position greater than 1cm from the previous frame, and calculated time spent moving as the proportion of time during which movement occurred. We divided the tank area into 2x2 cm grid sections, and defined exploration as the proportion of these grids the fish swam through during the 5 minute trial. Although exploration and time spent moving are related metrics, it is possible for an individual to obtain a high value for time spent moving and a low value for area used by moving back and forth in a small area of the tank.

Experiment two: Behavioral correlations in the presence of predators

Using the same focal individuals, we performed a second behavioral assay with a predator present in the experimental tank. We randomly assigned the 60 treatment group fish to one of three predator treatments: 1) native Green Sunfish (*L. cyanellus*), 2) invasive Rio Grande Cichlid (*H. cyanoguttatus*), or 3) novel Gulf Killifish (*F. grandis*). We also retested the 70 control group fish, without any predators present, in order to measure repeatability of behavior between consistent trial conditions. During the behavioral assays of treatment group fish we placed the predator in the experimental tank inside a clear, water-permeable enclosure (9x9x18cm), which provided the focal individual with both visual and chemical predator cues during the trial. We changed the arrangement of the opaque acclimation container and predator enclosure inside the testing tank between the first and second experiment to maintain a similar degree of novelty of the tank during the second trial. We tested all individuals between 4 and 6 weeks after experiment one.

Experiment three: Behavioral correlations and predation

The final stage of the study was a one-on-one predation trial using the same focal individuals. The treatment group fish were exposed to the same predator treatment they had been assigned during the behavioral assays with predators present. We filled 189.3 L predation tanks (45x90x40cm) with 25cm of water, and added gravel substrate, two sponge filters, one artificial plant and one flower pot (15cm diameter) to provide hiding places and structure. To standardize hunger levels, we did not feed predators for 24 hours preceding testing, and used each predator individual only once per day. Before the start of each trial we placed a clear, water-permeable, Plexiglas divider in the middle of the tank, and placed one focal mosquitofish on the empty side of the tank to allow 5 minutes of acclimation before direct exposure to the predator. We began the trial by removing the barrier and allowed the predator individual 20 minutes to interact with the focal individual. We recorded exact survival time, and also recorded survival as a binomial variable based on whether the focal individual was still alive at the end of this 20 minute trial. A single consistent observer watched trials through a mirror to prevent the observer from affecting the behavior of the test subjects, and we recorded the vertical position (top third of the tank, middle, or bottom) of the focal fish every 30 seconds. We counted the number of escapes from predator attacks before the fish was either consumed or 20 minutes had passed. We defined an escape as any biting attack by the predator that did not result in the death of the focal individual. Predation trials took place between 4 and 6 weeks following experiment two.

Statistical analyses

We used Spearman rank correlations to determine if the measured behaviors were correlated, and Principal Components Analysis (PCA: on the correlation matrix) to calculate behavioral scores for individual fish. We used the random skewers method to compare the correlation matrices from the PCA of the first and second experiments (Cheverud et al. 1993). We calculated consistency of behavioral score between the first two experiments using Spearman rank correlations (Cote et al. 2010, 2011). We calculated repeatability of behavioral scores using a linear mixed-effects model method (Nakagawa et al. 2010). Because of the problems caused by missing values in this method, we only included individuals who exited the chamber within 10 minutes during both the first and second experiments ($N_{Treatment} = 46$, $N_{Control} = 53$). For the third experiment we used exact logistic regression to test if behavioral scores or individual behaviors affected the individual's likelihood of survival. Additionally, we used a Cox proportional hazards model to assess the effect of prey behavioral score on survival time (Cox 1972). Finally, we used a Generalized Linear Model (GLM) with a Poisson distribution to discern the fixed effects of behavioral scores and predator type on the number of escapes observed during the predation experiment. For the GLM we included only individual prey that were consumed during the predation trial. Analyses were conducted in R2.15.0.

Ethical note

We followed ASB/ABS (2012) guidelines in designing and conducting this experiment. We chose to include direct predator exposure for this study because we were interested in fitness consequences of behavioral syndromes that are specifically the result of predator-prey interactions. We allowed prey individuals to visually and chemically sense the presence of the predator through a water-permeable barrier during the five minute acclimation period, and provided several potential refuges within the predation tank. After the 20 minute trial individuals not consumed were immediately removed, returned to group tanks, and kept at the lab for the duration of their lives. Our care and use of the fish in these experiments was approved by the Institutional Animal Care and Use Committee of Texas State University (protocol number 1111_0907_09).

RESULTS

Experiment one: Behavioral correlations in *G. geiseri*

Latency to emerge, time spent moving, and area used were positively correlated (Table 1). Latency to emerge, time spent moving, and area used loaded positively onto the first principal component, which explained 73.49% (Table 2). Subsequent components explained 20% or less of the variance, and had eigenvalues less than 1. However, because latency to emerge also loaded highly on the second principal component, this suggests this behavior is not as strongly associated with the behavioral syndrome. Therefore we performed subsequent analyzes using latency to emerge as a single predictor in addition to our analyses using behavioral score (PC1) as a predictor.

Experiment two: Behavioral correlations in the presence of predators

The behavioral correlations observed in the absence of a predator (experiment one) between latency to emerge, time spent moving, and area used were also present when the fish were tested in the presence of predators (Table 2). There were no significant differences among predator treatments in mean latency to emerge ($F_{136,139}$=0.93*, P*=0.42)*,* time spent moving ($F_{133,136}$=1.56*, P*=0.20), or area used ($F_{133,136}$=1.24*, P*=0.30). There was also no mean-level difference between behavioral assays with and without predators for latency to emerge ($F_{138,139}$=0.14*, P*=0.71)*,* time spent moving ($F_{135,136}$=0.11*, P*=0.37), or area used ($F_{135,136}$=2.899*, P*=0.09). Further, the structure of the correlations matrices from the PCA of experiments one and two were very similar (Random skewers correlation=0.97). We used component scores from individuals' behavior with predators present (experiment two) to calculate a behavioral score for each individual as they were the most recent measure of the individual's behavior, and because results from the first two experiments were so similar. Individual behaviors were non-normal but the subsequent behavioral scores were normally distributed. Individuals were consistent and repeatable in their behavioral score across the two experiments for both the treatment and control groups (Table 3).

Experiment three: Behavioral correlations and predation

Predators consumed 39 of the 60 treatment fish (native sunfish: 12, invasive cichlid: 9, novel killifish: 18). Behavioral score was not a significant predictor of an individual's survival in the predation trial (χ^2 = 0.40, P = 0.53), and neither was standard length (χ^2 = 0.51, P = 0.48). Additionally, a Cox proportional hazards model showed no effect of behavioral score on survival time, though survival time did differ among the predator species (predator: χ^2=17.66, P=0.0001, predator*behavioral score: χ^2=2.88, P=0.23, behavioral score: χ^2=1.49, P=0.22). We also found that the single behavior latency to emerge was not a significant predictor of survival time (predator: χ^2=14.4, P=0.0007, predator*boldness: χ^2=0.97, P=0.61, boldness: χ^2=0.95, P=0.33). However, proportion of time an individual spent at the bottom of the tank during the predation trial was a significant predictor of survival (χ^2 = 12.74, P < 0.001). There was no significant interaction between predator treatment and time at the bottom on survival (χ^2 = 0.94, P = 0.63).

We also examined the relationship between behavioral score and the number of attacks escaped during the predation trial. The model that included behavioral score, predator type (fixed effects), and an interaction term was significant (χ^2 =17.9, df=5, P = 0.003). There was a significant interaction between predator type and behavioral type (Figure 1, χ^2 = 9.24, df=2, P=0.01). Individual parameter tests from the GLM showed that all terms were significant except for the interaction of behavioral score with the invasive cichlid predator (see Table 4). We also ran a GLM using latency to emerge as a single predictor and found a non-significant trend that was similar to our analyses using behavioral score as a predictor, with the interaction of boldness and predator type approaching significance (χ^2 =5.69, df=2, *P*=0.058).

DISCUSSION

Although much work has been done on behavioral syndromes (Sih et al. 2004b), surprisingly few studies have illustrated a direct relationship between variation in individuals' behavioral tendencies and their ability to escape predators (Bell and Sih 2007; Smith and Blumstein 2010; Pruitt et al. 2012b). Our findings show that latency to emerge, time spent moving, and area used are correlated behaviors in *G. geiseri,* across different situations (with and without predators present). Individual *G. geiseri* were also consistent in their behavior across situations. Further, we found that individuals with higher behavioral scores escaped more attacks from *F. grandis,* a novel predator species. Taken together our results suggest that some behavioral types in this population may have a greater ability to escape this novel predator.

Experiment one: Behavioral correlations in *G. geiseri*

Similar to previous studies on *Gambusia* (Cote et al. 2010; 2011), we found that latency to emerge, time spent moving, and area used are positively correlated in *G. geiseri* (Table 1). Whether this behavioral syndrome is a shared feature of the entire *Gambusia* genus remains an intriguing notion for future comparative studies. Latency to emerge also loaded highly on the second principal component in our analysis, suggesting some variance in this behavior is not explained by the behavioral syndrome. Further testing could explore other variables that affect individual's latency to emerge.

Experiment two: Behavioral correlations in the presence of predators

Individuals showed rank order consistency in behavior over time, and behavioral correlations were present regardless of predator presence (Table 2, 3). In addition to individual consistency, we also saw no mean level differences in behavior with and without predators. These results could indicate that subjects failed to perceive the predator as a threat; however, we do not think this is the case for several reasons. First, our preliminary work on predator recognition in *G. geiseri* shows that they recognize and move away from visual and chemical cues of *L. cyanellus* in a similar laboratory set up (Blake et al. unpublished data). Here, our experimental design focused only on latency to emerge, time spent moving, and area used, and our experiment was not designed to capture other antipredator responses such as distance from cues, location in the water column, or shoaling behavior (Smith and Belk 2001; Rehage et al. 2009). Thus a lack of change in the three behaviors we measured does not necessarily indicate a lack of predator recognition by our prey. Further, our findings agree with Rehage et al. (2005), who found *G. geiseri* did not reduce their activity in the presence of a predator. We conclude that the correlations we detected among latency to emerge, time spent moving, and area used constitute a behavioral syndrome that is consistent within individuals across different situations.

