Environmental influences of behavior in two *Gambusia* species: public information use and behavioral consistency across ecological and evolutionary time scales.

DISSERTATION

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Abstract

Animals must gather information about the location and quality of resources while simultaneously using information to avoid predation. Individuals may then use the information they gather about the relative state of their environment to modify behavior in ways that increase fitness (e.g. by using the presence of foraging conspecifics to locate food, or by using the behavior of wary conspecifics to identify predation risk). Information may be gathered directly by the individual (private information) or by observing others (public information). The ecological factors that influence how animals use private and public information remain central questions to behavioral ecologists. In particular, recent work suggests that individuals often consistently differ from one another across contexts in behavioral traits and that these ‘personality’ differences may influence how they use information. In turn, differences in information about the environment may contribute to individual variation in behavior. For my dissertation, I examine how differences in predation risk affect how two species of mosquitofish (*Gambusia affinis* and *G hubbsi*) a) use private and public information, b) change consistency of behavior in response to differences in environmental context, and c) have behaviorally responded to divergence in ecological context across an evolutionary time scale. In chapter 2, I examine how the age of available environmental information and individual state (mass) alter how organisms value socially acquired information. Individuals did not value newer information over older information, but larger individuals were more likely to change foraging behavior after gaining public information about the location of food. In chapter 3, I found that high predation risk results in greater use of public information, even when the costs of acquiring private information about the environment are similar across environments. I suggest that, in high risk environments, individuals gain additional information such as the safety of food sources, by observing others. In chapter 4, I explore how inherent differences in behavior within a population (animal personalities) shape how the individuals use and gather environmental information. In contrast with my predictions, I found that individual differences in behavior only influenced learning. In chapter 5, I test how differences in predation risk influence consistency of behavior. I found that behavioral consistency (i.e. the repeatability of behavior) increased when predation risk was high, because of a combination of greater differences between individuals and lower variation within individuals. Finally, in chapter 6 I extended this finding to examine how long-term differences in ecological context shape individual variation in behavior over evolutionary time by using replicate populations of *G. hubbsi* that have evolved under high and low predation regimes. I did not find evidence of behavioral canalization in high risk populations, but I did find context-dependent behavioral consistency, similar to earlier work (chapter 5). Taken together, my results indicate that ecological context (specifically predation risk) influences public information use as well as behavioral consistency. Individuals behave more consistently and utilize available public information more when predation risk is high. This could have implications for both the cultural transmission of traits as well as long-term behavioral evolution.

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Fields of Study

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Chapter 1: Introduction

Throughout their lives, animals must gather resources while simultaneously avoiding predation. In order to increase their chances of successfully doing this, organisms gather information about their environment. Individuals may use the information to make decisions about resources such as mates (Witte and Massmann, 2002), territory quality (Redmond et al 2009) and foraging (Leadbeater and Chittka 2008). Individuals might then use the information they gather about the relative state of their environment to modify behavior in ways that increase fitness (e.g. by using the presence of foraging conspecifics to find food, or by using the behavior of wary conspecifics to identify the presence of predators) (e.g. Thorpe 1961). Additionally, recent work suggests that individuals often consistently differ from one another across environmental contexts in behavioral traits, and these 'personality' differences may influence how they use information (Trompf and Brown 2014). In turn, information about the environment may contribute to individual variation in behavior (Lindstedt chapter 5). Over evolutionary time, selection may likewise favor behavioral phenotypes that trade off the benefits of behavioral flexibility with the risks of costly mistakes (Edgall et al 2009). Taken together, the relationship among gathering information about the environment, behavioral modification in response to changes in environmental conditions, and the eventual evolution of behavior in response to differences in ecological context are all tightly linked. For my dissertation work, I explored how ecological context interacts with the acquisition and use of information about food (chapter 2) and risk (chapters 3-4), among- and within-individual variation in behavior in response to increased predation risk (chapters 4-5), and how a prolonged ecological context of predation ultimately leads to the evolution of behavior (chapter 6). I used 2 fish species in the genus *Gambusia (Gambusia affinis* and *Gambusia hubbsi)* to examine the interrelationships among environmental information, behavioral variation and behavioral evolution.

In chapter 2, I examine how the relative reliability of available public information (specifically, how old it is) relates to how individuals use it to make decisions. Previous work has demonstrated that individuals exploit the most reliable information available when two types of information conflict (Van Bergen et al 2004). In order to test this, I set up a conflict between previously acquired private information about a food source, and then provided individuals with conflicting public information that varied with respect to its age (i.e. some public information was recent and some public information was older). I predicted that individuals would rely on their previously acquired private information to make foraging decisions and forgo the available public information only when it was relatively old, and therefore outdated. I found that the age of the information provided did not seem to influence the foraging decisions of the individuals in my study. However, I did determine that the mass of the individuals influenced their use of public information with heavier individuals using public information more to make foraging decisions than lighter individuals. It is possible that smaller individuals may be using public information to identify the highest density of individuals (e.g. Koops and Abrahams 1999) in order to avoid conspecific aggression.

In chapter 3, I further explore the effects of environment on public information use by examining whether an increase in environmental risk (i.e. predation risk) influences whether an individual uses available public information to make foraging decisions. Extensive theory suggests that individuals should utilize public information when the costs of acquiring private information increase (Boyd and Richerson 1988). Additionally, there is some empirical evidence that animals may use public information more when the costs of obtaining private information increase (Webster and Laland 2008). In chapter 3, I examine whether individuals would still utilize public information in riskier habitats even when the costs of obtaining private information were equal. I tested the "concurrent information hypothesis" in which individuals gain information about one aspect of the environment (e.g. predation risk) by paying attention to conspecifics for a potentially different reason (e.g. to gain information of food location) In order to test this, I set up a conflict (similar to chapter 2) between previously acquired private information about foraging quality and conflicting public information. Importantly, individuals acquired all private information about the location of food under similar high costs (in non-vegetated tanks) prior to testing. This was done in order to control for any costs of obtaining private information at the time of testing: all individuals had already paid equal costs of gaining private information prior to being exposed to public information. During testing, I modified the predation risk of the experimental tank by altering the aquarium vegetation to either be highly vegetated (low risk) or non-vegetated (high risk). I found that individuals used public information more in the non-vegetated trials than in the vegetated trials, despite having similar private information costs for both risk treatment. This is preliminary support for the “concurrent information hypothesis”.

In chapter 4, I examine how inherent, consistent among-individual differences in behavior (i.e. “animal personalities”) contribute to differences in public information use. There is evidence that differences in sociality may lead to differences in public information use (Trompf and Brown 2014). Here, I examine how among-individual differences in sociality and exploration contribute (if at all) to variation in public information use. I presented individuals with the same paradigm as in chapters 2 and 3 in which previously acquired public information conflicted with available public information about the location of food. Additionally, I examined how individuals in my population varied in sociality (defined as the time spent near a social group) and exploration (defined as the time taken to explore a novel tank). I then examined how these differences in among-individual behavior correlated with differences in public information use. I predicted that more social individuals would use public information relatively more than asocial individuals. Likewise, I predicted that more exploratory individuals would rely more on private information to make foraging decisions. Despite my predictions, I did not find a correlation among any of the personality traits I measured and public information use.

In chapter 5, I examine how inherent among- and within- individual variation in behavior interacts with differences in environmental context and leads to behavioral modification. I use a behavioral reaction norm (BRN) framework (Dingemanse et al 2009) in order to investigate how the consistency of behavior varies with environmental context, specifically predation risk. I examined how the consistency of three behavioral traits (sociality, exploration, boldness) would vary in response to an increase in environmental risk. I predicted that behavioral variance due to error (e) would decrease in the non-vegetated treatments, because the costs associated with making behavioral “mistakes” would be greater in high predation risk environments. I found that behavioral consistency increased in the non-vegetated treatments, but examination of the variance contributions revealed that error only decreased for one trait (sociality). Among-individual variance (I) increased for the other significantly consistent trait (exploration). I suggest that this may be a result of selection favoring high behavioral variation within a population.

Finally, in chapter 6 I examine how behavioral consistency varies with differences in selection. Theory suggests that strong, prolonged stabilizing selection should lead to the canalization of traits (Waddington 1942). Canalization occurs when traits become buffered against environmental or genetic change. Here, I examine whether populations that vary in their strength of selection exhibit differences in the canalization of three behavioral traits. Since canalization buffers traits against environmental change, I measure canalization by measuring the consistency of the traits across different environmental contexts. I measured behavioral consistency among 4 different populations of Bahamas mosquitofish (*Gambusia hubbsi*) from 4 different blue holes that varied in their predation regime (2 populations were predator-present and 2 population were predator-absent). I predicted that individuals from the high-predation populations would exhibit behavioral consistency across 2 different environmental contexts (vegetated and non-vegetated) as a result of behavioral canalization. I measured three behavioral traits (sociality, boldness, activity) across these 2 predation regimes for the low and high predation individuals. Despite my predictions, I did not find evidence that behavioral canalization had occurred in the high predation populations. I suggest that this may be due to disruptive or directional selection which would preclude the evolution of canalization. Likewise, this may be due to a lowering of among-individual variance (I) which might indicate population-wide expression of an ‘optimal’ behavioral trait value for high predation populations.

Relatively little is known about how animals gather and use public information. Here I tease apart how the relative influences of attention paid to conspecifics and costs of gathering personal information may lead to public information use. Likewise, there has been almost no work on how environment influences behavioral consistency despite the recent eruption of animal personality work. My work is among the first to directly examine how the environment affects behavioral consistency. Finally, though there has long been interest in trait canalization among evolutionary biologists, my work is among the first attempts to document behavioral canalization in the wild. Together, these various experiments directly examine how the environment can influence behavior on both an ecological and evolutionary time scale.

Chapter 2: Variation in social information use: the influences of information reliability and mass on decision making in a group-living fish *Gambusia affinis*

Abstract

The effect of the reliability of available social information was assessed by examining whether the age of social information changes its effects on a foraging decision in a group-living fish *Gambusia affinis*. Individuals switched their patch preference when faced with social information that conflicted with personal information in general (p=0.045); however, the age of the social information did not significantly influence preference for feeding patch (p=0.097). The mass of decision-makers was positively correlated to their use of available social information, with heavier individuals exhibiting a greater difference in patch preference than lighter individuals (p=0.033), suggesting that large and small fish trade-off the benefits of information acquisition and the costs of competition from conspecifics differently.

Introduction

Animals can gather information about the location of resources or risks in several ways. Individuals may directly sample the environment and make decisions based on this personal experience (gather “personal information”), or they can observe the decisions made by others and adjust their behaviour according to these decisions (gather "social information") (Danchin et al 2004). Previous work has demonstrated that when personal information can be obtained at low cost, it is typically preferred over social information when the two types conflict (Nordell and Valone, 1998; Van Bergen et al 2004, Jonker et al 2010 (but see Mery et al 2009). However, as the costs associated with gathering personal information increase, individuals often switch their preference to exploit available social information instead (Day et al, 2004; Kendel et al 2004, Webster and Laland 2008, Campobello and Sealy 2011). The potential costs associated with using personal information are varied and include factors such as potential exposure to predation risk, as well as increased acquisition time leading to missed forging opportunities (Boyd and Richerson, 1985, 1988); using social information however may mitigate some of these costs by allowing faster and more efficient decision-making (Pitcher et al 1982, Valone 1989) or can minimize an individual's direct exposure to predation while still allowing for information gathering (Coolen et al 2003)

Additionally, under some conditions social information may be more accurate than personal information (Ward et al 2011, Morand-Ferron and Quinn 2011, Bouliner et al 2008, Canonge et al 2011). This may occur for several reasons, including when the social information is produced by older, more experienced individuals (Dugatkin and Godin 1993), or when the available social information is more current (i.e. up-to-date) than the personal information. Van Bergen et al (2004) found that nine-spined sticklebacks *Pungitius pungitius* (L.) relied more on social information and less on personal information, as the elapsed time since their last personal experience with a food patch increased.   In frequently changing environments, information that is older may be less useful to potential information gatherers than newer information. In addition, gathering social information in general may be favored when environmental variability is relatively high, as individuals can acquire relevant information without bearing the costs of direct interaction with the environment (Boyd and Richerson, 1985). Thus, if accurate personal information can be acquired at low cost it may be beneficial to an individual to simply ignore available social information (Templeton & Giraldeau 1996); however, social information should become increasingly used as the costs of gathering and using personal information increase, particularly when environmental predictability is low. Additionally, older information may be less beneficial to use, since it may be outdated when an environment changes often. So in this way, individuals should assess the reliability of information by considering both its source (social vs personal) as well as its age (newer or older), to determine its usefulness.