Our consistency and repeatability values are similar to earlier studies on latency to emerge, time spent moving, and area used in *G. affinis* over a period of several months (Cote 2011). Our findings also agree with Sih et al. (2003), who found behavior to be consistent within individual salamander larvae across predator situations. Our results showing consistency of behavior within individuals regardless of predator presence is contrary to the hypothesis that predators could induce a change in behavioral type through behavioral plasticity of individuals. Rather, our results are consistent with the hypothesis that *G. geiseri* exhibit behavioral syndromes that may limit their ability to change their behavior in the presence of predators.

Experiment three: Behavioral correlations and predation

We found that behavioral type influenced the number of predator attacks that a prey individual escaped. Individuals with high scores on the behavioral type axis exhibited more escapes during predation trials with our novel predator, *F. grandis*. Our analyzes using latency to emerge as a predictor showed a similar non-significant trend, with individuals who emerged sooner escaping more from *F. grandis.* In contrast to the results for *F. grandis*, individuals with *low* behavioral scores on the behavioral type axis escaped more attacks by *L. cyanellus* (Figure 1). Although the trend for prey exposed to *H. cyanoguttatus* predators was similar to that for *L. cyanellus*, we hesitate to make any conclusive claims about the relationship of behavioral score to escapes from *H. cyanoguttatus* due to fewer overall numbers of prey consumed by *H. cyanoguttatus* during the study. Nevertheless the finding that predator species influences the direction of the relationship between behavioral score and escapes supports our hypothesis that these different predators interact in distinct ways with prey behavioral syndromes.

Despite behavioral type affecting the number of attacks prey escaped from, we saw no effect of behavioral type on likelihood of survival of the trial or on survival time. Although our results may at first seem counter intuitive, behavioral type need not predict survival time in order for behavioral type to predict ability to escape. We argue that number of escapes is a better representation of the ability to evade predators than survival time, because survival time can be affected by many factors, including variation between trials in a predator's latency to detect or attack prey. Previous research has pointed out the shortcomings of measuring only survival time, and recording the number of attacks survived provides more information on the role of escape ability in predator-prey interactions (Smith and Blumstein 2010).

An alternative interpretation of our findings is that bold individuals were more likely to be attacked by *F. grandis* than shy individuals. It is possible that bold individuals may have had a higher encounter rate and/or approached predators sooner, and future studies could examine these variables. However, our finding that fish with high behavioral scores were able to escape more attacks still stands. We used only individuals that were eventually consumed in our final analysis, thus, a low number of escapes indicates that these individuals were consumed after only a few interactions with the predator, while individuals with a high number of escapes were able to successfully elude predators many times before being consumed. Thus, we interpret a positive relationship between behavioral score and number of escapes from *F. grandis* to mean that fish with higher behavioral scores were better able to escape when they were attacked.

Although the interaction of predator species and behavioral type of prey is clear in our findings, further work is needed to determine whether the degree of novelty of the predator is responsible for this effect, rather than differences in hunting strategy of the predators. We observed differences in behavior between the different predator types, including differences in latency to attack. The novel predator, *F. grandis,* was most likely to attack quickly, and was the most active even when prey were not present. In contrast, we observed that the native *L. cyanellus* and invasive *H. cyanoguttatus* spent more time hiding and performed more ambush attacks. *Lepomis cyanellus* is generally considered a sit-and wait predator, while cichlids, especially the genus *Hericthys,* are known for having a great degree of variety and plasticity in their foraging behaviors (Werner and Hall 1977, O' Swanson et al. 2003). *Fundulus grandis* is a top minnow, and although this species feeds throughout the water column on a variety of prey, *F. grandis* may be morphologically more adapted to feed at the water surface than centrarchid predators like *H. cyanoguttatus and L. cyanellus* (Rozas and Lasalle 1990). In fact, the feeding behavior and habitat use of *F. grandis* is somewhat similar to *G. geiseri,* which could increase the likelihood of *F. grandis* encountering and preying upon *G. geiseri.* Further investigation is needed to ascertain whether the effect of predator type in our results was due to the contrasting hunting behavior of the specific predator species used, or a generalizable effect in the novelty of the predator.

It is also possible that differences between individual predators of the same species may play a role in the predator-prey interactions in our study. Emerging research suggests that individual predators of different behavioral types may interact in distinct ways with individual prey behavioral types (McGhee et al. 2013). Because our experimental design focused on between-species differences, we did not collect information on behavioral or morphological differences between individual predators. Complex interactions between individual predator and prey behavioral types could have contributed to the lack of an effect of prey behavioral score on survival in our study. Our results add support to the hypothesis that differences between predator individuals or predator species could contribute to the continued existence of multiple behavioral types in a population (Smith and Blumstein 2010; Wolf and Weissing 2010; Pruitt et al. 2012b).

Proportion of time spent at the bottom of the predation tank was a significant predictor of survival, but the interaction between predator treatment and time at the bottom was not significant. This result suggests that the same behavior (water column position) was associated with survival regardless of predator species. We also noted that most fatal attacks occurred in the top zone of the water column, which reinforces our finding that remaining in the bottom zone is an effective strategy for *G. geiseri* to evade predation from the species we used in this experiment. Due to the constraints of filming trials from above, our behavioral assays in the first two experiments did not measure water column use, so it is unclear whether an individual's water column use is correlated to the other behaviors we measured. Due to the importance of water-column use in predicting survival of predators in our experiment, future work should explore whether this is a consistent behavioral trait for prey individuals.

To our knowledge, this is the first study to show that the likelihood an individual will escape a predator attack correlates with the behavioral type of these same individuals measured in another context. The existing literature on the fitness consequences of behavioral syndromes has focused largely on reproductive success, and although studies on fish have tended to find negative correlations between survival and boldness (Smith and Blumstein 2008), only a few studies have explored predation specifically as a mechanism for fitness consequences of behavioral types (Bell and Sih 2007; Smith and

Blumstein 2010; Pruitt et al. 2012b). The fact that the direction of the effect of behavioral score on escape from predator attacks depends on predator species suggests that multiple predators could select for the maintenance of several prey behavioral types. Further, our study shows the importance of integrating research on behavioral syndromes and invasion ecology. Our results show that novel predators may favor different behavioral types than native predators, which could have important implications for prey populations that experience invasion. Although behavioral type did not predict survival in one-on-one laboratory predation trials, in a natural environment surrounded by a shoal of conspecifics, ability to escape predators may have an effect on an individual's *relative* likelihood of survival. Further predation studies in more naturalistic settings are required to explore this possibility.

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Table III. 1

Spearman correlations for behaviors tested in behavioral assays without predators present ($N = 72$)

	Time spent moving	**Area Used**
Latency to emerge	**0.425**	**0.389**
	$P < 0.001$	$P < 0.001$
Time spent moving		**0.701**
		$P < 0.001$

Table III. 2

Principal components analysis of behavioral assays without predators, and with predators present (*N* = 72)

	Without Predators		Predators Present	
	Component Loading (PCI)	Component Loading (PCII)	Component Loading (PCI)	Component Loading (PCII)
Latency to emerge	0.495	0.865	0.508	0.845
Time spent moving	0.621	-0.291	0.594	-0.486
Area used	0.607	-0.410	0.624	-0.224
Variance Explained	73.49%	20.39%	79.48%	17.92%
Total Variance Explained		93.89%		97.40%

Table III. 3

Rank order consistency and repeatability of behavioral score measured in behavioral assays without predators, and with predators present ($N_{Treatment}$ = 46, $N_{Control}$ = 53)

Behavioral Score	**Rank order consistency**	**Repeatability**
Treatment Groups	$R_s = 0.402$, $P = 0.006$	$R_M = 0.324 \pm 0.134$ $P = 0.031$
Control Group	$R_s = 0.313$, $P = 0.009$	$R_M = 0.404 \pm 0.123$, $P = 0.010$

Behavioral score is from the first principal component representing latency to emerge, time spent moving, and area used. For the control group, no predator was present in either of the two trials. Consistency was calculated from Spearman rank correlations, and repeatability was calculated using a linear mixed-effects model.

Table III. 4

Effects of prey behavioral score on escapes from predator attacks

	Parameter Estimate	χ^2	***P***	**Lower CL**	**Upper CL**
Intercept	1.53	79.05	**<0.001**	1.27	1.75
Behavioral Score	-0.20	4.83	**0.03**	-0.42	-0.02
Predator (invasive *H. cyanoguttatus*)	-0.43	5.22	**0.02**	-0.86	-0.06
Predator (novel *F. grandis*)	0.33	6.44	**0.01**	0.07	0.61
Predator (invasive *H.cyanoguttatus*) x Behavioral Score	-0.30	3.53	0.06	-0.69	0.01
Predator (novel *F. grandis*) x Behavioral Score	0.31	9.24	**0.002**	0.11	0.54

Parameter estimates from a generalized linear model using a Poisson distribution, with fixed effects for behavioral score, predator type, and behavioral score*predator type ($N =$ 39).

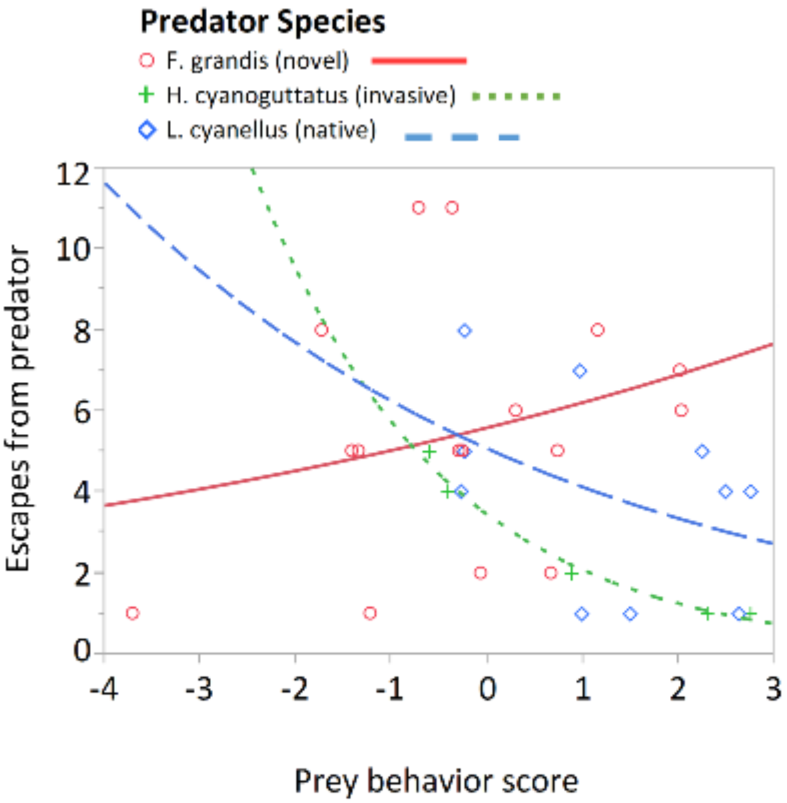

Figure III. 1

Relationship between behavioral score of prey individual and escapes from predator attacks, from a Generalized Linear Model using a Poisson distribution (*N* = 39).

CHAPTER IV

CONSPECIFIC BOLDNESS AND PREDATOR SPECIES DETERMINE CONSEQUENCES OF PREY PERSONALITY

Abstract

Prey personality can influence their predation risk in complex ways. We first tested the personality of individual common roach (*Rutilus rutilus*), a species that shows individually consistent difference in boldness. We then tested these roach in pairs with two predator species to ascertain how the behavior of each affected its survival. We found that the personality type of prey can affect their survival of predators in complex ways. When a paired bold and shy prey fish interacted with a perch predator, they were consumed in almost equal numbers. However, pike predators ate more shy fish, and prey body size and emergence time both contributed significantly to which prey fish was eaten. Our findings support the idea that multiple predators with differential selection on prey personality could help maintain variance in personality type in the prey population. Further, for social species like shoaling fish, the ultimate consequences of an individual's personality can depend upon the personality of its nearby conspecifics.

Introduction

Selection by predators can be a strong driver of evolution for a variety of prey traits, but is not always simply directional. Predators can in fact increase and maintain polymorphisms in prey species (Losey et al. 1997; Bond and Kamil 2002). Predators can maintain variation in the prey population via frequency dependent selection (Merilaita 2006), and through differences in selection by multiple predator types (Torres-Dowdall et al. 2014).

Polymorphisms within a population can include morphological or behavioral traits, or a combination of the two. Recently the study of polymorphic behavior within a population has been discussed in the framework of behavioral syndromes or personality (Wilson and McLaughlin 2007). Animal personality is characterized by individuals that consistently differ from their conspecifics in one or more important behavioral traits (Sih et al. 2004a; Reale et al. 2007). Individually distinct personalities or behavioral types have been found in many species, across many taxa, from birds (Dingemanse et al. 2003) to fish (Conrad et al. 2011) and even invertebrates (Briffa et al. 2008). Behavioral syndromes can impact(Wolf and Weissing 2012) predator-prey interactions (Quinn and Cresswell 2005; Bell and Sih 2007), social group selection (Pruitt and Riechert 2011), and sexual selection (Schuett et al. 2010). In particular, behavioral traits associated with risk taking, usually termed boldness, can be expressed in different individuals of the same species along a shy-bold continuum (Wilson et al. 1993; Ward et al. 2004; Brown et al.

2005). Boldness traits can correlate with other ecological processes such as invasion (Cote et al. 2010) and migration (Chapman et al. 2011).

Boldness as a personality trait is especially important to interactions between predator and prey. In fact, selection by predators can be a factor in the evolutionary origin and maintenance of bold behavioral syndromes (Bell and Stamps 2004; Bell and Sih 2007). Selection pressure can occur because the boldness of individual prey can affect their behavioral interactions with predators and survival of predator attacks (Smith and Blumstein 2010; Blake and Gabor 2014). As with other types of polymorphisms, predators could promote the maintenance of behavioral variance within a prey population. For example, multiple prey personality types could be maintained through selection by multiple predators of different hunting strategies that selectively consume prey of different behavioral types (Smith and Blumstein 2010; Pruitt et al. 2012; Wolf and Weissing 2012; Blake and Gabor 2014).

The current study builds upon previous research on personality and predation in the common roach, *Rutilus rutilis*. Roach are a freshwater cyprinid fish that display variation along the shy-bold continuum that is repeatable for individuals over time (Chapman et al. 2011). Further, bold roach are more likely to be eaten by cormorant (*Phalacrocorax carbo* sp.) predators (Hulthén 2014). In this experiment we examined whether the boldness of roach prey influenced their survival of predation by piscivorous fish in the lab. By exposing pairs of bold and shy roach to either pike (*Esox lucius*) or perch (*Perca fluviatilis*) predators, we were able to ascertain whether these two fish predators interact with prey behavioral type in the same way as each other, and as the previously researched cormorant predators (Hulthén 2014). Perch and pike predators could selectively consume prey of different behavioral types as they contrast in their hunting behavior and how they attack prey, with perch being a more active predator, and pike a sit-and-wait predator (Hart 1997; Turesson and Bronmark 2004). We hypothesized that differences among predators in their preference, or lack thereof, for consuming certain behavioral types of roach could contribute to the maintenance of behavioral variation in the prey population, and the persistence of both bold and shy variants.

Methods

Collection and maintenance

We collected juvenile roach, *Rutilus rutilus*, ($\overline{x}$±se,77 ± 12 mm in standard length (SL)) from Lake Krankesjön, a 3.4- km^2, shallow, macrophyte-rich lake in southern Sweden (55°41'54.0"N 13°28'02.9"E). We used electrofishing and dip nets to collect fish before annual migration in September and October of 2015, and transported them back to the lab at Lund University for testing. Fish acclimated to the lab for at least one week. Following acclimation, and three to five days before behavioral testing, we tagged fish to allow for repeated identification of individuals using surgically implanting passive integrated transponder-tag (PIT-tag) (Texas Instruments, TRPGR30ATGC, Plano, Texas, USA; 134 kHz, 12 mm long, 2.12 mm diameter). During acclimation and testing we housed fish in two 400l glass aquaria. In addition to the experimental individuals, we maintained a smaller 75l glass aquarium of 30 individuals of the same size class collected at the same time that served as novel shoalmates for sociability trials. We fed all roach frozen *Daphnia* once per day and maintained them on a 14:10h light cycle at 17°C.

Behavioral type assessments

We assessed behavioral traits of the roach (N=68) using both sociability and boldness assays. The terms and methods used in the study of animal personality throughout the literature, and behavior in a novel environment is sometimes included in a wider definition of an exploratory syndrome (Reale et al. 2007). Here we measured boldness using the well-established emergence test, and follow the methods and terminology used in previous research on this species (Chapman et al. 2011). We placed an individual into a closed PVC refuge box (28 × 20 × 20 cm) within a novel, circular PVC arena (60 cm diameter, 10cm depth of water). After a 20 min acclimation period, we remotely opened the door to the refuge box using a pulley system to allow the focal fish the opportunity to emerge into the arena. We recorded the time to emerge for each fish, with a ceiling value of 1200 s if the fish had not left after 20 min. We repeated this test twice for each individual, on two consecutive days, and shifted the orientation of the refuge box within the tank between tests to maintain a similar degree of novelty. We performed these tests in a temperature controlled room set to 17°C using aerated water that had been allowed to come to temperature overnight. We recorded all trials using USB webcams (Logitech, HD Pro C920, Lausanne, Switzerland) mounted above the tanks. We recorded emergence times from these videos, and determined the mean emergence across the two days for each individual. We conducted these trials between 800 and 1800 h in October and November of 2015.

Following the emergence test we also assayed sociability twice using a shoaling preference test. The shoaling test occurred in another circular PVC tank (65 cm diameter, 10cm depth of water), with a smaller clear cylindrical tank (15cm diameter, depth of water 10cm) affixed into the middle. We placed two novel conspecifics in the smaller central tank to act as a stimulus shoal, and placed the focal fish into the outer ring of the tank. We then monitored the amount of time the focal fish spent in shoal preference zone during a 15 min trial period. The shoal preference zone was defined as a circular region within 7cm of the central shoal enclosure, approximately one body length for the fish in our experiment. We recorded videos of all trials, and imported these as image stacks into ImageJ to yield one image per second. We used ImageJ to isolate the location of the focal fish in each frame, selected the shoal preference zone as the area to be analyzed, and calculated the number of frames the fish was found in the preference zone. This number equaled the number of seconds out of the 15 min trial that the focal fish spent shoaling, and we used this value to represent sociability. We conducted these trials between 800 and 1800 h in October and November of 2015.

Predation trials

After we tested prey twice for both emergence time and shoaling preference we matched prey individuals into pairs for predation trials. We matched prey considering both SL and emergence times so that prey would be of similar size but contrast in their behavioral type. All paired fish were within 15 mm in SL with a mean difference of 4.9±3.8 mm between paired individuals. Although emergence time, and boldness as a trait generally, vary continuously along a gradient of behavior within a population, for this experiment we paired fish using artificial behavioral categories so that one prey individual in the pair was distinctly "bold" relative to the other "shy" fish it was paired with. The two paired fish differed by at least 5 min in their emergence time, with a mean difference in emergence time between paired fish of 604±220 s. All fish that were classified as "shy" did not emerge from the refuge box at all during at least one of the two testing days. After we determined prey pairings, we randomly assigned each prey pair to one of the two predator treatment groups, perch (*Perca fluviatilis*) or pike (*Esox lucius*), resulting in 17 predations trials for each predator species.

We collected predators from Lake Krankesjön between May and September 2015 and all had acclimated to the lab for at least three weeks before testing began. Before and after testing predators were housed in groups of 6 individuals in large glass aquaria (600l) and fed a diet of frozen larval *Chironomid* and live fish prey (*Rutilus rutilus*). Pike predators were 200-300 mm in SL and perch predators were 180-250 mm in SL. During the course of the experiment, we housed the predators in the experimental tanks between trials to minimize disturbance. The tanks were circular, 75 cm in diameter and 115cm tall, filled with only 20 cm of water to prevent predators from jumping out of the tanks. To further encourage normal behavior, each predation tank contained three predator individuals during the trial. We used 6 different individuals of each predator species during the experiment, and predators were starved 3 days between trials to standardize hunger.