Western mosquitofish *Gambusia affinis* Baird and Girard 1853 inhabit a wide range of variable environments where food patch reliability is likely to be unpredictable and temporally variable as *G. affinis* typically feed on prey that either falls onto the surface of the water or is itself freely moving in the environment (reviewed in Pyke 2005). Therefore, *G. affinis* is a good system to examine how the age of information may change the benefits associated with using it. The objective of this study was to test the hypothesis that individuals use currently available social information differently than older social information, and to test the prediction that, when personal and social information about the location of food are in conflict, currently available social information will have a greater effect on foraging location than will older social information.

Methods

This experiment was conducted using captive-bred female western mosquitofish (n=36) in 38 L tanks. Both training and experimental tanks were covered with a different substrate type on each half of the tank floor, a small sized (approximately 2-3 mm) black and white gravel on the left side, and a larger (approximately 0.5-1 cm) earth-toned gravel (i.e tan and brown) on the right side. Prior to testing, individuals were fed once daily for two weeks exclusively over one of the two substrate types (individuals were randomly assigned to one of the substrate types). A total of four fish were housed in each of two training tanks with a total of eight fish being tested in each of four total experimental periods. Following this training period, all trained individuals were tested in the experimental tank in order to establish whether a preference was developed and would carry over to a new tank. All fish were marked with unique caudal fin clips in order to keep track of individuals over the course of the experiment. Additionally, mass was measured for all experimental individuals.

Experimental Tanks were approximately 38 L and had substrate type and placement which was identical to the training tanks. Two social groups of three individuals were present in the experimental tank for all trials. The individuals in the social groups were selected to be approximately the same size as the focal individuals. These social groups were confined to two transparent cylinders on either side of the tank (see figure 1) During trials, focal individuals were first placed in a transparent enclosure (approximately 13 cm x 9 cm) for 2 minutes prior to starting the trial for an acclimation period. Following the acclimation period, a single brine shrimp was dropped over both substrate types, the enclosure was lifted, and focal individuals were allowed to forage freely in the tank. For all trials, the three female conspecifics in each of the holding cylinders remained enclosed. In all cases, the time spent over each of the two substrate types was measured, and the trial was stopped after three minutes of foraging. All individuals were exposed to the "no social information" trial first (see below) then fish were randomly assigned to either the "older" or "current" social information treatments (see below). Experimental individuals were food deprived for 24 hours prior to testing to standardise hunger levels.

No social information: Individuals were first tested with two social groups present in the experimental tank, neither of which was fed during the trial. This treatment was used to establish the patch preference of the focal individual after they had been trained, and is based only on past personal information. To avoid changes in 'baseline' preference resulting from exposure to socially acquired information, this trial was always performed first.

Fish were returned to their training tank following the baseline preference measurement. Original patch preference was reinforced over the next five days by feeding individuals exclusively over their original substrate type. This was done to reduce any effects on feeding preferences that may have been established by the trial itself. After the five day period, individuals were once again tested in the experimental tank. In all trials, the social groups were always present.

Current social information: For this treatment, the social group was fed during the trial while the focal fish was foraging (figure 1, A). This treatment was considered "current social information". In order to generate a conflict of information between that which the individual has gathered personally and the available social information, only the social group that was located over the substrate type *not* preferred by the focal fish was fed. For example, if the focal fish had been trained to feed over black-and-white gravel, the social group over the neutrally coloured gravel was fed.

Older social information: For this treatment, the social group was fed and allowed to finish eating and resume normal swimming behaviour within their cylinders before the focal individual was released from the holding compartment and allowed to forage (figure 1, B). Since the holding compartment was transparent, the focal individual was able to observe the behaviour of the social groups as they foraged within their cylinders. Since the food had already been consumed by the time the focal individual began foraging, this information was older (and more likely to be outdated) in regards to the location of food. The focal individual was then allowed to forage freely and again the proportion of time spent over each substrate type was calculated for the 3 minute trial. The social groups were fed at the beginning of the acclimation period and had mostly finished eating by the end of the two-minute acclimation such that focal individuals were confined to the holding compartment for a similar amount of time regardless of treatment.

Differences among the treatments and between the two groups were calculated using a repeated-measures ANOVA. The between-subject factors was the age of the information (old or current), and the within-treatment factor was the presence (or absence) of conflicting social information (again either “current” or “older” depending on the experimental group). Mass was also included as a covariate. For analyses, only individuals who spent more than 55% of their time over their trained substrate during the “no social information” trials were used as this was interpreted to mean that the individuals had established a preference for a substrate type (n=23). All proportions were arcsin square-root transformed prior to analysis. Since all individuals were tested only twice (with no social information and with conflicting information) we did not control for order-effects in our analysis.

Results

Across treatments, individuals altered their patch use when they had been exposed to either current or older social information when this social information conflicted with personal experience (RM ANOVA: $F_{1,22}=4.52$, $p = 0.045$). Both the new and old social information influenced patch preferences roughly equally (figure 2) (RM ANOVA: $F_{1,22}=3$, $p = 0.097$). Mass had a significant effect on how individual patch use changed with social information, with larger individuals altering their patch preference more than smaller individuals (RM ANOVA: $F_{1,22}=5.14$, $p = 0.033$; figure 3)

Discussion

Individuals in this experiment changed their feeding-patch preferences when conflicting social information about food availability was presented. Individuals increased their use of the patch where the social group was fed, despite their pre-existing preference for the opposite patch. However, despite the predictions that individuals should use old and new social information differently, a significant difference between the effects of the two information types on foraging decisions was not observed; indeed, a greater change in foraging location when older information was presented was observed. These results suggest that individuals were either unable to perceive a difference between older and current information, or that the net benefits of using older and current information for foraging decisions did not differ. It seems unlikely that individuals were unable to perceive differences between information types, however, they changed their substrate preferences based on the social information available despite their previously established patch preference. This indicates that they likely perceived it as more informative. It seems more likely therefore, that mosquitofish do not value the old and new information that was presented in this experiment differently. Perhaps in this experiment, the reliability difference of the information (approximately 3-5 minutes) was not enough to change how informative it was in regards to food availability.

The mosquitofish responded differently to available social information depending on their size, with smaller individuals changing their preference less than larger individuals. In fact, many of the smallest individuals spent more time over their trained patch when conflicting social information was available than when no social information was available at all (see figure 3). We suggest that this relationship may be the result of the effects of size on competitive ability in interference competition; larger individuals may be more successful at displacing smaller individuals from food, and therefore are more willing to enter resource patches that are already in use from conspecifics. A positive relationship between body size and successful resource competition has been observed across taxa including invertebrates (Menge 1972), amphibians (Mathis 1990), reptiles (Schuett 1997), fish (Sabo and Pauly, 1997), and mammals (Tannerfeldt et al 2002). Smaller individuals may be more likely to incur costs associated with interacting with larger individuals, either because of inferior feeding abilities (Schoener 1983, Young 2004), or possibly because they are often the recipients of increased aggression in size heterogeneous populations, a pattern observed regularly in fish (Sakakura and Tsukamoto 1996, Sakakura and Tsukamoto 2002, Papandroulakis et al 2005, Moran 2007).

When social information is in the form of local enhancement (where the information about the location of a resource is indicated by the presence of conspecifics that are also exploiting the resource), the foraging activity of others provides information about the location of food as well as the location and number of competitors. Our results suggest that how individuals use this information may differ with characteristics that influence competitive ability, such as size. Thus, the use of personal and social information in decision-making depends not only on the consequences of each for finding information about the location of resources, but also the consequences associated with expected social interactions. In populations where likelihood of direct interaction with other individuals are high, and competitive ability differs among individuals, there may be substantial intrapopulation variation in how individuals use social information.

Chapter 3: Influence of public information on a foraging decision increases under perceived predation risk despite minimal costs of private information acquisition

Abstract

Animals use information that they have acquired personally ('private information'), or social information that they have gained from either the presence ('social cues') or behaviors ('public information') of others. The costly information hypothesis predicts that individuals should use public information when the costs of gathering private information are high. Recently, it has been shown that use of public information may increase when risk of predation is high, as predicted by the costly information hypothesis. However, risk of predation may also influence use of public information if individuals increase their reliance on public information about predators, facilitating the social transmission of information that is not directly related to predation as a byproduct. These hypotheses are not mutually exclusive, and both predict that the contribution of public information to a foraging decision should be increased as perceived predation risk increases. To test these hypotheses, we trained 36 captive-bred female *Gambusia affinis* (western mosquitofish) to obtain food in a specific patch under standardized conditions. We then presented individuals with conflicting public information about patch quality, by feeding a group of fish over an untrained patch while leaving a matched group over the trained patch unfed, in both non vegetated tanks (high risk) and tanks with simulated vegetation (low risk). We found that individuals used the patch that was demonstrated by successfully feeding conspecifics to make foraging decisions more when in a non-vegetated tank, and less when in a vegetated tank ($p<0.05$). These results are consistent with both the costly information and by-product hypothesis. However, because private information was acquired under standardized conditions we suggest that this results better support the "concurrent information hypothesis". These results suggest that multiple mechanisms could be involved in the acquisition and use of information by individuals in response to environmental risk.

Introduction

Animals can gather the information about their environment in one of two ways: one is by sampling it through direct interaction with the environment (gathering "private information"). The other source of information is that which is gathered by observing others ("social information") (Danchin et al 2004). Social information can generally be divided into two categories: ‘‘social cues,’’ in which information about a resource is gained from the decisions of other individuals, and ‘‘public information,’’ in which it is gained from the direct observation of others utilizing the resource (Danchin et al 2004, Coolen et al 2005). This distinction is important in that the observer is likely to acquire information of differing quality depending on the nature of that information. In the case of social cues for example, individuals may get information about the location of a resource based on the presence of conspecifics (i.e. local enhancement, Thorpe 1964), but get no information on the quality of the resource. This is qualitatively distinct from public information in which individuals gather information about the resource from direct observation of the behavior and success of others while they exploit the resource (Valone 1989).

Whether an individual uses information gathered from observing other individuals or actively pursues acquiring private information from their environment likely depends on a suite of costs and benefits to using and acquiring each. Private information is often thought to be the most accurate since it requires direct sampling from the environment, and research suggests that private information should be preferred as more reliable when it can be accurately gathered, and that public information should be used preferentially only as the accuracy of personal information diminishes (Nordell and Valone 1998; Templeton and Giraldeau 1996, Van Bergen et al 2004). Additionally, private information is likely to be more up to date since there is potentially less elapsed time between an organism's experience and their subsequent decision; previous work has demonstrated that individuals often ignore public information until their own private information becomes outdated or unreliable (Pongrácz et al 2003, Van Bergen et al 2004).

Although arguably the most accurate kind of information to use, the acquisition of private information can be costly. Individuals must personally experience the environment in order to gather private information. Individuals may have to travel some distance in order to gather enough private information to make a decision, which in turn exposes them to any environmental risks, such as predation (Boyd and Richerson 1988). These costs might be mitigated by acquiring public information instead of private information. Since an organism only needs to be able to observe others in order to gather public information, it may be possible for them to gather information without exposing themselves to environmental risks, as well as minimize the time and energy associated with gathering it (Morris 1992).

In contrast, relying solely on public information has costs as well. Copying errors or misinterpretation of cues and/or signals can lead to incorrect or inappropriate information for the observer (Giraldeau et al 2002). Furthermore, information cascades can lead to widespread misinformation in social groups in which individuals rely heavily on public information (Giraldeau et al 2002). Additionally, public information may become outdated in rapidly changing environments (Boyd and Richerson 1985, 1988; Feldman et al 1996). Moreover, public information may be costly to use for decision-making because decisions made by others may not have the same advantages for all observers. For example, foraging patches might be selected based on an individual's state, which will not necessarily be the same for all observers (Lindstedt & Hamilton 2013).

If private information is more accurate but expensive to acquire, and public information is potentially less accurate but relatively cheaper to acquire, this may create a trade-off between precision and expense for individuals who are gathering and using information. Boyd and Richerson 1985 proposed a "costly information hypothesis", which suggests that when information is particularly costly to obtain, individuals will take advantage of the relatively less expensive form of information, which is typically that provided by other individuals (public information or even cheaper, social cues).

Therefore, as environmental risks such as predation risk increase, so too should an individuals' reliance on public information.

The costly information hypothesis has seldom been directly tested, and these have yielded conflicting results. Galef and Whisken (2006) tested food preferences in Norway rats (*Rattus norvegicus*) in the presence of a predator. In their experiment, they failed to find that predation risk influenced the food preferences of observers who were presented with food from demonstrators. Webster and Laland (2008), however, found support for the costly information hypothesis. In their experiment, they determined that minnows (*Phoxinus phoxinus)* fed more from a patch which was demonstrated by a social group when predation risk was high. In both of these experiments the information provided to the observers was the simpler form of public information, social cues: individuals gained information about the resource simply from the presence of the demonstrators. In both cases the observers were not able to witness the success of the demonstrators on the patches. This is important, since information about the environment beyond the resource itself is potentially conveyed with public information use over simpler, social cues alone. In this way, the "costly information hypothesis" alone may not be sufficient to explain the relationship between public information use and environmental risk (see below).

There is an alternative (but not mutually exclusive) explanation for public information use in a risky environment. Since the gathering of public information involves the direct observation of the behavior of others, it may also provide information about environmental conditions unrelated to the resource itself, such as predation risk (Johnsson and Sundström 2007). In order to illustrate this point, consider an organism foraging in a risky environment. It may be paying particularly close attention to the public information provided by other foragers in the environment in order to maximize its own foraging success. In so doing, this individual may additionally get information about the riskiness of the environment simply by observing the behavior of the other individuals. Similarly, an individual paying attention to conspecifics in order to gain information about the location of predators may gain additional information about the location of food. Thus, increased attention to foraging success of others may be a byproduct of the need to gather information about predation risk. As a result of this increased attention, individuals may concurrently get information that a resource is both available *and* safe to exploit. This “concurrent information hypothesis” predicts that public information will be used more in risky environments because it potentially gives information about the risk of an environment in addition to information about the resource itself. Individuals who forgo the acquisition of available public information when predation risk is high (and instead gather private information) may be missing out on additional information about the environment. In this way, selection may favor the use of public information over private information when predation risk is high, which may be unrelated to the cost of obtaining private information.

Here we examined whether public information use increased in a risky environment despite low (and equal) costs of private information acquisition by testing the concurrent information hypothesis in the western mosquitofish (*Gambusia affinis*). *G affinis* are a highly social, non-territorial poeciliid that spend much of their time shoaling (Al-Daham et al 1977), making them a particularly suitable organism in which to test public information use. We tested the prediction that individuals would spend more time on a publically demonstrated food patch that was different from that in which they had been trained to expect food when in a non-vegetated (perceived high risk) tank than in a vegetated (perceived low risk) tank. Although no predator was present, we used the presence or absence of vegetation as a proxy for predation as previous work demonstrated that fish experience similar plasma cortisol changes with direct exposure to predation as they did from an absence of aquarium plants (Woodley and Peterson 2003). Importantly, we attempted to control for differences in the costs of obtaining personal information between the two environments by training all individuals in non-vegetated tanks prior to testing. This allowed individuals to amass private information about patch quality in a high risk environment before experimentation; during testing the individuals already had gained private information about patch quality and it was similar for both the non-vegetated and vegetated treatments. We predicted that individuals would use the publically demonstrated patch relatively more in high risk despite minimal costs of obtaining additional private information.

Methods

Training protocol: establishing a food patch preference

We used captive-bred female *G. affinis* (n=36) kept in 38 L tanks. We used only one sex (females) in order to control for any effects of male/female interaction. All fish were marked with unique caudal fin clips in order to keep track of individuals over the course of the experiment. Additionally, we measured mass of all experimental individuals.

In order to keep the number of individuals training in one tank relatively small, we trained individuals in groups of four in two training tanks. Prior to testing, we established a food patch preference with the fish by feeding them in one of two available patches. All individuals were trained in non-vegetated tanks. We covered both training tanks with a different substrate type on each half of the tank floor, a small sized (approximately 2-3 mm) black and white gravel on the left side, and a larger (approximately 0.5-1 cm) neutral colored gravel on the right side, which we used as different foraging "patches". Additionally, we used a unique landmark (one of two different types of aquarium plants with their leaves removed) associated with each side as an additional cue to distinguish between the two foraging patches. For training, we randomly assigned one side/substrate as a feeding patch. Individuals were fed once daily for two weeks exclusively over the feeding substrate (either exclusively over the right side or exclusively over the left side). Following this two-week training period, we tested all trained individuals for food patch preference in a new tank in order to determine whether a preference had been established and would carry over to a new tank (for detailed explanation, see below).

General experimental protocol

We food deprived all experimental individuals for 24 hours prior to testing to standardize hunger levels. For all experimental trials, we used tanks which were identical in size and substrate setup as the training tanks. In addition, experimental tanks included two transparent cylinders, one over each substrate type. We placed a group of three unfamiliar female conspecifics in each cylinder during trials. We then placed a focal individual in a third transparent enclosure (approximately 13 cm x 9 cm) that was placed in the middle of the tank, over both substrates (Figure 4 a). Following a 2 minute acclimation period for all treatments (see below), we dropped a single brine shrimp (*Artemia spp.*) over both substrate types, lifted the enclosure, and allowed the focal individual to forage freely in the tank. For all trials, the three female conspecifics in each of the holding cylinders remained enclosed. In all cases, we measured the time spent over each of the two substrate types, and the trial was stopped after three minutes of foraging. We exposed all individuals to three total treatments: the “equivalent public information” trial, which was always first (see below), then both the “vegetated” (Figure 4 b) and “non-vegetated” treatments (figure 4 c) (see below) in random order. There were always five days of training between all trials

Treatment 1: Equivalent public information: Individuals were always tested with this treatment first. Two social groups were present in the experimental tank, neither of which was fed during the trial. By not feeding either group, we aimed to provide equal public information to the focal fish about environmental conditions and patch quality (in this case, food was not successfully found by the social group in either patch). The purpose of this treatment was used to establish whether the focal individual had established a foraging patch preference based on the training. To avoid changes in ‘baseline’ preference resulting from exposure to socially acquired information, this trial was always performed first.

Fish were returned to their training tank following the baseline preference measurement. Original patch preference was reinforced over the next five days by feeding individuals exclusively over their original substrate type in order to reduce any effects on feeding preferences that may have been established by the trial itself.

Treatment 2: Differing public information about patch quality in a non-vegetated (high risk) and vegetated (low risk) environment: Five days following the first trial ("equivalent public information"), we tested individuals in the same experimental tank, but now provided the focal individuals with differing public information on patch quality. As with the "equivalent public information" treatments, two clear cylinders containing three females each were placed over each substrate type. However, unlike the "equivalent public information" treatment, we provided food (Hikari© First Bites baby fish food) to one of the confined social groups during the trial, by feeding them just prior to the release of the focal individual. In order to set up a conflict between the private information already acquired and the newly provided public information, we only fed the social group located over the substrate type over which the focal individual was not trained.

We allowed the focal individual to observe the social group feeding for the duration of the trial. Because of the small granule size of the food, it is unlikely that the focal fish would be able to directly observe floating granules from its enclosure, and would therefore only observe the foraging behavior of the social group. When we fed the social group, we placed our hands over both social groups, but only released food over one, which controlled for any behavioral effect that the fish may exhibit from having hands placed over them.

As before, the time spent over each substrate type was measured. Individuals were returned to the training tank following the 3 minute trial. Individuals were trained for 5 days before the next trial. All individuals were tested using this protocol in both a non-vegetated (high risk) and vegetated (low risk) environment (see below) in order to determine whether their foraging preferences differed between the environmental contexts.

<u>Non-vegetated (high risk):</u> For this treatment, the tank was kept absent of aquarium plants, except for the two landmarks used in the training tanks. Substrate type, landmark type and placement were identical to the training tanks. Previous work has demonstrated that *G. affinis* prefer tanks with planted aquarium plants (Casterlin and Reynolds, 1977) and *G affinis* regularly hide among aquarium plants when frightened (pers obs). Additionally, Woodly and Peterson (2003) demonstrated that simply an absence of aquarium vegetation was adequate to cause an increase in cortisol levels in longnose killifish, (*Fundulus majalis*) which mimicked levels when a predator was present. Therefore we used a lack of aquarium vegetation as a proxy for predation risk.

<u>Vegetated (Low risk):</u> This treatment was carried out exactly like the “non-vegetated” treatment, however, for this treatment we planted the experimental tanks with aquarium plants prior to testing. Plants were placed along the outside edge of the tanks so that the

focal individual could always see the social groups from its holding container and other central areas of the tank.

Statistical analysis

First, we performed a binomial test to determine whether use of the trained patch and demonstrated patch differed from a probability of 0.5 (i.e., 50% of the time in the trained patch and 50% in the demonstrated patch) in each of the three treatments. We scored individuals as a 0 if they spent more than 50% of their time over their trained patch and a 1 if they spent more than 50% of their time over the demonstrated patch (in our dataset, no individuals spent exactly 50% over each patch so there was always a preference to score). We conducted a binomial test for patch preference for the entire dataset (all individuals) as well as for our smaller subsets of individuals (those that spent at least 55% and 65% of their time over the trained patch during the "equivalent public information" trial).

We conducted a repeated-measures ANOVA using the proportion of time spent over the trained patch as the dependent variable. These data were not normally distributed, so all data were arcsine square root transformed to achieve normality prior to analysis. Treatment ("equivalent public information", "vegetated (low risk)" and "Non vegetation (high risk)") was the within-subjects factor. No between-subjects factors were included since all individuals were exposed to each treatment. We also included mass as a covariate, but removed this if the effect was not significant. In order to assure that we used only individuals that had a successfully learned a feeding preference during training, we used only individuals that used their trained side during the "equivalent public information" trials at least 55% of the time (n=19), which we used as our operational definition of an established patch preference (Lindstedt and Hamilton 2013). In addition, we also used a subset of fish that had a *strong* preference for their trained patch (> 65% of time spent on the trained patch) (n=14).

Results

The results of the binomial test revealed a significant preference for the trained patch in the "equivalent public information" (all fish, p=0.006) and "vegetated" treatments (all fish, p=0.002; 55% cutoff, p=0.001, 65% cutoff, p=0.001). In the non-vegetated treatment, use of the trained and demonstrated patch did not differ significantly from a probability of 0.5 for each (all fish, p=0.122; 55% cutoff, p =0.359; 65% cutoff, p=0.454). (figure 5).