To begin the predation trial, we placed the paired fish in two acclimation chambers (10 cm diameter) that allowed prey to sense both visual and chemical cues from the predators, one fish per chamber. After the 5 min the acclimation period we lifted the acclimation chambers out of the tank to expose the prey directly to the predators. The predation tank contain a patch of artificial eel grass 20 cm x 30 cm in which fish could hide. During the first 6 hours following the introduction of the prey fish, we checked the tanks every hour to determine if the either of the prey fish had been eaten. We concluded the trial when the first fish was eaten and removed the uneaten prey individual. Most prey fish were eaten during the first 6 hour period of monitoring, but some trials continued overnight, and we checked these the following morning, approximately 20 h after the start of the trial.

Statistics

We first assessed consistency of behavior using Spearman rank correlation to test the consistency of boldness and sociability as personality traits for these prey individuals. We tested for a relationship between emergence time and body size using linear regression. For predation analyses the experimental unit was the prey pair (n=17 per predator treatment). Because the consumption rates of bold and shy fish were nearly equal for perch, we conducted analyses on the two predators separately to understand what factors may have influenced more consumption of shy fish than bold by pike. We also calculated hypothetical selection coefficients (s = 1- relative fitness) for the shy and bold phenotypes, treating each predator treatment group like a population. To analyze pike predation trials, we ran a logistic regression with the fish that was eaten as the response variable (Shy/Bold), and using the emergence time and SL of the shy and the bold fish in each pair, as well as an interaction term for the emergence time of the two prey as predictor variables. We also calculated relative difference in emergence time for each pair by subtracting the bold fish's emergence time from that of the shy fish and dividing by the emergence time of the shy fish. We calculated relative difference in body size similarly using the SL of the paired prey fish.

Results

We found that sociability was not consistent within individuals and hence we did not include this parameter in our further analyses (N=68, ρ =0.08, P=0.81). Emergence time was consistent within individuals over the two trials (N=68, ρ =0.37, P=0.001). There was no significant relationship of body size (SL) to emergence time ($F_{1,62}$=0.87, P=0.35).

During predation trials, predator species and prey behavioral type jointly influenced the outcome of the predator-prey interaction. There was a significant interaction between predator species and the behavioral score of the shy prey individual that affected whether the shy or bold prey fish was eaten (N=17, χ^2=7.09, P=0.007). Perch predators consumed shy and bold fish in almost equal numbers across the 17 prey pairs (Shy=8, Bold=9). Treating the whole treatment group as a hypothetical prey population, this would give a selection coefficient of s=0.113 against bold fish exposed to perch predators. The relative difference in emergence time did not vary between trials in which the shy prey was eaten and when the bold prey was eaten (Fig 1b).

In contrast, pike predators consumed more shy fish from the prey pairs (Shy=11, Bold=6). This yields a selection coefficient of s=0.453 against shy fish exposed to pike predation. A logistic regression of the pike predation data showed that there was a significant effect of the SL of the shy fish (N=17, χ^2=4.32, P=0.037), the SL of the bold fish (N=17, χ^2=8.08, P=0.004), and a significant interaction of the two prey fish's emergence time (N=17, χ^2=5.32, P=0.021), all affecting which of the two fish was eaten by the pike. When there was a larger relative difference in emergence times between the paired prey fish, the bold fish was more likely to be eaten by the pike (Fig 1a). The relative difference in SL between the two fish was also higher when bold fish were eaten by pike (Fig 2a).

Discussion

We found that the personality type of prey can affect their survival of predators in complex ways. Previous research on the common roach has shown that bold fish are more likely to be consumed by cormorants than shy fish (Hulthén 2014), but we have shown that this same effect does not necessarily carry over to other predators. When a paired bold and shy prey fish interacted with a perch predator, they were consumed in almost equal numbers. However, when we placed prey pairs with pike predators more shy fish were consumed, and prey body size and emergence time both contributed significantly to which prey fish was eaten.

First, we did not find any significant effects of prey personality or body size on which prey fish were consumed by the perch predators. This contrasts with previous work that showed cormorant predators ate more bold roach (Hulthén 2014), as well as with research on other species in which prey personality does have an effect on their survival when exposed to predators (Smith and Blumstein 2010; Pruitt et al. 2012). However, previous research has also suggested that predators may vary in how they interact with prey personality, both within (Pruitt et al. 2012) and across predator species (Blake and Gabor 2014). Thus a shy prey individual may be more likely than its conspecifics to be eaten by one predator, but more likely to escape from another predator. Our results here support the idea that variance among different predators in how they selectively consume particular prey types, could contribute to the evolutionary stability of multiple prey personality types (Schreiber et al. 2011; Wolf and Weissing 2012).

In addition, even a small difference in predators consuming one prey behavioral phenotype over another, could lead to evolutionarily significant selection coefficients. For example, although perch predation in this study was not statistically significantly affected by prey personality type, a selection coefficient of s=0.113 against bold prey would quickly lead to real evolutionary consequences in the wild. Although the present study design is a very small sample and not a true test of selection in a prey population, considering the evolutionary impacts of even small, statistically non-significant differences in predation rates of prey personality types is still a useful exercise. Further, the consequences of a larger and significant effect of prey personality type on pike predation could potentially lead to strong selection pressures in the wild, as illustrated by our calculation of s=0.453 against shy fish.

We found that when roach prey interacted with pike predators, their emergence time and body size jointly influenced whether the shy or bold fish was eaten. For pike predators, relative differences between the paired fish in both SL and emergence time were higher when the bold fish was eaten, as compared to when the shy fish was eaten. We found that the body size of both prey individuals influenced the outcome of the pike predation trials. This coincides with previous research showing that predators are highly attuned to prey body size, but that their preference may change depending on the context of the prey population (Sogard 1997; Gliwicz et al. 2010; Rodgers et al. 2015).

Previous studies have mostly examined how the personality of a single prey individual influences its survival, but social context may be particularly important to the study of personality in social animals like shoaling fish. The expression of personality can be influenced by the social context of the focal individual, and social context may also influence the ultimate impact of personality (Webster and Ward 2011; Keiser et al. 2014; Wey et al. 2015). If the behavioral types of two or more shoalmates interact to influence individual survival rates, this supports the idea that predator selection on prey personality may be context and/or density dependent. This finding also raises the question of whether a prey individual could decrease its predation risk by spending time in proximity of conspecifics with a certain behavioral type. There is already evidence that fish exhibit shoaling preferences for conspecifics with certain characteristics (Krause et al. 2000; Cote et al. 2012), and further research should explore how shoaling preferences could influence the impact of personality on individual predation rates.

The influence of personality on predation risk could also have wider ecological implications for this species. The common roach exhibits partial migration behavior, in which some individuals within a population migrate annually and some do not. In this species partial migration is influenced by trade-offs between predation and food availability that prompts 50-80% of fish to migrate from high predation lake habitats to adjoining streams each winter (Bronmark et al. 2008; Skov et al. 2010). In addition, the likelihood an individual will migrate can be affected by its body condition and its behavioral type (Brodersen et al. 2008; Chapman et al. 2011). Bold fish are more likely to migrate, while shy fish are more likely to remain resident in lake habitats (Chapman et al. 2011). If bold and shy fish also differ in their susceptibility to multiple predators, this could influence each individual's costs and benefits of migrating away from the predator-rich lake habitat. However, the relative risk from each predator species is variable, and this could further contribute to the dynamic nature of partial migration for this species, especially as increasingly numerous cormorant populations spread into roach habitat (Steffens 2010).

In conclusion, we have shown that the ultimate consequences of prey personality can depend on the personality of nearby conspecifics. Further, the effect of prey personality on survival can vary by species of predator encountered. The complex and dynamic nature of the selective pressures on prey personality could in fact contribute to the origin and maintenance of this behavioral variation.

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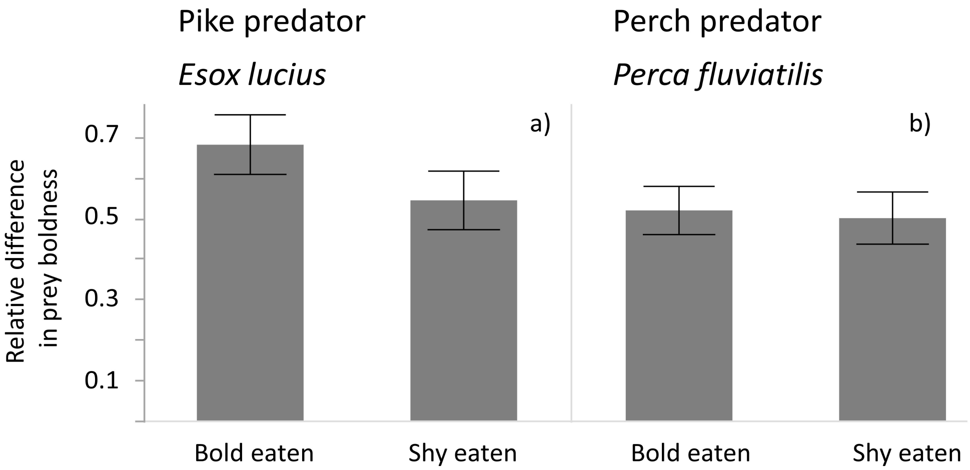

Figure IV. 1

Relative difference in boldness (emergence time) of paired prey when either the shy or the bold fish was eaten by one of two predator species, pike (*Esox Lucius*), or perch (*Perca fluviatilis*).