For our repeated measures ANOVAs, the data for the 55% cutoff subset violated Mauchly's test of sphericity (W=0.897) so we used the Huynh-Feldt correction. Our data for the 65% cutoff however did not violate the assumption of sphericity (W=.608). When we examined the within-subjects contrasts, we found that there was a significant difference between the equivalent public information and non-vegetated treatments RM ANOVA, all learners: $F_{1,18}=5.782$, $p<0.05$; strong learners: $F_{1,13}=8.926$, $p<0.05$) but not between the equivalent public information and vegetated treatments (RM ANOVA: $F_{1,18}=0.115$, $p>0.05$) or between the non-vegetated and vegetated treatments (RM ANOVA: $F_{1,18}=2.585$, $p>0.05$). However, we failed to find an overall effect in the repeated measures analysis for all learners (> 55% time spent over trained patch in the equivalent social information trials) (RM ANOVA: $F_{2,17}=2.729$, $p=0.079$), suggesting the results from using these data should be viewed with caution. For strong learners (> 65% time spent over the trained patch) there was a significant overall effect of the within-subjects factor on patch use (RM ANOVA: $F_{2,12}=3.714$, $p<0.05$; figure 6). We did not find a significant effect of mass within subjects for the 55% cutoff, for either within-subjects (RM ANOVA $F_{2,16}=0.509$, $p>0.05$) or between subjects (RM ANOVA: $F_{1,17}=0.363$, $p>0.05$). Additionally, we did not find an effect of mass for the 65% cutoff either within-subjects (RM ANOVA: $F_{2,13}=0.982$, $p>0.05$) or between-subjects (RM ANOVA: $F_{1,14}=1.037$ $p>0.05$).

Discussion

We found that western mosquitofish established a foraging preference when trained. This preference remained when provided public information that conflicted with their private information in vegetated tanks, but was lost in non-vegetated tanks. Further, fish that had developed a strong preference for the trained side changed their foraging decisions in the face of conflicting available public information more so in non-vegetated tanks than in vegetated tanks. These results support earlier work by Webster and Laland (2008) which demonstrated that individuals increasingly utilize public information as environmental risks increase. Similarly to our experimental setup, Webster and Laland created a conflict between private information that individuals had previously gathered about the presence of food in a feeding apparatus and contradictory public information about the presence of food in an alternate feeding apparatus in minnows (*Phoxinus phoxinus*). Individuals were trained to receive food from only one of two apparatuses, but a social group demonstrated food in the opposite feeding apparatus during experimentation. However, an important distinction in our experiment is that we presented the focal individual with *two* identically sized social groups during each trial-a social group in each of the two foraging patches and only fed the group confined to the "poor quality" patch. This was important because this provided the focal individuals with public information about patch quality (and environmental conditions) while we kept social cues such as the presence and number of conspecifics consistent between patches. Further, all individuals were earlier trained in a non-vegetated tank. Therefore, any high costs associated with acquiring private information had already been experienced in the training period, and were experienced by all individuals.

Our results suggest that the costly information hypothesis alone may not explain variation in information use among different environmental contexts. Individuals may also be using public information in risky environments simply as a byproduct of paying more attention to conspecifics in a risky environment, or because they can gain information about the safety of the resource. Although the concurrent information hypothesis that we tested suggests a different mechanism from the costly information hypothesis, these are not mutually exclusive, so it is possible that individuals make decisions about which information they use based on a variety of contributing factors (e.g. the cost of acquiring personal information as well as a by-product of paying attention to conspecific foragers). Additionally, these results suggest that caution should be used when attributing public information use to costly private information acquisition, as public information may still convey information even when the costs of obtaining personal information are minimal.

Our results differed from earlier work by Galef and Whisken (2006) who failed to find that individuals were more likely to use public information when environmental risk was high. One key difference in our experiment was that individuals directly demonstrated the presence of food to the focal observer while the focal individual foraged. In Galef and Whisken's experiment, the information was not in the form of visible foraging conspecifics, but rather was the scent of food on a conspecific demonstrator (social cues). It might be that the presence of concurrently foraging conspecifics is a stronger or more reliable form of public information than the presence of a food odor on a conspecific, as previous work has demonstrated that individuals are less likely to utilize public information as the elapsed time from exposure to it increases (Van Bergen et al 2004).

This experiment demonstrates preliminary support for our "concurrent information" hypothesis. This hypothesis indicates several potentially important things about public information. First, our results reveal that information is likely not compartmentalized, but rather that information about one aspect of the environment may append itself to another (e.g. information about predation may be available from watching individuals forage). Individuals that do not (or cannot) pay attention to the behavior of others may be forgoing information about multiple aspects of the environment, for example information about the location and quality of resources may additionally convey information about how safe it is to exploit that resource (or vice versa). Importantly, observers might inadvertently get information about the environment that might not have been of interest to the observer originally (e.g. if a hungry individual pays more attention to foraging conspecifics simply in order to get information about the location of food and unintentionally gets information about the risk of the environment). Furthermore, the concurrent information hypothesis is not necessarily limited to foraging behavior. If, for example, individuals are paying close attention to conspecifics in order to gather information about mate quality, the behavior of those prospective mates may convey information about risk (Koga et al 1998, Warner and Dill 2000). Another important implication of the concurrent information hypothesis is that it suggests that social individuals (individuals in the population that are inherently more social than others, due to "personality differences" (Sih,et al 2004)) are expected to be more informed about various aspects of their environment than non-social individuals, simply as a result of having more opportunities to gather information because of close proximity to (or paying closer attention to) conspecifics (see chapter 4). This potentially creates within-population variation in how informed the individuals are, which is directly linked to differences in phenotype (and specifically genotype, as there is increasing evidence that personality traits are heritable, see Van Oers et al 2005).

Despite extensive theory regarding how and when public information should be utilized, clear, empirical patterns remain inconsistent (Kendal et al 2005). Several contributing factors such as the relative reliability of the alternative types of information (Van Bergen et al 2004), the various states of the individuals involved (Lindstedt and Hamilton 2013), and the composition of information providers in a group

(Rogers, 1988; Giraldeu et al 2002; Laland 2004) all appear to influence public information use. Here we demonstrate that variation in the use of public information may be related to individuals’ opportunities to gain information about multiple aspects of the environment. Finally, these results suggest something important about variation in sociality and risk: individuals that pay more attention to others in risky environments are also getting more environmental information than those that pay less attention. Therefore it is possible that large-scale biological processes such as cultural transmission (the transmission of learned behaviors) may be more likely when environmental risk is high.

Chapter 4: No effect of personality on public information use in a group-living fish (*Gambusia affinis).*

Abstract

Animal personalities (behavioral differences among individuals in a population that are consistent across different environmental contexts) occur widely and influence behaviors such as propensity to disperse, likelihood of parasitic infection, and invasion success. Another aspect of animal behavior that may be correlated with personalities is how an animal acquires and uses information in their environment. Here we tested whether two ecologically relevant personality traits (sociality and exploration) were correlated with the use of private and public information in a group-living fish (*Gambusia affinis*). We trained fish to feed exclusively from one of two patches, and then presented them with conflicting public information about the location of food. Separately, we measured three personality traits in two environmental contexts (vegetated and non-vegetated tanks). We did not find significant correlations between any of the personality traits and either public or private information use. This suggests that individuals of varying propensities for exploration and sociality may value public information equally, despite inherent personality differences. We did find a significant correlation between

sociality in the non-vegetated treatments and exploration in the vegetated treatments. This gives preliminary support for the presence of behavioral syndromes in our population.

Introduction

Animal personalities, behavioral differences among individuals in a population that are consistent across different environmental contexts (Sih 2004), have been documented in a wide diversity of taxa, including insects (Nemiroff, and Despland 2007), amphibians (Smith and Doupnik 2005), birds (Dingemanse et al 2002), mammals (Michelena et al 2008) and fish (Cote et al 2010), (for a review see Bell et al 2009). Animal personalities are an important source of behavioral variation seen both within and among populations of the same species, and importantly, are correlated with variation in other aspects of behavior. There has been a recent surge of research examining whether differences in personalities are linked with differences in other aspects of behavior and ecology such as propensity to disperse (Cote et al 2010 a, b), likelihood to be parasitized (Barber & Dingemanse 2010; Kortet et al. 2010) and invasion success (Sol et al. 2002, Duckworth and Badyaev 2007). Such differences may scale up to influence the distribution of a species. This insight has shed light on how the inherent behavioral differences of individuals in a population may be drivers of large-scale biological processes.

Among the ecologically relevant personality traits are sociality (how much time an individual spends near conspecifics (Cote et al 2012)) as well as exploration (how readily an individual explores a novel environment (Cote et al 2010). These are both traits that vary among individuals in a population (Sih et al 2012) and are ecologically and biologically significant. For example, a strong pattern has emerged that indicates that individuals who make up the "invasion front" of a newly invasive species tend to be more exploratory, less social, more aggressive and bolder (Sih et al 2012). Potentially related to invasion and dispersal, another aspect of behavior that may be linked to these traits is how animals use and gather information about their environments. Organisms that enter novel environments gather information about the new conditions in order to increase their likelihood of success (Dall et al 2005, Cote and Clobert, 2007, Clobert et al 2009, Chaine et al 2013). Animals can gain information about their environment by observing others (gaining "public information") or they can directly sample the environment (gaining "private information") (Dachin et al 2004). If social individuals pay more attention to conspecifics, then it is likely that they are also more likely to acquire (and use) public information to make decisions. Indeed, work in guppies (*Poecilia reticulata*) suggests that social individuals are more likely than asocial individuals to utilize public information (Trompf and Brown 2014). Likewise, if exploratory individuals tend to approach and sample novel environments by exploration, they may be more likely to gather (and use) private information to make decisions. Importantly, in addition to intra-population differences in personality, there is also intra-population variation in which type of information individuals utilize when making decisions, even when they have access to both (Lindstedt and Hamilton 2013, Lindstedt chapter 3). What contributes to this variation may be underlying morphological differences among individuals such as mass (Lindstedt and Hamilton 2013) or perceived differences in predation risk (Lindstedt chapter 3); however, how (if at all) inherent differences in personality lead to observed variation in information use among individuals remains largely unexplored

Here we examined whether two ecologically important personality traits (sociality and exploration) correlated with variation in public information use in the western mosquitofish (*Gambusia affinis*). *G. affinis* is a highly social poeciliid that was introduced across much of its range (Welcomme 1992). These characteristics make it a particularly good model species for studying the link between sociality and exploration and how they relate to public information use. Both sociality and exploration exhibit significant consistency in a high predation risk context (Lindstedt and Hamilton, chapter 5). Additionally, these traits exhibit the characteristics of personality in *G. affinis* (Cote et al 2010). We tested the hypothesis that more social individuals would weigh public information more heavily to make foraging decisions, and that individuals that were more exploratory would weigh previously acquired private information more to make foraging decisions. We predicted that individuals that spent more time near a social group would also spend relatively more time over a socially demonstrated feeding patch than do less social fish, when public information conflicted with their private information about the location of food. Likewise, we predicted that individuals that explored a novel tank faster (more exploratory) would spend relatively more time feeding over a patch on which they had already gathered private information regarding food richness than would less exploratory individuals. We tested this by creating a conflict between previously acquired private information about the richness of two feeding patches, and publically demonstrated information about the richness of food on the opposite patch.

Methods

We used 19 captive bred female western mosquitofish (*Gambusia affinis*) for this experiment. Individuals were selected at random from a laboratory population, and were subsequently housed in groups of four for the length of the experiment (4 weeks). All individuals were kept on a 12 hour light:dark cycle. In order to determine whether ecological context influenced behavioral traits, we tested individuals for all traits (public information use and personality traits) in both a non-vegetated and vegetated treatment. Previous work has demonstrated that *G. affinis* prefer tanks with planted aquarium plants (Casterlin and Reynolds, 1977) and *G affinis* regularly hide among aquarium plants when frightened (pers obs). Additionally, Woodley and Peterson (2003) demonstrated that simply an absence of aquarium vegetation was adequate to cause an increase in cortisol levels in longnose killifish, (*Fundulus majalis*) which mimicked levels when a predator was present. Therefore we expected that presence or absence of vegetation would manipulate perceived predation risk, with non-vegetated tanks perceived as having a higher risk of predation than vegetated tanks

Information use: tank setup

In order to test public information use, we created a conflict between private information and public information about the relative quality of two foraging patches, as in Lindstedt chapter 3. First, we trained individuals to associate a particular tank side and substrate with food. We covered housing tanks with a different substrate type on each half of the tank floor, a small sized (approximately 2-3 mm) black and white gravel on the left side, and a larger (approximately 0.5-1 cm) neutral colored gravel on the right side, which we used as different foraging “patches”. Additionally, we used a unique landmark (one of two different types of aquarium plants with their leaves removed) associated with each side as an additional cue to distinguish between the two foraging patches.

Information use: training protocol

All individuals were trained in non-vegetated tanks. For training, we randomly assigned one side/substrate as a feeding patch. Individuals were fed once daily for two weeks exclusively over that feeding substrate (either exclusively over the right side or exclusively over the left side). A total of four fish were housed in each of two training tanks with a total of eight fish being tested at one time. Following this two-week training period, we tested all trained individuals in a series of experimental trials in a different tank, as explained below. We food deprived all experimental individuals for 24 hours prior to testing to standardize hunger levels. For all experimental trials, we used tanks which were identical in size and substrate setup as the training tanks. In addition, experimental tanks included two transparent cylinders, one over each substrate type. We placed a group of three unfamiliar female conspecifics in each cylinder during trials. We then placed a focal individual in a third transparent enclosure (approximately 13 cm x 9 cm) that was placed in the middle of the tank, over both substrates. All fish were marked with unique caudal fin clips in order to keep track of individuals over the course of the experiment.

In order to determine whether a preference had been established and would carry over to a new tank, we performed a "patch preference test" first (more information below). (Note: patch preference test is the same test as 'equivalent public information' from Lindstedt and Hamilton chapter 3).

Information use: testing protocol

Patch preference test: In order to test patch preference in focal fish, we allowed them to freely forage in the presence of two confined social groups in an experimental tank. First, we confined a focal fish in the transparent holding compartment for 2 minutes in order to allow for acclimation. Following a 2 minute acclimation period for all treatments (see below), we dropped a single brine shrimp (*Artemia spp.*) over both substrate types, lifted the enclosure, and allowed the focal individual to forage freely in the tank. For all trials, the three female conspecifics in each of the holding cylinders remained enclosed.   In all cases, we measured the time spent over each of the two substrate types, and the trial was stopped after three minutes of foraging. Following this treatment, the focal individual was returned to their holding tank.

Public vs private information trials: In order to determine whether environmental context would impact correlations between public information use and personality, we modified perceived predation risk by altering vegetation. Individuals always completed the "patch preference test" treatment first, and were randomly assigned to complete either the "non-vegetated" or "vegetated" treatments next (all individuals were tested in all three). There were always five days of training between all trials.

Non-vegetated (high risk): For this treatment, the tank was kept free of aquarium plants, except for the two landmarks used in the training tanks. Substrate type, landmark type and placement were identical to the training tanks. Just as with the other treatments, we confined the focal individual to a holding enclosure for 2 minutes prior to testing, and we had 2 social groups confined in the tank. We lifted the enclosure and allowed the individual to forage after 2 minutes. As opposed to the "patch preference test" above, in this trial, we fed the focal group located over the *opposite* patch over which the focal individual was trained prior to testing (e.g. if the focal individual was trained over the left-hand patch, we fed only the social group over the right-hand patch). This set up a conflict between the focal individual's previously acquired private information, and conflicting public information about the location of food. We recorded the proportion of time the focal individual spent over each of the respective patches and ended the trial after 3 minutes of foraging.

Vegetated (Low risk): This treatment was carried out exactly like the "non-vegetated" treatment, however, for this treatment we planted the experimental tanks with aquarium plants prior to testing. Plants were placed along the outside edge of the tanks so that the focal individual could always see the social groups from its holding container and other central areas of the tank. Experimental procedure was identical to the "non-vegetated treatment" above.

Personality traits

All personality trials were conducted during the same month as the public information trials. We tested each of the two personality traits (sociality and exploration) twice in each of two treatments (vegetated and non-vegetated). All personality trials were held 1 week apart. Individuals were randomly assigned to either the "vegetated" or "non-vegetated" treatment for that trait, and the second trial of the same treatment was conducted 1 week later.

Testing procedure: sociality

Sociality was measured as the time a focal individual spent near conspecifics (Cote et al 2010). We first confined three non-experimental individuals in a transparent cylinder on one side of a 38L tank. We then placed a focal individual in the tank and allowed the individual to freely swim for at least 3 minutes prior to the beginning of the trial in order to acclimate. Trials began only when the focal individual was within two body lengths of the social group in order to control for differences in starting position after the three minute acclimation period. Sociality was calculated by determining the amount of time the individual spent near (≤ two body lengths) the social group during a ten minute trial. After ten minutes, the individuals were returned to their training tanks.

Testing procedure: Exploration

Exploration was measured as the time a focal individual took to explore a novel environment. We confined a focal individual into a transparent cylinder on the far end of a 38 L tank and allowed the individual to acclimate for three minutes prior to starting the trial. After three minutes, the cylinder was lifted and the individual was allowed to freely swim in the tank. The fronts of experimental tanks were divided into six equally sized zones (8cm x 6 cm). Exploration was calculated as the amount of time spent until the individual had entered five of the six tank zones. For low risk treatments, plants were always planted in the periphery of the tank so as not to impede movement of the focal individual while it swam in the tank. Individuals were given ten minutes to explore the zones; individuals that took longer than ten minutes were marked as to which zones they entered. Individuals that did not enter at least 5 zones were not used in data analysis.

Statistical analysis

We transformed our data prior to analysis since they were not normally distributed (sociality: square-root transformed, exploration: ln transformed). Additionally, we arcsin square-root transformed the proportions of time spent over the trained patch from the public information trials. We ran Pearson's correlation tests for all pairs of our public information and personality tests. Elsewhere, (chapters 2 and 3) we restricted data to "learners" (individuals that spent at least 55% of their time over the trained patch in the 'patch preference' trial) and "strong learners" (individuals that spent at least 65% of their time over the trained patch during the 'patch preference' trial). Here, we used all individuals, as we were interested in determining whether either of the personality traits of interest (sociality and exploration) were correlated with both private information use by itself (i.e., whether fish learned the location of food during training, which is measured in the 'patch preference' trial) and the conflict between public and private information (which is measured in the ‘vegetated’ and ‘non-vegetated’ social information trials). Additionally, we were interested in determining whether either of the personality traits influenced how individuals learned, as this was what we tested in the ‘patch preference test’.

Results

We did not find significant correlations between any of the personality traits we measured and use of the trained patch in trials where public information conflicting with private information was presented (all results listed in Table 1). There was a significant positive correlation between exploration in the vegetated tank and sociality in the non-vegetated tank (r=0.532, n=16, p=0.034, figure 7), but there was not a significant correlation between any other pair of behavioral traits, or between any of the public information trials and either of the personality traits. We did find a significant positive correlation between the amount of time an individual spent over their trained patch during the “patch preference test” trial and the amount of time they spent near the social group in the non-vegetated (high risk) treatment (r=0.472, n=16, p=0.048, figure 8). Likewise, we found a significant positive correlation between the amount of time an individual spent over the trained patch in the “patch preference test” and the time spent to explore the tank in the non-vegetated (high risk) treatment (r=-0.547, n=16, p=0.028, figure 9).

Discussion

We predicted that individuals that spent more time near a social group (in both the vegetated or non-vegetated treatments) would spend more time over the publically demonstrated patch (in either the vegetated or non-vegetated treatments) while foraging for food than would less social individuals. Likewise, we predicted that individuals that spent less time exploring a novel tank would also spent less time over the publically demonstrated patch while foraging for food than would more exploratory individuals. Despite our predictions, we did not find any significant correlations between either sociality or exploration and the extent to which an individual foraged over a publically demonstrated food patch, when private and public information conflicted. Taken together, our results suggests that though individuals may vary in how exploratory or social they are, these behavioral traits seem to have little influence on how much individuals value (or utilize) available public information.

Our results conflict with earlier work by Trompf and Brown (2014) in which they found that sociality was positively correlated with public information use in guppies (*P. reticulata*). Our experimental protocols were similar, so methodological differences are likely to not account for the differences we observed. However, when we examined the mass of focal fish we found a negative (non-significant) relationship among focal individuals and sociality: larger individuals tended to be less social than smaller individuals (r=-0.4, p=0.09, n=19; figure 10). Earlier work in the lab determined that smaller individuals were also less likely to utilize public information (Lindstedt and Hamilton 2013). Taken together, our results suggest that smaller individuals may spend more time near the social group (perhaps for safety) but are less likely to utilize public information (perhaps to mitigate the costs of conspecific aggression). Indeed, work on guppies suggests that aggression increases as the number of foragers on a patch increases (Koops and Abrahams 1999). Therefore in our population, sociality and public information use may not be correlated due to different causes of attraction of the social group (safety vs public information about food) to individuals based on their mass.

Although asocial individuals spend less time near a social group, we did not measure how *attentive* they are to the social group. It may be that asocial individuals spend less time near the social group in order to mitigate conspecific aggression, however they are equally attentive as the 'social' individuals to the behavior of nearby conspecifics. Likewise, although more exploratory individuals did not utilize their private information more to make foraging decisions, it may be that exploration per se has little to do with attention to conspecifics.

We found significant correlations between the "patch preference test" treatment and both exploration and sociality. In the case of sociality, there was a positive correlation between sociality (in the non-vegetated treatment) and use of the trained patch the "patch preference test" (i.e. more social individuals spent a larger proportion of time over their trained patch in the "patch preference treatment"). These results are somewhat unclear to interpret as we did not have any predictions about how sociality should vary with an overall ability to learn in non-social settings (which is what we tested in this treatment). There is some evidence that sociality may affect learning ability (e.g. the cultural intelligence hypothesis Van Schaik and Burkart 2011); however Trompf and Brown (2014) found no effect of sociality on learning ability in guppies (*P. reticulata*).

Exploration, however, exhibited the opposite relationship with learning in which individuals that explored the tank faster (were more exploratory) in the vegetated treatments, spent less time over their trained patch in the “patch preference test” treatment (i.e. more exploratory individuals did not learn their patch as successfully as less exploratory individuals). This contradicts our preliminary hypothesis that exploratory individuals would value private information more. Additionally, it suggests that more exploratory individuals may be less able to learn. Recent work by Jones and Godin (2010) with convict cichlids (*Amatitlania nigrofasciat*) demonstrated that fast explorers reacted more slowly to an attack from a simulated predator. They suggest that this may result from different personality types exhibiting differences in how they partition their time (specifically between foraging and vigilance). It may likewise be that fast explorers forage more by directly sampling their environment and spend less time learning about their environment through vigilance. This might lead to the pattern we observed between fast explorers learning less efficiently about the presence of food on a patch.

We did find some preliminary evidence of a ‘behavioral syndrome’ in our population. Behavioral syndromes occur when different individually consistent behaviors are correlated with one another (Sih et al 2004). We found that exploration in the vegetated tanks was positively correlated with sociality in the non-vegetated tanks. This might be explained if we consider that individuals who are more wary of risky environments might likewise be highly social in a non-vegetated tank, as they may be seeking protection from others. Indeed, minnows gather in larger shoals when approached by a predator (Magurran and Pitcher 1987). It could be that these same wary individuals are only more exploratory when predation risk is relatively low (i.e. in the vegetated tanks).

Despite a lack of influence of personality on public information use, there still may be influences of other inherent behavioral differences on public and private information use. Indeed, some preliminary work suggests that boldness affects learning and public information use (*P. reticulata*, Trompf and Brown 2014) and boldness and activity are predictors of learning in cavies (*Cavia aperea*) (Josep et al 2013). We did not examine boldness here, since boldness did not show consistency across environmental contexts given our assays in our population (Chapter 5), which indicated it did not meet the criteria for an ‘animal personality’. Further exploration of the influence of other common personality variables (e.g., aggressiveness, boldness) on learning and information use is needed.