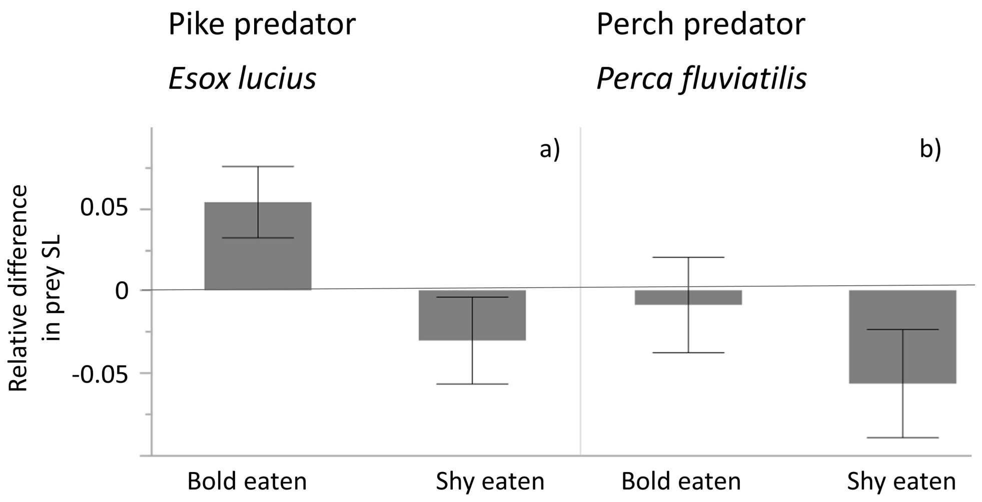

Figure IV. 2

Relative difference in standard length (SL) of paired prey when either the shy or the bold fish was eaten by one of two predator species, pike (*Esox Lucius*), or perch (*Perca fluviatilis*).

CHAPTER V

BEHAVIOR-MORPHOLOGY ASSOCIATIONS IN TWO SPECIES OF MOSQUITOFISH

ABSTRACT

Questions: Do individual personality traits correlate with individual morphological traits, as might be expected if correlational selection favors particular trait combination or if the traits share pleiotropic effects? If morphology-personality correlations arise via correlational selection from predators (e.g. risky behaviors associated with anti-predator morphologies), then does a generalist species coexisting with predators show stronger correlations than a specialist species experiencing strongly reduced predation pressure?

Methods: We examined the relationship between personality (boldness, activity, exploration) and morphology (body size and shape) within two sympatric species of mosquitofish from Comal springs and river in New Braunfels, Texas. *Gambusia affinis* is a widespread generalist species, while *Gambusia geiseri* is a spring-adapted endemic.

Conclusions: We found no significant correlation between personality and body shape for either species. This finding does not support predator-driven correlated evolution of morphology and animal personality. For both species we found that body size had an allometric relationship with body shape.

INTRODUCTION

Inter-individual variation within species can reveal important insights about the evolution of phenotypic diversity. Two major categories of traits have received considerable attention in this regard: behavior and morphology. Yet, we currently have a poor understanding of their potentially joint evolution as integrated traits. Two particular aspects of each trait category that we might especially expect to exhibit individual-level associations owing to their potential for functional co-dependence are animal personality traits, such as boldness, and antipredator morphologies, such as body forms that enhance escape performance.

Behavioral ecologists refer to consistent differences in individual behavioral traits as behavioral syndromes or personality. Personality traits are partially heritable (Dingemanse et al. 2009a), and important in many aspects of ecology (Sih et al. 2012). Personality is particularly important in predator-prey relationships, correlating with predation levels at the population level (Biro et al. 2004; Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007), as well as affecting the nature and outcome of direct behavioral interactions among predator and prey individuals (Dugatkin and Alfieri 2003; Smith and Blumstein 2010; Pruitt et al. 2012; Sih et al. 2012; Blake and Gabor 2014).

Analogously, inter-individual variation in morphology within a species can play a significant role in the ecology and evolution of organisms. For example, selection by predators promotes morphology related to escape ability (O'Steen et al. 2002; Langerhans et al. 2004; Langerhans 2007). For prey fish, body depth and length of the caudal region is directly related to performance of fast-start swimming maneuvers used in escaping predators (Langerhans et al. 2004; Langerhans 2009b).

Several studies in ecology and other fields have examined links between individual physical and behavioral characteristics. For example, the proactive-reactive behavioral axis has been linked to physiological stress responses, as well as different life stages (Wingfield 2003; Benard 2004). Other studies have found that bold, active individuals tend to have higher foraging and metabolic rates (Biro and Stamps 2008; Biro and Stamps 2010; Careau et al. 2010; Careau and Garland 2012). Antipredator morphology and behavior have been linked for several species of snails (DeWitt et al. 1999; Rundle and Bronmark 2001). Additionally, bold behavior and morphology related to escape ability, can both be selected for by predators (O'Steen et al. 2002; Langerhans et al. 2004; Bell and Sih 2007).

Connecting inter-individual variance in behavioral and morphological traits can advance our understanding of underlying genetic and physiological pathways that give rise to these traits. There are several ways for correlations between behavior and morphology to arise, in particular between antipredator behavior and antipredator morphology. One possibility is that individuals make behavioral decisions based on awareness of their physical state (Luttbeg and Sih 2010). Behavioral syndromes have sometimes been linked to the idea of trait compensation; bold individuals exhibit riskier behavior because they are physically superior in escaping predation than physically less-protected, shy individuals (DeWitt et al. 1999; Luttbeg and Sih 2010). Trait compensation and trait co-specialization are usually defined as relationships between physical and behavioral traits that are mechanically independent (Rundle and Bronmark 2001). However, when links between behavioral and physical traits are advantageous, it is also possible for correlations among physical and behavioral traits to become mechanistically linked through correlational selection that could favor shared or linked genetic or developmental processes.

In this study we explore the relationship between behavior and antipredator morphology. We examined the relationship between body shape and behavior in two species of mosquitofish, one a spring-adapted endemic and one a widespread generalist. Boldness, exploration, and activity are repeatable personality traits for these species (Cote et al. 2010; Blake and Gabor 2014). We examine whether boldness correlates with morphological indicators of predator-escape ability. If morphological and behavioral traits of individuals are correlated, these traits may be linked via correlational selection for complimentary traits, and/or through pleiotropic developmental cascades. If a correlation is observed, we predict that the link between behavior and morphology would be stronger in the generalist species, because they inhabit environments with more predators, and likely experience stronger selection on antipredator traits.

Study species

Gambusia geiseri is endemic to the San Marcos and Comal springs in Central Texas, USA. Its congener, *Gambusia affinis* is a widespread species, its native range stretching from Alabama to some parts of New Mexico, south into Mexico, and as far north as Illinois, and has also become invasive in other introduced habitats. In central Texas, *Gambusia affinis* and *G. geiseri* are sympatric in parts of their native ranges, although *G.*

geiseri always dominates in spring headwaters and does not usually extend very far downstream. The downstream areas where *G. affinis* dominates harbor much higher densities of piscivorous fish predators than the upstream spring habitats. Both species are small (<65mm), mostly insectivorous, and live-bearing.

Collection and laboratory maintenance

We used adult, wild-caught *G. geiseri* (17-31mm) collected with seine nets from Comal Springs, Comal County, TX (29-42'47" N, 098-08'13" W) in January 2013. We collected *G. affinis* from the Comal River at a nearby site downstream from the headwaters (29-42'15" N, 098-07'49" W) in February 2014 (140 fish for each species). We transported fish back to the laboratory in insulated five gallon buckets. We used only female fish for this study because they are more numerous at our collection sites and their larger body size makes them easier to inject with elastomer tags for individual marking (see below). We allowed fish to acclimate to laboratory conditions for between 5 and 12 weeks. Once we began testing, we maintained mosquitofish in groups of twenty in 38L tanks on a 14:10h light cycle at 25-27C and fed flake food (Ocean Star International) *ad libitum* once a day.

Experimental protocol

The terminology and methodology of testing boldness, activity, and exploration varies throughout the literature on behavioral syndromes, and behavior in a novel environment is sometimes included in a wider definition of an exploratory syndrome (Reale et al. 2007). Here we follow the methods used in previous work on *Gambusia* sp. (Cote et al. 2010; Blake and Gabor 2014). At least one week before testing, we injected individuals with an individualized tag using up to two colors of elastomer (Northwest Marine Technology, WA, USA). Mortality from elastomer tagging was less than 1.0% and generally occurred within the first 24 hours after injections. We conducted a single behavioral assay to measure all three behaviors in a shallow, opaque plastic tank (52 × 35 cm) with cameras mounted above the tanks to film each trial. We filled the tank with 8 cm of water, so that movement was primarily horizontal, and water temperature ranged from 25-27°C. We placed a mosquitofish in an opaque container (9 × 9 × 18 cm) and allowed the fish to acclimate for 5 minutes before we opened the door of the container, allowing the subject the opportunity to emerge into the larger plastic tank. We calculated boldness as the log of the quotient of the time it took the fish to exit over the maximum time allowed to exit (10 minutes) the container into the novel tank environment. Thus, a higher value for *boldness* indicates that the fish entered the novel tank environment sooner. We calculated *activity* as the proportion of the 5-minute observation period the individual spent moving, and *exploration* as the proportion of the tank area explored (see (Blake and Gabor 2014) for more detailed methods).

Following behavioral assays we photographed each individual by placing the fish in a small Plexiglas tank (60 × 20 × 40 mm) just large enough to house the fish while restricting movement. Using *tpsDig* (Rohlf and Slice 1990) we digitized eleven morphological landmarks, using the same observer for all photos (Fig. 1). We also recorded standard length (SL) as the distance from the snout to the end of the caudal peduncle.

Although we tested the behavior of 140 fish for each species, some fish did not emerge from the container during the behavioral trials. We used multiple regression to test for differences in shape between those that emerged and those that didn't for each species. Following this we did analyses on the whole data set for boldness and shape, and separate analyses on boldness, activity, and exploration only on fish for which we had complete data, giving a final n=80 for each species. We used *tpsRelw* to perform a generalized Procrustes Analysis. This analysis produced relative warps, which are principal components of the covariance matrix of partial warp scores, and were computed using the unit centroid size as the alignment scaling method. We then used *tpsPLS* to perform a partial least squares analysis to test for covariance among shape and behavior variables. For the partial least squares analysis we used only 80 individuals from each species due to missing behavioral data from individuals that did not emerge from the container during the emergence test. The PLS examined covariance of a matrix of all shape variables (relative warps from the 11 landmarks) and a matrix of all behavioral variables (boldness, activity, and exploration) (Rohlf and Corti 2000). In addition, we explored the potential for allometric relationships by including centroid size in multivariate regression models. A power analysis (α=0.05, p=0.8) confirmed that our sample size of 140 was large enough to detect correlations above r=0.11. The regression analysis was performed in in R 3.0.2.