Chapter 5: Behavioral consistency is influenced by environmental context in western mosquitofish (*Gambusia affinis*)

Abstract

Behavior is often thought to be highly variable both within and among individuals; however, many recent studies have shown that individual behavior can be remarkably consistent within and across environmental contexts. Here, we explored whether the consistency of behavior itself varied across environmental contexts within an individual. We hypothesized that in high predation risk environments, mistakes are more likely to reduce fitness, and therefore, selection may favor decreased expressed variation (high consistency) of behavior. Using western mosquitofish (*Gambusia affinis),* we utilized a within-subjects design to repeatedly measure three behavioral traits (sociality, exploration and boldness) in each of two vegetated (low risk) and non-vegetated (high risk) tanks for all experimental individuals. We found that individuals exhibited high behavioral consistency for both sociality and exploration in non-vegetated tanks, but not in vegetated tanks. Individuals did not exhibit high consistency for boldness in either environment. Examination of the variance components of consistency revealed that the higher consistency in non-vegetated environments was not solely due to lower error variance, but also higher variance due to among-individual differences (I). Our results demonstrate that selection may favor lower error expression in high risk environments.

Additionally, these results indicate that among-individual variance increased in high risk environments, suggesting selection may favor populations with numerous behavioral phenotypes in high risk, perhaps as an outcome of social niche construction or as a consequence of predation pressure resulting in highly variable behaviors.

Introduction

Behavioral ecologists have traditionally focused on within-individual behavioral variation resulting from phenotypic plasticity of behavioral traits, such as how individuals adjust their behavior in response to predation risk (Lima and Dill 1990), changes in behavior in response large-scale ecological disruption like urbanization (Sol et al 2013, Sih 2013) and modification of parental care in response to offspring need related to parent-offspring conflict (Trivers 1974, Godfray 1996). Recently however, it has also become clear that *among*-individual variation is meaningful in ways other than simply being existing variation upon which selection can act. One important component of among-individual variation is that associated with “animal personalities”, or mean behavioral differences among individuals within a population, that are consistent within individuals across situations or contexts (Sih et al 2004). The behavioral variation associated with personalities may make up a large proportion of overall observed behavioral variance (>30%, Bell et al 2009) and appears to play important roles in large scale biological events such as invasion success (Duckworth and Badyaev 2007) and dispersal (Fraser et al. 2001, Cote et al 2010) (For review, see Sih et al 2012).

Understanding within and between individual variation has been especially important in the field of predator/prey dynamics. For many species, phenotypic plasticity can also alter life history and/or morphology leading to permanent, fixed, antipredator traits that develop under risk of predation (Miner et al 2005). For instance, individuals may develop armor (Grant and Bayly 1981), morphologies more capable of escape (Relyea 2004) or different life history trajectories (Nunes et al 2014) in response to predation cues during development. In addition to fixed, non-changing traits, phenotypic plasticity allows individuals to adjust flexible traits like behavior to predation risk as well. Behavioral responses to changes in perceived predation risk include changes in sociality such as increases in group size (Caraco et al 1980) and changes in group structure (Abrahams and Colgan 1985; Magurran and Pitcher 1987), decreased activity (Li and Li 1979 ,Godin and Sproul 1988), changes in foraging behavior (Price et al 1984) and changes in parental care (Ghalambor et al 2013) (for a review see Lima and Dill 1990). In this way, phenotypes are modified in ways that will increase fitness in a context-appropriate way.

In order to understand the relationships between among- and within-individual behavioral variation, and how these ultimately relate to environmental conditions (e.g. variation in predation risk), a recently conceived framework of "Behavioral Reaction Norms" (BRN) has been developed (Dingamense et al 2009). Just as traditional reaction norms (RN) examine genetically determined phenotypic differences across environmental gradients among individuals in a population (Sarker 1999), behavioral reaction norms analogously examine changes in behavioral phenotypes across an environmental gradient. Within the behavioral reaction norm framework, among-individual differences in behavior (I) are the different animal personalities present in a population for any given trait (e.g., sociality- with some individuals being more social and others being less social across contexts). Environmental variation (E) represents variation in behavioral traits caused by the environment (environmentally induced plasticity). For example, individuals in a population may become more or less social across some environmental gradient (e.g. predation risk). The interaction of environment and individual behavior (I x E) represents the differences in plasticity in the population. Individuals may differ in how much their sociality changes in response to changes in predation risk; some individuals may change a great deal while others may change very little.

The final contribution to behavioral variation, is the error term (e). In general terms, error is observed variation not attributable to any of the sources mentioned above. Specifically, the error term may reflect random noise, environmental variation that is uncorrelated with the environmental gradient, and noise in the expression of the behavioral trait. The contribution of error to variance may be important, particularly in risky environments. The error term (e) itself may interact with the other variance components. Individuals or environments may be more or less ‘noisy’. If variation in noisiness exists among individuals, selection may act on the expression of ‘noise’, for example by increasing canalization of the trait in environments in which variation from an adaptable ‘optimum’ is costly (Waddington, 1942; West-Eberhardt, 2003). As an individual engages with its environment, it may express a range of values for a given behavioral trait (e.g. sociality figure 11.A). If an individual’s mean behavioral expression is ‘optimal’ given its state, it is likely that behaviors that diverge from the mean will carry fitness costs. In a risky environment the costs of these mistakes, or the range of behaviors that result in high costs, are likely greater (figure 11 B), leading to a truncated distribution of behavioral expression in high risk environments (figure 11 C). If however, an individuals' mean of behavioral expression is maladaptive, the opposite pattern of increased variation may be observed (Baldwin effect, Baldwin 1902).

We suggest that, analogously, the expression of error may vary *within* individuals, so that the error term for an individual's behavior varies depending on its environment. As a consequence, we hypothesize that if an individual is observed in different environments, the observed behavioral consistency will be greater in environments in which the costs of making mistakes are greater. In terms of behavioral consistency, (defined as the proportion of the total variance attributed to among-individual variance), a high error term leads to individual variation making up a smaller proportion of total variation, and thus reduces consistency. It is important to note however, that since consistency is a proportion, either a lower error (e) *or* a higher among-individual variance term (I) could both lead to higher consistency. Here we aim to examine the relationship between behavioral consistency and predation risk by measuring several behavioral traits (sociality, exploration, boldness) repeatedly across different predation regimes, in the western mosquoitofish (*Gambusia affinis*). Sociality, boldness and exploration are all traits that are sensitive to changes in predation risk (e.g. Lima and Dill 1990, Cote et al 2013) making them an ideal tool to study how changes in risk may lead to changes in behavioral consistency. We test the hypothesis that variance due to error (e) will be smaller in high predation risk than in low predation risk. We predicted that repeated measures of these traits will be more similar in non-vegetated treatments (high predation risk), as a result of a lower error term (e) leading to high consistency, than in vegetated (low predation risk) treatments.

Methods

We conducted this experiment using captive-bred female western mosquitofish (*Gambusia affinis*) (n=26) in 38-L tanks. We used only one sex (females) in order to control for any results driven by mating behavior. Individuals were housed in groups of four. Fish were kept on a 12 hour dark:light cycle. All fish were given a unique caudal fin clip for identification throughout the duration of the experiment.

All individuals were tested twice in both a vegetated and non-vegetated tank (two non-vegetated trials and two vegetated trials for a total of four trials for each behavioral trait for each individual figure 12 A-B).Vegetation consisted of artificial aquarium plants of varying shape. Plants were placed randomly throughout the tank, except for the boldness and exploration trials. For the boldness and exploration trials, all plants were planted around the outer edge of the tank so as to not obscure the view of the object from the focal fish. All tests were conducted in 38 L experimental tanks. Previous work has demonstrated that *G. affinis* prefer tanks with aquarium plants (Casterlin and Reynolds, 1977) and *G affinis* regularly hide among aquarium plants when frightened (pers obs). Additionally, Woodley and Peterson (2003) demonstrated that simply an absence of aquarium vegetation was adequate to cause an increase in cortisol levels in longnose killifish (*Fundulus majalis*), which mimicked levels when a predator was present. Therefore we expected that presence or absence of vegetation would manipulate perceived predation risk, with non-vegetated tanks perceived as having a higher risk of predation than vegetated tanks.

We collected data on three behavioral traits that exhibit large amounts of among-individual variation (i.e. characteristics of animal personality) in *G.affinis*: sociality, boldness, and exploration (e.g. Cote et al 2010; 2011), and that have been shown to display high consistency over repeated trials (Bell et al 2009). Specific protocol for data collection of each behavior is explained below. All behavioral tests were conducted weekly, and repeated measures of the same behavior and risk regime were conducted one week apart (e.g. if an individual was measured in high risk for sociality on one day, they were measured again for sociality in high risk one week later). We allowed one week to elapse between trials in order to minimize any acute state-effects on individuals. We randomly assigned individuals to be exposed to either high or low risk treatments first. All individuals were measured for all three behavioral traits and all individuals were measured in both risk regimes.

Exploration

Exploration was measured as the time a focal individual took to explore a novel environment (figure 12 C). We confined a focal individual into a transparent cylinder on the far end of a 38 L tank and allowed the individual to acclimate for three minutes prior to starting the trial. After three minutes, the cylinder was lifted and the individual was allowed to freely swim in the tank. The fronts of experimental tanks were divided into six equally sized zones (8cm x 6 cm). Exploration was calculated as the amount of time spent until the individual had entered five of the six tank zones. Individuals were given ten minutes to explore the zones; individuals that took longer than ten minutes were marked as to which zones they entered. Individuals that did not enter at least 5 zones were not used in data analysis. After an individual either entered 5 tank zones or 10 minutes had elapsed, the focal individual was returned to the stock tank.

Sociality

Sociality was measured as the time a focal individual spent near conspecifics (Ward et al 2004). We first confined three female non-experimental individuals in a transparent cylinder on one side of a 38L tank (figure 12 D). The social group was always composed of unfamiliar individuals in order to control for any variation in affiliative effects due to familiarity. We then placed a focal individual in the tank and allowed it to swim freely for at least 3 minutes prior to the beginning of the trial in order to acclimate. Trials began only when the focal individual was within two body lengths of the social group in order to control for differences in starting position after the three minute acclimation period. Sociality was calculated by determining the amount of time the individual spent near (≤ two body lengths) the social group during a ten minute trial. After ten minutes, the individuals were returned to their stock tanks.

Boldness

Boldness was calculated using a modification of the standard novel object paradigm (Thomson et al 2012). We confined an individual into a transparent cylinder for three minutes on the far end of the tank and allowed the individual to acclimate for three minutes prior to starting the trial. After the three minutes, we lifted the cylinder and the focal individual was allowed to swim freely. Boldness was calculated as the time an individual took to approach a novel object within one body length (figure 12 E). The novel objects were created using plastic building blocks, and were rearranged using the same pieces after each run so that the configuration of the object differed for each trial, while the overall volume, surface area and coloration did not change. After an individual either approached the object within one body length, or 10 minutes had elapsed, the focal individual was returned to the stock tank.

Statistical analysis

Because the trait values were not normally distributed, we transformed the data (sociality: square-root transformed, boldness and exploration: ln transformed). First, we determined whether there was an overall effect of risk on the three traits, by running a repeated-measures ANOVA with two within-subject factors for each of the three traits. The within-subject factors were the trial number (either 1 or 2) and the risk level (high or low).

In order to determine if individual identity had an effect on behavior, we ran a likelihood ratio test between two models, one containing individual identity as a random effect and one without. We performed likelihood ratio tests for models including all data (vegetated and non-vegetated trials) and for each vegetation regime separately, for each behavioral trait. In order to measure internal behavioral consistency, we tested whether the intraclass correlation (Cronbach's Alpha) for the repeated measures for each of the three behavioral traits differed from zero, for both the non-vegetated and vegetated trials separately, as well as the vegetated and non-vegetated trials combined. Cronbach's Alpha (α) values of 1 would indicate absolute consistency ($\frac{I}{(I+e)}=1$) (where I is among-individual variance and e is variance due to error), and all values are tested against the hypothesis that $\alpha = 0$. To test whether consistency differed among treatments, we compared the intraclass correlation coefficients for vegetated and non-vegetated trials, using a modified *t*-test accounting for among-treatment correlations (Feldt et al 1987).