Results

Gambusia geiseri

There was no significant difference in body shape between fish that emerged during the behavioral assay and those that did not (n=140, F=0.44, P= 0.98). For the 80 fish used in the PLS analysis, the singular values for the covariance of first two pairs of shape and behavior variables were 0.003 and 0.003. There was not a significant correlation between the first pair of PLS scores (shape vs. behavior), or the second pair (n=80, r=0.18, *P*=0.992, r=0.33, *P*=0.12). There was also no significant correlation of just boldness alone with shape (n=140, r=0.24, *P*=0.79). Thus, behavioral traits were not correlated with body shape for *G. geiseri* (Fig 2). There was no significant interaction between centroid size and behavior on the body shape, though body shape did vary based on centroid size alone (Table 3).

Gambusia affinis

There was no significant difference in body shape between fish that emerged during the behavioral assay and those that did not (n=140, *F*=1.02, *P*=0.44). For the 80 fish used in the PLS analysis, the singular values for the first two pairs of variables were 0.006 and 0.003. There was not a significant correlation between the first pair of PLS scores (shape vs. behavior), or the second pair (n=80, r=0.27, *P*=0.67, r=0.33, *P*=0.15). There was also no significant correlation of just boldness alone with shape (n=140, r=0.22, *P*=0.15). Thus behavioral traits were not correlated with body shape for *G. affinis* (Fig 3). There was no significant interaction between centroid size and behavior on the body shape, though body shape did vary based on centroid size alone (Table 3).

Discussion

We found no significant correlation between behavior and body shape for either species of *Gambusia*. This lack of correlation does not support a co-evolutionary relationship between morphology and behavior in these populations. In a scenario where a link between behavioral and morphological traits is advantageous for fitness, one might expect these two traits to become linked through selection. However we found no correlation, which does not add any support for this co-evolutionary hypothesis. Alternately, it is possible that the physiological or genetic mechanisms behind the expression or development of these behavioral and morphological traits constrain them from being positively linked even if it were advantageous (Ketterson and Nolan 1999; Stirling et al. 2002). Additionally, it may be more likely to detect a correlation between behavioral traits and performance of escape maneuvers, especially if the correlation arose through pleiotropy or gene linkage, rather than a correlation of behavior with body morphology arising from a subtle linkage disequilibrium (Kern et al. 2016).

In addition we saw no difference in the relationship of behavior to body shape between the specialist and the generalist species, despite their different level of exposure to predation. In addition, both populations exhibit behavioral correlations among boldness, activity, and exploration, and previous work shows these correlations are usually stronger when predators are present (Bell 2005; Bell and Sih 2007). In fact due to spreading ranges of introduced piscivorous fish into the spring in recent years, there may be less difference in the predation regime between the habitats used by these two species than there was historically (Hubbs et al. 1978).

We also examined relationships between body size, behavior and body shape. We saw no significant interaction of behavior and body size on the shape of either species. Relationships between body size and behavior have been found in some studies (Sinn and Moltschaniwskyj 2005), but not in others (Wilson et al. 2010). We did find that body shape varied significantly by body size, suggesting allometric changes in shape dimensions across different body sizes (Table 3). Allometric changes in body shape across different body sizes have been observed in many animals including other fish species (Wainwright et al. 2002; Shingleton et al. 2007; Albert and Johnson 2012).

In conclusion, we did not find a correlation that would support coevolution of behavioral and morphological traits. However, our results do not rule out the possibility that a correlation between body shape and behavior could occur through plastically, especially in organisms with inducible antipredator morphology (Domenici et al. 2008). Further research is need in this area to discern when and why behavioral and physically traits of individuals may be adaptively, mechanistically, or functionally related.

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Table V. 1 Loadings of behavioral variables for *G.geiseri* from PLS analysis.

Behavioral variable	**PLS 1**	**PLS 2**
Boldness	0.3938	0.6182
Activity	0.3253	- 0.5985
Exploration	0.8597	- 0.5096
Variance Explained	76.73	14.32
Cumulative variance explained	76.73	91.05

Table V. 2 Loadings of behavioral variables for *G.affinis* from PLS analysis

Behavioral variables	**PLS 1**	**PLS 2**
Boldness	0.120	0.989
Activity	0.749	- 0.030
Exploration	0.651	- 0.147
Variance explained	55.11	38.37
Cumulative variance explained	55.11	93.48

Table V. 3 Results of a multivariate regression analysis testing the effects of behavior on all body shape variables with body size (Centroid) as a covariate (n=80 for each species).

	Gambusia geiseri		***Gambusia affinis***	
	F	*P*	*F*	*P*
Boldness	1.38	0.17	1.02	0.46
Activity	1.10	0.37	0.65	0.85
Exploration	0.89	0.59	0.67	0.84
Centroid	5.14	<0.001	9.31	<0.001
Boldness*Centroid	1.00	0.47	0.98	0.50
Activity*Centroid	0.77	0.75	0.65	0.86
Exploration*Centroid	1.04	0.44	1.06	0.41

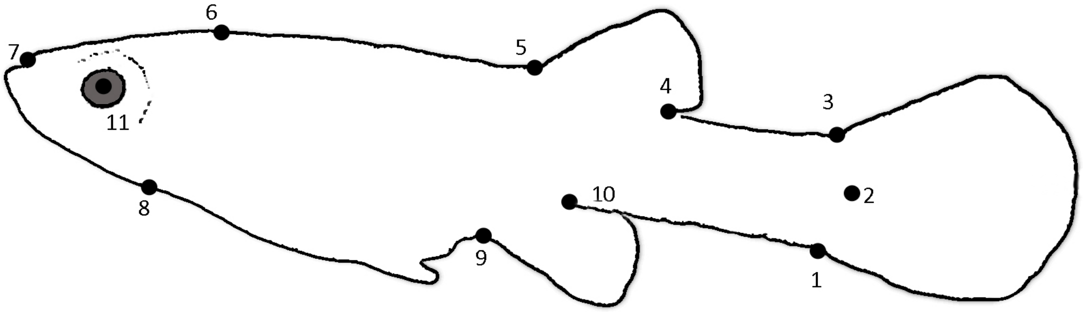

Fig V. 1: Morphology landmarks
Locations of the 11 landmarks digitized in tpsDig.

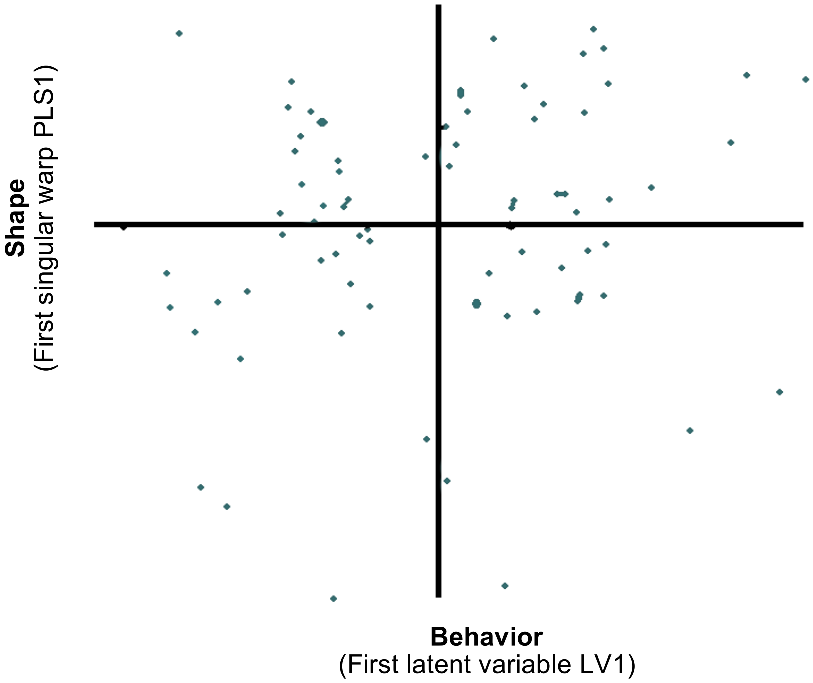

Fig V. 2: Shape and behavior, *G. geiseri*
There was no correlation between shape and behavior vectors from a PLS analysis (N=140, r=0.18, *P*=0.992).

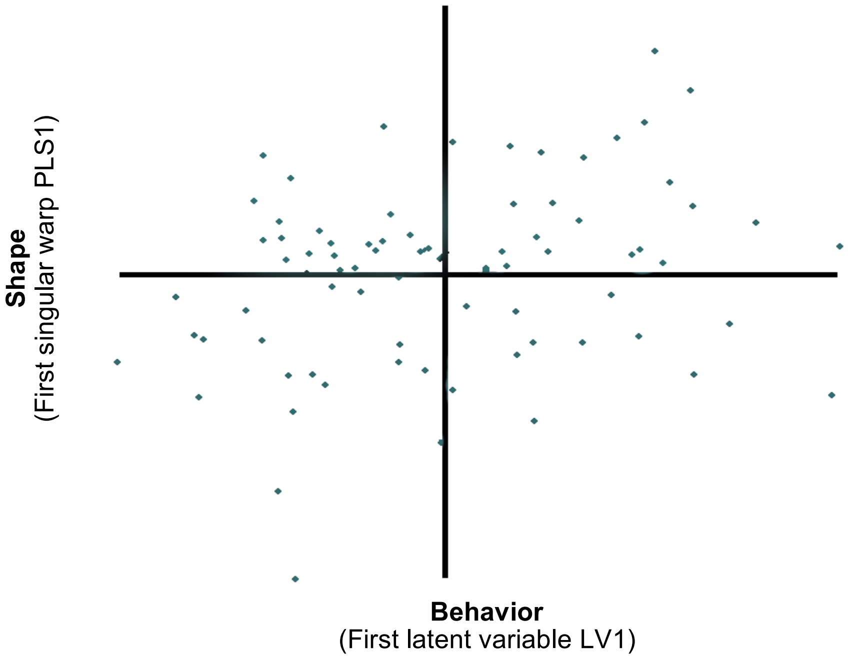

Fig V. 3: Shape and behavior, *G. affinis*
There is no correlation between shape and behavior vectors from a PLS analysis (N=140, r=0.27, *P*=0.67).