Finally, we determined the relative variance contributions of the error (e) and among-individual (I) sources to overall consistency by running a generalized linear mixed model including individual identity as a random effect. We examined the model output to determine the variance attributed to individual and the variance attributed to error (residual error).

Results

We found that exploration was significantly faster in non-vegetated than in vegetated (RM ANOVA*:* $F_{1,16}$=17.691, p=0.001). The time to approach a novel object (boldness) was significantly faster in vegetated than in non-vegetated (RM ANOVA: $F_{1,15}$=11.435, p=0.004) . Sociality did not exhibit an overall effect of treatment (RM ANOVA*:* $F_{1,18}$=0.96, p=0.76) (Figure 13).

Results from our likelihood ratio test revealed that when both vegetation regimes were included, individual identity was a significant predictor of behavior for 2 of the 3 behavior traits: exploration (χ^2=4.05, df=1, $p<0.05$) and sociality (χ^2=11.96, df=1, $p<0.05$), but not boldness in which the likelihood ratio test estimated a negative ratio ($p>0.05$).

Results from the intraclass correlation tests revealed that overall, consistency was higher in the non-vegetated treatments (Table 2). The differences in consistency revealed that consistency was significantly higher for the non-vegetated treatments than the vegetated treatments for exploration (t=2.16, df=15, $p < 0.05$; figure 14). Behavioral consistency was not significantly different between the non-vegetated and vegetated treatments for sociality (t=1.66, df=17, $p > 0.05$), however consistency was significantly greater than 0 in non-vegetated tanks (α= 0.768, $p<0.05$) but not significantly different from 0 in vegetated tanks (α= 0.516, $p>0.05$; figure 14). The Cronbach's alpha statistic for boldness was not significantly different from zero for either treatment, and there was not a significant difference in consistency between the treatments (t=1.19, df=14, $p>0.05$).

Finally, examination of the relative variance components revealed that among-individual (I) variation increased in the non-vegetated treatments for both exploration and sociality, and the error term (e) decreased for sociality (Table 3).

Discussion

As predicted, we found that consistency of behavior differed between non-vegetated and vegetated tanks. Consistency of exploratory behavior was significantly higher in the non-vegetated (high risk) environment than in the vegetated environment. Although for sociality, consistency did not differ significantly between the non-vegetated and vegetated treatments, behavioral consistency was not significantly different from 0 in the vegetated treatments but was in the non-vegetated treatments. The differences between environments (E) (high for boldness and exploration, relatively lower for sociality) indicates that the environmental effect on behavior was a large source of variation for only two traits: exploration and boldness; predation risk did not cause population-wide changes in behavior for sociality. When considered together, these results suggest that individuals in our population exhibit high levels of among-individual variation (I), and environmentally induced plasticity in the traits (E) seems to be a large contributing factor to variance for only exploration and boldness. Additionally, when we examined the relative contributions of (I) and (e) to behavioral consistency, we found that the error term (e) decreased for one trait (sociality) as we predicted, but not for exploration.

We found a smaller error term in non-vegetated tanks for sociality, as predicted if the noisiness of behavioral expression is reduced in risky environments. The mechanism giving rise to this effect is unknown. It may be attributable to differences in underlying physiology. Indeed, glucocorticoid steroid hormone levels are often associated with a stress response (primarily cortisol in fish). Several studies have revealed that fish respond to stress by increasing circulating cortisol levels (for a review, see Barton 2002), and naive fish that are exposed to predators exhibit an increase in plasma cortisol levels (Winberg et al 1993, Bell et al 2007). Additionally, Schjolden et al (2005) conducted an experiment in which they examined behavioral differences in two lines of rainbow trout (*Oncorhynchus mykiss*), one that was selected for high cortisol response and one that was selected for low cortisol response. They determined that the high cortisol response individuals behaved more consistently in their movement during a 12 minute period than did the low cortisol individuals. Taken together this suggests that the presence of high levels of circulating cortisol as a result of high predation risk may result in lower behavioral variation (increased consistency) in high predation environments.

We did not find that the error term was lower in non-vegetated tanks for exploration, despite the increase in consistency in these treatments. Surprisingly, for both exploration and sociality, among-individual variation (I) increased in the non-vegetated treatments. This suggests that in high risk environments, individuals in our population became more *dissimilar* to each other. This could indicate that selection favors individuals with highly variable behavior such that they exhibit divergent phenotypes under high risk environments. Indeed, recent work suggests that individual behavior becomes more unpredictable in high risk conditions (e.g. Domenici et al 2008), indicating that predators may be less successful when they are unable to predict a prey animal's behavior. In this way, individuals within a population may become more dissimilar to one another in order to avoid capture by predators (however this is contrary to other work such as Cote et al 2013, where individual behavior becomes more similar when predation risk is high). Alternatively, large among-individual variation (I) in behavioral expression may indicate that our population experiences an amplification of social niche construction in high risk environments. Social niche construction occurs when individuals in a population express differing behavioral phenotypes in order to reduce competition within a population (Bergmüller and Taborsky 2010), and changes in risk may induce social niche construction in *G. affinis*.

In contrast to the patterns we observed for both sociality and exploration, we did not observe a significant intraclass correlation for boldness. It could be that boldness is a trait that is robust to changes in predation risk, and therefore is not responsive when predation risk is altered. This seems unlikely, however, since previous work has demonstrated that boldness is affected by predation risk in another fish species, the rainbow trout (*Oncorhynchus mykiss*) (Thomson 2012), and additionally we observed environmentally induced plasticity in boldness (though the results should be interpreted with caution). It is more likely, therefore, that our assay for boldness did not accurately assess boldness. We observed that mosquitofish often used the object as cover in non-vegetated tanks, which may interact with their reaction to it as a novel, potentially threatening object (pers. obs.).

Contrary to earlier work (such as that by Elgar 1986 and Ekman 1987) predation risk did not significantly influence the population mean for sociality. Even though previous work has demonstrated some effect of risk on sociality, this relationship is somewhat inconsistent (Lima and Dill 1990). Mosquitofish are highly social (Al-Daham et al 1977) so it is possible that they remain in close proximity to their social group, regardless of risk context. In this way, sociality may be robust to context, and may remain consistent regardless of predation risk. This would support earlier work from Cote et al in which they found evidence that sociality was a personality trait in *G. affinis.* We did find an influence of vegetation on both exploration and boldness. The time taken to explore the tank and time to approach a novel object were shorter in the non-vegetated environment than in the vegetated environment. In the case of exploration, it is possible that individuals explored the tank faster in the non-vegetated treatment because they were searching for a hiding refuge. The increase in apparent boldness under high predation risk again may be explained if individuals were using the novel object as structure under which they could hide.

Our results suggest that the recent surge of personality research should use caution when interpreting the results of experiments that test the repeatability of behavior, particularly when testing individuals across risk regimes, as predation risk itself could affect the consistency of the behavior. This is because there may be an interaction between environment and error (E x e) that may complicate the interpretation of the environment (E) term, as well as the individual by environment interaction (I xE). Indeed, recent work by Toscano et al (2014) demonstrated a similar (E x e) interaction in which the consistency in refuge use in mud crabs (*Panopeus herbstii*) increased in the presence of a predator cue. These results suggest that simply taking a single measure in each of two risk treatments may not give an accurate assessment of behavioral repeatability, and therefore may have implications for anyone interested in quantifying the presence of animal personalities in a population. Additionally, it may be important to specifically examine consistency *within* as well as *across* treatments. We recommend that researchers interested in examining personalities in their population should measure traits of interest multiple times across environmental gradients, and should consider including an environment by error (E x e) interaction term in their models.

The results from this study are among the first to demonstrate that consistency of behavior may be highly context-dependent. Recently, we have observed similar patterns regarding the context-dependence of behavioral consistency with wild caught individuals of a different species in the genus as well (Lindstedt and Hamilton, chapter 6). These results suggest that simply examining population-wide changes in behavioral means may not be fully appropriate when considering the effect of environment on behavior. Our results suggest that the complex interaction of environment, inherent differences in individual behavior, and how individuals adjust their behavior in response to environment are all key components to understanding the expression and, ultimately, evolution of behavior.

Chapter 6: Lack of inter-population divergence in behavioral canalization despite differences in predation regime in the Bahamas mosquitofish (*Gambusia hubbsi*)

Abstract

Trait canalization occurs when expressed phenotypes are similar across environmental gradients, and is expected to occur under a) prolonged unchanging selection pressures and b) under stabilizing selection. Canalization of relatively “rigid” traits like body size and developmental pathways have been extensively demonstrated, but the role canalization plays in the evolution of reasonably flexible traits like behavior remains to be understood. Here we examine behavioral canalization by measuring the behavioral consistency for three behavioral traits (activity, boldness, and sociality) for Bahamas mosquitofish (*Gambusia hubbsi*) from blue hole populations that differ in their predation regime (some co-occur with a predator fish *Gobiomorus dormitory*). We tested the hypothesis that the relative strength of selection in high predation populations would be stronger and thus more likely to result in behavioral canalization than in low predation populations. We collected individuals from the field and repeatedly measured the three behavioral traits across two environmental contexts: non-vegetated and vegetated tanks. We predicted that individuals from high predation populations would exhibit relatively higher consistency for the traits across the two environmental treatments than the low predation populations. We found overall behavioral consistency to be low for boldness

and exploration. Sociality exhibited significant differences in consistency within a predation regime and within a treatment, but overall consistency did not differ significantly between predation regimes. Consistency was relatively low for all traits suggesting that behavioral canalization has not evolved in this system. Selection may instead favor highly unpredictable behavior, particularly in the high predation environments.

Introduction

Longstanding theory suggests that flexible traits should become less plastic and more canalized (Waddington 1942) under continued stabilizing selection (Gavrilets and Hastings 1994, Rouzic et al 2013) and when selection remains unchanged over evolutionary time (Waddington 1961). Trait canalization occurs when traits become robust to environmental or genetic variation, such that similar phenotypes are expressed across varying environmental conditions. Canalization likely acts as a buffer to ensure the expression of selectively advantageous traits despite environmental or genetic perturbations (Schmalhausen, 1948). Although trait canalization has been of interest to biologists for decades, a recent surge of both theoretical and empirical work has again stimulated a dialog among scientists, particularly about the role of trait canalization in the evolution and maintenance of complex genomes.

Canalization been documented in the expression of a variety of traits. Most experimental work has been conducted on relatively ‘rigid’ traits including various aspects of development such as body segmentation (Houchmandzadeh et al 2002, Luccetta et al 2005) and genital formation (Gleason et al 2002, Braendle and Felix 2009) as well as morphological traits such as body size (Walzer and Schasberger 2014). Despite a vast body of theoretical and experimental work however, only recently has canalization started to be documented in the wild (e.g. Edgell et al 2009, Svensson et al 2014).

Studies on behavioral canalization are limited. Although there are abundant examples of behavioral consistency, or so-called 'animal personalities', the links between this consistency and canalization are unclear. Conceptually, the links between strong, stabilizing selection and behavioral consistency seem apparent. If, for example, a population experiences a history of predation, selection may favor the expression of an "optimal" trait value, thus cleaving the ends of the distribution of behavioral variation within the population leading to stabilizing selection on the trait. The strength of stabilizing selection is believed to lead to canalization (Wagner et al 1997). In *Gambusia affinis*, work in our lab determined that individual behavioral variation in sociality is decreased (consistency is increased) within individuals when predation risk is high (Lindstedt chapter 5). Although we did not examine behavioral consistency as a result of selection, this change in within-individual behavioral expression in response to predation risk could be an evolutionary 'first step' towards canalization. Further evidence for behavioral canalization in response to predation comes from Edgell et al (2009). They examined behavioral canalization between two wild populations of flat periwinkle snail (*Littorina obtusata*) in response to an invasion of the European green crab (*Carcinus maenas*) (a snail predator). They found that behavioral consistency was higher across two treatments differing in risk (risky and not risky) in the populations where crabs and snails had co-occurred for longer. This suggests that an evolutionary history of predation leads to the canalization of behavioral traits.