CHAPTER VI

CONDITION-DEPENDENT MODIFICATION OF BOLDNESS

Animal personality is defined by consistent behaviour of individuals, but even animals that exhibit distinct personalities show some degree of intra-individual variation. Examining the source of this intra-individual variation is integral to understanding the mechanisms and adaptive significance of personality. In this study I clip the fins of individual fish to temporarily compromise their physical condition, and test whether bold personality traits can be condition-dependent. Untreated individuals in this and previous studies of *Gambusia geiseri* show consistency and repeatability of personality traits including boldness, defined as an individual's latency to exit a refuge into an unknown environment. However, individuals with reduced physical condition via clipped caudal fins were not repeatable in their boldness. Further, the direction of change in an individual's boldness following caudal fin clipping treatment varied widely, suggesting individually-specific responses to the condition change. My findings provide insight into the source of intra-individual variation in personality, and suggest a more nuanced view of the origins and the ecological consequences of animal personality.

Keywords

antipredator morphology; behavioural syndromes; boldness; condition-dependence

1. Introduction

Consistent individual differences in behaviour, termed animal personalities or behavioural syndromes, have important impacts on the behavioural ecology of many organisms across diverse taxa (Sih et al. 2004a). Consistency of behaviour within an individual is a defining aspect of personality, yet individual animals with identifiable personality traits are not completely and indefinitely consistent. Single behaviours and/or correlations among behaviours can range in their stability over ontogeny (Bell and Stamps 2004), and relative plasticity of behaviour itself can be a unique characteristic of individuals (Nussey et al. 2007; Dingemanse et al. 2010). At the same time, personalities often have a repeatable, heritable component (Bell et al. 2009; Dingemanse et al. 2009b). Personality traits like boldness can lead to important fitness consequences, like higher foraging rates or greater exposure to predators (Biro et al. 2004; Dingemanse et al. 2009b). Thus personality occupies a subtle evolutionary space, being responsive to selection as well as allowing for plasticity within an individual. Understanding the shared roles of genes, environment, and plasticity is increasingly key to advancing the study of personality (Dingemanse et al. 2012; Herczeg and Garamszegi 2012). In this light, examining the source of intra-individual variation is an important part of understanding the mechanisms and adaptive significance of personality.

One likely source of intra-individual behavioural variation is condition-dependence, in which individuals modify their behaviour based on their current physical condition (Wolf et al. 2007; Luttbeg and Sih 2010). Behavioural decisions based on variable physical parameters like body temperature can affect antipredator behaviour

(Martin and Lopez 1999; Heithaus et al. 2007). Further, individuals in poor condition are often more willing to forage under high predation risk, and this can impact their distribution within a habitat (Martin and Lopez 1999; Heithaus et al. 2007). Thus there is evidence that risk-taking behaviour can be condition-dependent. In personality research risk-taking behaviour is often termed boldness, and the shy-bold continuum is perhaps the most studied personality axis in animals (Sih et al. 2003b; Ioannou et al. 2008).

In this experiment I explore the hypothesis that bold-shy personalities can be modified based on current physical condition. I use fin clipping to experimentally create a condition change that reduces swimming ability of fish. In the wild, fin condition can be compromised by either attacks from predators or diseases that cause fin rot. Damage to the caudal fin can be especially limiting, as fish use fast-start swimming to escape predators, and performance of fast-starts and survival of predators is directly related to the shape and length of the caudal region of prey fish (Langerhans et al. 2004; Langerhans 2009b). Thus experimentally altering the condition of the caudal fin represents an increase in potential vulnerability, and a lowered condition for that individual. By examining changes in boldness following this condition change I can determine what role condition-dependent behaviour has in explaining intra-individual variation in personality traits.

The Largespring mosquitofish (*Gambusia geiseri*) exhibits consistent individual differences in behaviour that are correlated together in the form of a behavioural syndrome (Blake and Gabor 2014). Thus, in the present study I predicted control fish without any change to their physical condition would exhibit consistent boldness upon repeated testing. In contrast, I hypothesized that individuals with reduced physical condition via fin clipping would respond with changes in boldness following treatment. If boldness of treated fish changes after experimentally reducing condition, this would provide support for the hypothesis that condition-dependent behaviour can be a source of variation within individual personality. Reducing boldness following caudal fin clipping would likely be the most adaptive response to compromised swimming ability and increased vulnerability to predation. Inability to modify boldness following fin clipping could be maladaptive, as bold individuals who maintain bold risk-taking behaviour following this condition change would be at increased risk of predation from their lowered swimming ability.

2. Materials and methods

2.1 Study species and laboratory maintenance

Largespring mosquitofish (*G. geiseri*) are endemic to the San Marcos and Comal Springs in Central Texas (Hubbs 1991; Thomas et al. 2007). *Gambusia geiseri* exhibit individually consistent and repeatable behavioural correlations for activity, exploration and boldness (Blake and Gabor 2014). I used adult *G. geiseri* (17-31mm) collected with seine nets from Comal Springs, Comal County, TX (29°42'47" N, 098°08'13" W) in January 2013. During 5 weeks of acclimation to the laboratory and between testing, I maintained mosquitofish in groups of twenty in 38L tanks on a 14:10h light cycle at 25-27°C and fed flake food (Ocean Star International) *ad libitum* between 1630h - 1730h each day.

2.2 Experimental protocol

One week before testing, I injected individuals with elastomer tags (Northwest Marine Technology, WA, USA). Mortality from elastomer tagging was less than 1.0%

and occurred within the first 24 hours after injections. I tested 140 *G. geiseri* in May-June 2013 and all testing was conducted between 0800 h – 1500 h. I conducted boldness assays in a shallow, opaque plastic tank (52x35 cm). I filled the tank with 8cm of water, so that movement was primarily horizontal, and water temperature ranged from 25-27 C. I placed a mosquitofish in an opaque container (9x9x18 cm) and allowed the fish to acclimate for 5 min before I opened the door of the container. I calculated boldness as the log of the proportion of maximum time allowed to exit (10 minutes) over the time to when the fish exited an opaque container into a novel tank environment (Cote et al. 2010; Blake and Gabor 2014). Thus, a higher value indicates that the fish entered the novel tank environment sooner (bold) and lower values indicated that the fish took longer to enter the tank (shy).

After initial behavioural assays I divided these fish into one of three treatment groups: (1) anal fin clip, (2) caudal fin clip, or (3) control. I randomly assigned 40 fish each to the anal fin and caudal fin clipping group, with one individual misplaced before testing began, giving an actual sample size of n=41 for the caudal fin clip group, and leaving n=59 for the control group. For fin clipping treatments I removed approximately 20% of the fin using a razor blade sanitized with ethanol. The caudal fin provides thrust for fast-start swimming, while the anal fin does not contribute to swimming power (Webb 1977; Webb 1982; Careau and Garland 2012). Thus caudal fin clipping compromises condition by reducing swimming ability, while the anal fin clipping group controls for any negative health impacts of cutting fin tissue, without reducing swimming ability. Fish can regrow fin tissue in as little as two weeks, so behavioural assays were timed to allow for recovery from stress of handling but avoid fin regrowth. Control group fish were also handled and returned to their tanks for consistency among treatments. Three to four days following treatment, I retested behaviour of all individuals using the same boldness assays above.

2.3 Ethical note

Clipping of fish fins is routinely used as a humane, non-destructive method for obtaining DNA from fish, and has also been used in the past to study functional morphology of fish fins (Webb 1977; Webb 1982; Wasko et al. 2003). There was no mortality from the fin clipping procedure in this experiment, fish with clipped fins were able to perform normal foraging and shoaling behaviours, and the lifespan of the subjects in the laboratory following the regrowth of their fins was not reduced. Fin clipping exposes subjects to minimal harm, and creates the most accurate approximation of reduced condition that would occur via predation attempts or fin disease in the wild. The research described in this manuscript was conducted following the Guide for the care and use of laboratory animals (NIH 2011) and was approved under IACUC protocol 0404-0424-05.

2.4 Statistics

Boldness was calculated as the log of the proportion of maximum time allowed to exit over the emergence time to improve normality. I calculated repeatability of behaviour and estimated variance components for within and between individual variance using a restricted maximum likelihood model (Nakagawa and Schielzeth 2010; Biro and Stamps 2015). Because the direction of behavioural change following treatment varied among individual fish, I conducted analysis using the absolute change. I calculated absolute change in boldness as the absolute value of post-treatment boldness minus initial boldness for each individual. I used ANOVA to assess differences in absolute change in boldness among treatment groups and a Levene’s test for unequal variance among treatment group. I also used a Levene’s test to confirm that variance in boldness before caudal fin treatment and variance in boldness after treatment were not significantly different. I conducted analyses in JMP Pro 11.

3. Results

Boldness was significantly repeatable across the two tests for the control and anal fin clip groups, but not for the caudal fin clip group (Table 1). While anal fin clip and control group fish did not significantly change their behaviour over repeated testing, fish with caudal fins clipped showed a high amount of within fish variance, reflecting that they changed their behaviour following the treatment. The direction of change varied, with some individual fish increasing their boldness and some decreasing their boldness following caudal fin clipping. Fish with caudal fins clipped showed overall higher absolute changes in their boldness, and also exhibited significantly higher variance in their behavioural changes than control and anal fin clip groups (Figure 1).

4. Discussion and conclusions

My findings show that boldness can be condition-dependent, and further that responses to condition changes can vary in their direction among individual fish. I demonstrated that a change in fin condition can eliminate repeatability in a species that otherwise exhibits consistent personality traits. Notably, only clipping of the caudal fin, produced a condition-dependent behavioural change, while fish with their anal fin clipped exhibited repeatability in their boldness before and after treatment. In fish, the length of the caudal region is directly related to performance of fast-start swimming manoeuvres and survival of predators (Langerhans et al. 2004; Langerhans 2009b). In the present study, change in boldness occurred after modification of the primary fin used in antipredator manoeuvres, but not as a general response to injury of any fin. This suggests that the caudal fin's role in fast-start swimming ability was of significance in eliciting the observed behavioural response. My findings that physical traits associated with predator-escape ability are related to boldness at the individual level compliments previous research that has pointed to predation as a driving force in shaping the evolution of boldness in prey at the population level (Bell and Stamps 2004; Biro et al. 2004; Bell and Sih 2007; Dingemanse et al. 2007).

The change in boldness elicited by caudal fin clipping coincides with previous work showing that risk-taking behaviour can be dependent on current condition (Martin and Lopez 1999; Heithaus et al. 2007; Briffa and Twyman 2011; Langard et al. 2014). Individuals may modify their predator-avoidance behaviour when their low condition also decreases their potential escape performance. Prey with lower health condition may respond to their increased vulnerability by increasing the length of approach distance or sensitivity of their antipredator response (Martin et al. 2006). Major predators of *G. geiseri* are mostly piscivorous fish, and fast-start swimming is an important escape manoeuvre in evading attack from these larger fish (Langerhans 2009a; Blake et al. 2014; Blake and Gabor 2014). Thus it is possible that the condition-dependent behaviour observed in the present study was a reaction to caudal fin clipping increasing vulnerability to potential predation. However, my results also showed that some individuals behaved counterintuitively in response to reduced condition by increasing their boldness, potentially further increasing their vulnerability to predators.

While the demonstration of condition-dependent behaviour is not unique, the present study complicates the relationship between physical condition and behaviour by empirically demonstrating that not all individuals will respond to a condition change in the same way. It is possible that neither increased boldness nor decreased boldness is uniformly beneficial to all individuals following a condition change. Future research should explore in more depth the initial boldness and consistency of individuals with multiple tests before, and multiple tests after a condition change to better understand how initial behavioural type or boldness could affect the direction of the behavioural response to reduced condition.

Further, the observed high variance in the direction of behavioural changes in response to reduced condition could be consistent with an asset-protection model, in which individuals with high assets behave cautiously, and individuals with low assets behave with a “nothing-left-to-lose” strategy (Wolf et al. 2007; Luttbeg and Sih 2010). For example, if some fish began this experiment with lower physical condition (fewer assets) than others, a further reduction in condition could bring those individuals below a threshold that requires more risk taking to ensure survival. In fish with higher initial assets, their clipped caudal fin may represent only a temporary reduction in swimming ability (until their fin grows back), and the most adaptive response for these fish could be to wait to perform risky behaviour until they return to a higher physical condition. Similarly, variance in initial swimming ability before fin clipping treatments may also affect the most adaptive response of each individual to a reduction in swimming ability. Future studies could explore how initial differences in condition or swimming ability and between-individual variation in initial condition could influence the effects of reducing condition on individuals' willingness to take risks.

Many researchers have posited that personality and morphological/physiological characteristics could have underlying correlations due to genetic linkages or physiological cascades (Biro and Stamps 2008; Biro and Stamps 2010; Careau and Garland 2012). However, my findings suggest that one must consider whether correlations between physical traits and personality could arise through condition-dependent behaviour alone, before claiming underlying genetic linkages as an explanation for correlations between physical and personality traits. Further, if boldness is condition-dependent, future research should further explore how boldness could serve as an honest indicator to potential predators or competitors of an individual's ability (Smith and Blumstein 2010; Fabre et al. 2014). For instance, if boldness is consistently associated with superior escape performance, behaving boldly around predators may help discourage would-be predators before they attack (Fitzgibbon and Fanshawe 1988; Godin and Davis 1995).

In summary, I have showed that boldness can be condition-dependent, and that condition-dependent responses vary wildly in strength and direction among individuals. It is important to note that although my findings suggest a more nuanced view of behavioural syndromes, condition-dependence is not mutually exclusive with heritable personality. However, it can be elucidating for behavioural syndromes studies to more explicitly partition variance in behaviour into fixed genetic effects caused by gene pleiotropy, linkage dis-equilibrium or maternal effects (between-individual variance), and variance resulting from plasticity and environmental effects (within-individual variance) (Dingemanse et al. 2012). My results and those of others raise the question of whether endogenous behavioural characteristics, or the specific circumstances of a current situation are the stronger driver in the moment-to-moment behaviour of an individual, a puzzle with a long history in human psychology research as well (Fleeson 2001; Funder 2001). Taken together, these findings suggest that individual responses to situational changes can complicate the nature and interpretation of personality. This, in turn, could have notable effects for our understanding of how individual-level variation impacts the ecology and evolution of behaviour.

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Tables

Table VI.1 Variance components and repeatability (REML) of boldness measured once before and once after treatment.

	Anal Fin Clip (N=40)	**Caudal Fin Clip** (N=41)	**Control** (N=59)
Between individuals	31.8%	3.5%	23.2%
Within individual	68.3%	96.5%	76.8%
Repeatability	$R^2=0.48$, $P<0.001$	$R^2=0.07$, $P<0.001$	$R^2=0.38$, $P<0.001$

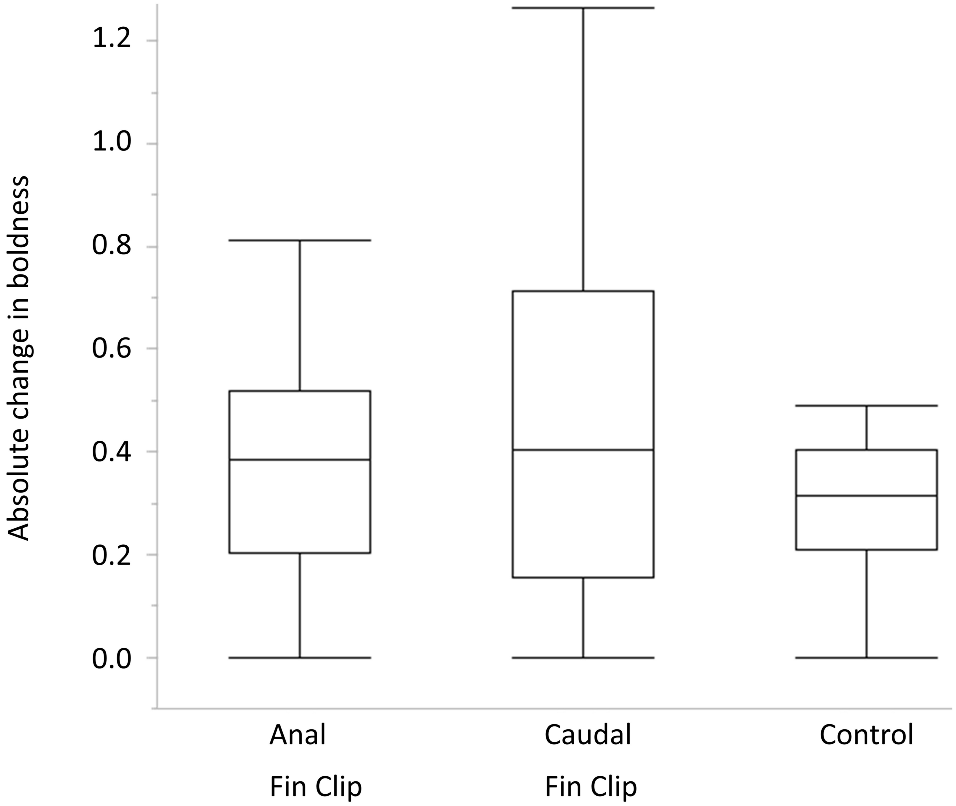

Fig. VI. 1 Absolute change in boldness is the absolute value of the difference between an individual's post-treatment boldness minus their initial pre-treatment boldness. Absolute change in boldness differed in both mean and variance among groups (ANOVA: $F_{2,119}$ = 3.89, $P = 0.023$. Levene's: $F_{2,119}$ = 13.16, $P < 0.0001$)

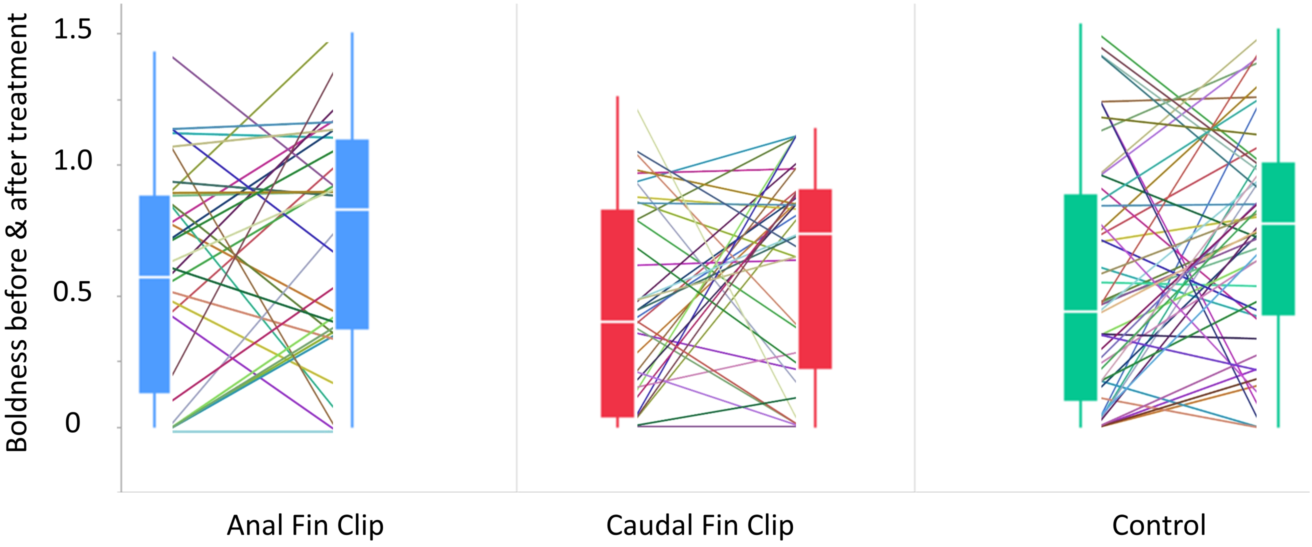

Fig. VI. 2 Boldness before and after treatment for each treatment group, with outlier box plots and reaction norms for each individual fish.

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