The blue hole cave system on Andros Island in The Bahamas, provides a model system in which to study how an evolutionary history of predation influences behavioral canalization. Andros Island is covered by a widespread system of blue holes, or water-filled caves which vary in their fish population composition (Langerhans and Gifford 2009). While virtually all of the blue holes were colonized by the Bahamas mosquitofish (*Gambusia hubbsi*), a much smaller proportion of the blue holes were colonized by a predatory fish, the bigmouth sleeper goby (*Gobiomorus dormitory*) (R.B. Langerhans unpubl data). This has created a system in which multiple blue holes have both *G. hubbsi* and their predator *G. dormitory*, while other blue holes have only *G. hubbsi*. These replicate populations therefore offer powerful insight into how differences in predation lead to inter-population phenotypic divergence in behavior and morphology. Previous work has demonstrated strong phenotypic divergence among these populations in response to predation regime including differences in morphology (Langerhans et al 2007), life histories (Downhower et al, 2000, Riesch et al 2013), locomotor performance (Langerhans 2009, 2010), genital shape (Langerhans 2005, Heinen-Kay and Langerhans, 2013) and behavior (Heinen et al 2013).

We used *G hubsii* from predator-present and predator-absent blue holes to test the hypothesis that divergent selection resulting from differences in predation regime would lead to evolution of behavioral canalization in the high predation populations more so than in the low predation environments, since selection like likely to be stronger. Since the result of canalization is that traits are buffered against environmental changes, we can test the presence of behavioral canalization by examining how the consistency of behavioral traits responds to different environments; behavior should remain consistent across environmental conditions if canalization has occurred. We tested this by repeatedly measuring three behavioral traits (sociality, boldness, activity) across two different environmental contexts: vegetated and non-vegetated tanks. We chose to test individuals in vegetated and non-vegetated tanks in order simulate a risky (non-vegetated) and less risky (vegetated) environment. Sociality, boldness and activity level are all behavioral traits shown to respond to differences in predation (Lima and Dill 1990, Cote et al 2013). Additionally, previous work has demonstrated a difference in sociality among the predation regimes of another poeciliid, the guppy *(Poecilia reticulata)* (Magurran 1991). Finally, since consistency is defined as the proportion of total variance attributed to among-individual variance ($\frac{\boldsymbol{I}}{(\boldsymbol{I}+\boldsymbol{e})}$, where I is among-individual variance and e is variance due to error), we also examined how the various variance components (either among-individual or error) contributed to overall consistency both between predation regime as well as for each blue hole. We predicted that the behavioral consistency of three behavioral traits would be significantly higher across these two experimental conditions for high predation populations than low predation populations.

Methods

Study system

Blue holes are water-filled vertical caves found in some carbonate banks and islands (Mylroie et al.1995), and Andros Island, The Bahamas harbors the greatest density of blue holes on earth. Blue holes were previously air-filled caves, filling with water during the past 17,000 years (Fairbanks 1989) as rising sea levels lifted the freshwater lenses of the island (freshwater aquifers floating atop marine groundwater), which flooded the caves with water. This created a unique replicate set of environments eventually colonized by aquatic organisms. Three particular species comprise the bulk of fish inhabitants in the Bahamas blue hole system: the small livebearer, Bahamas mosquitofish (*G. hubbsi*, 89 % occurrence), the small pupfish, sheepshead minnow (*Cyprinodon variegatus*, 38 % occurrence), and the larger predatory eleotrid, bigmouth sleeper (*Gobiomorus dormitory*, 27 % occurrence) (R.B. Langerhans unpubl data). All existing genetic evidence indicates isolation among the blue hole fish populations (Schug et al. 1998 ; Langerhans et al 2007; Riesch et al. 2013).

Collection

Individuals were collected from each of four blueholes, two high predation blue holes (Cousteau (Cou) and West Twin (Wtwn)), and two low predation blue holes (East Twin (Etwn) and Hubcap (Hub)) between the dates of 7/14/12-7/20/12 and transported back to Forfar field station (International Field Studies; 24.898205, -77.930410) where they were housed separately according to blue hole (in water from their native blue hole). Individuals were housed at Forfar field station for the entirety of the experiment (7/20/12-

8/1/2). Individuals were fed a similar diet of freeze dried bloodworms while in the field station. Housing tanks had aerators and filtration. Experiments were all conducted during natural daylight hours.

Experimentation

A total of 55 experimental individuals were chosen at random from the population of collected individuals from the 4 blue holes (Etwn: 16, Hub: 15, Wtwn: 11, Cou: 13). In order to identify individuals, detailed descriptions and drawings were taken of each individual, and only four individuals from each blue hole were tested at one time. Once selected, all experimental individuals were housed in temporary tanks used only while individuals participated in the experiment.

All individuals were measured for three behavioral traits (sociality, boldness and exploration; more information below) across two environmental treatments: vegetated and non-vegetated tanks. We chose vegetated and non-vegetated treatments in order to simulate a risky (non-vegetated) and not risky (vegetated) environment. Woodly and Peterson (2003) demonstrated that simply an absence of aquarium vegetation was adequate to cause an increase in cortisol levels in longnose killifish, (*Fundulus majalis*) which mimicked levels when a predator was present. Therefore we felt that an absence of vegetation was a good simulation of predation risk. All individuals were measured twice in each treatment, for a total of four trials for each behavioral trait.

Sociality

Sociality was measured as the time a focal individual spent near conspecifics (Ward et al 2004). In order to test how behavioral traits responded to changes in environmental conditions, we tested individuals in both a vegetated tank (in which we planted artificial aquarium plants) and a non-vegetated (in which tanks were open) tank. For low risk treatments, plants were always planted in the periphery of the tank so as not to impede movement of the focal individual while they swam in the tank. All individuals were tested twice in each of these treatments. Each individual was randomly selected to complete either the non-vegetated or vegetated treatment first.

First, we confined three non-experimental individuals originating from the same population in a transparent cylinder on one side of an experimental tank (~ 20 L). We then placed a focal individual in the tank and allowed the individual to freely swim for at least 3 minutes prior to the beginning of the trial in order to acclimate. Sociality was calculated by determining the amount of time the individual spent near (≤ two body lengths) the social group during a five minute trial. The individuals used for the social group were randomly collected from the pool of individuals housed at the field station from each blue hole such that each focal individual only saw a social group from their own blue hole. After testing, focal individuals were returned to their temporary tanks. Individuals were then tested in the opposite treatment type (either vegetated or non-vegetated) in the afternoon. We used the same testing protocol for individuals in the afternoon session, but with a different social group of fish (which were again selected at random from the same blue hole populations in the lab). The second (and final day) of testing was identical to the first, with individuals being tested a second time in both treatments (vegetated and non-vegetated). All individuals tested for sociality were concurrently tested in the activity paradigm as well (see below).

Activity

Activity trials immediately followed the sociality experiments. Activity level was calculated as the number of times an individual switched among six equally sized zones (8cm x 6 cm) that were marked on the experimental tank. We confined a focal individual into a transparent cylinder on the far end the experimental tank and allowed the individual to acclimate for three minutes prior to starting the trial. After three minutes, the cylinder was lifted and the individual was allowed to freely swim in the tank. Individuals were given five minutes to explore the tank; individuals that never switched into any zones were marked as a 0. We removed individuals from the tank as soon as they entered all six tank zones. Since some individuals ended the activity trials sooner than others, activity was calculated as a rate (switches/second). As with the sociality trials, all activity trials were conducted in both a vegetated and non-vegetated tank condition. Individuals were randomly selected as to which treatment (vegetated or non-vegetated) they were tested in first. Once the first trial was finished, individuals were returned to their temporary holding tanks. Individuals were then tested again in the afternoon in the opposite (vegetated or non-vegetated) treatment that they completed in the morning.

Boldness

Boldness was measured as the time it took for an individual to enter any zone after the holding cylinder had been lifted. Time to exit a holding compartment has been used as a measure of boldness in *Gambusia affinis* (Cote 2010). Boldness was measured during the activity trials, and was calculated twice for both vegetated and non-vegetated tanks, similarly to the other traits.

Statistical analysis

Since the trait values were not normally distributed we log transformed the data. First we determined whether there was an overall effect of current risk or predation regime on the three traits by running a repeated-measures ANOVA for all three traits with treatment (either vegetated or non-vegetated) as the within-subjects factor, and 'predation regime' and 'blue hole identity (predation regime)' as the between-subject factors.

In order to measure behavioral consistency, we tested whether the intraclass correlation (Cronbach's Alpha) for the repeated measures for each of the three behavioral traits differed from zero, for both the non-vegetated and vegetated trials separately within a predation regime (either high or low predation), as well as the vegetated and non-vegetated trials combined. Additionally, we calculated intraclass correlation coefficients within each blue hole-treatment combination. Cronbach Alpha values of 1 would indicate absolute consistency ($\frac{I}{(I+e)}$ =1), and all values are tested against the hypothesis that $\alpha = 0$. To test whether consistency differed among treatments within a predation regime, we compared the intraclass correlation coefficients for vegetated and non-vegetated trials, using a modified T-test accounting for among-treatment correlations (Feldt et al 1987). In order to calculate if the intraclass correlation coefficients differed between predation regimes, we used a modified F-test according to Feldt et al. We also calculated whether there was a significant difference among the intraclass correlation coefficients among blue holes by using a modified chi-squared test, also from Feldt et al 1987.

Finally, we determined the relative variance contributions of the error (e) and among-individual (I) sources to overall consistency by running a generalized-linear mixed model including individual identity as a random effect, for every predation-regime/treatment combination and every blue hole/treatment combination. We report the variance attributed to individual and the variance attributed to error (residual error).

Results

All RM ANOVA results listed in table 4. We found that there was a difference in boldness between the high and low predation populations when we pooled them, with time to exit the first zone being significantly higher in high predation populations (1.8483 ± 0.1168, mean ± 95% CI) than in low predation populations (1.518 ± 0.1019, mean ± 95% CI). Additionally, we found a significant effect of vegetation on boldness, with time to exit the first zone being significantly faster in the non-vegetated treatments (1.52 ± 0.1098, mean ± 95% CI) than in the vegetated treatments (1.7978 ± 0.1058, mean ± 95% CI) for all individuals. There was a significant effect of blue hole identity on boldness as well. We also found a significant difference in activity between high and low predation populations (when we pooled them) with activity being significantly lower in high predation populations (-0.0493 ± 0.1541, mean ± 95% CI) than in the low predation populations (0.2155 ± 0.0329, mean ± 95% CI). Additionally we found that vegetation had a significant effect on activity with activity rates being significantly higher in the non-vegetated treatments (0.1657 ± 0.0378, mean ± 95% CI) than in the vegetated treatments (0.0896 ± 0.1274, mean ± 95% CI) when we included all individuals. There was no effect of blue hole identity on activity level. There was no effect of predation regime, blue hole identity, or risk on sociality (table 4).

When we examined the intraclass correlation values within the pooled predation regimes (high or low) we found that overall behavioral consistency to be relatively low for all traits from high predation populations, and the consistency *across* the treatments did not differ significantly from 0 for any of the three behaviors (table 5). Additionally, there was not a significant difference in overall consistency across treatments between the pooled low and pooled high predation individuals ($F_{55,24}=0.498$, $p>0.05$). Examining only the pooled high predation individuals, within treatments the consistencies of boldness and activity did not differ significantly from 0 in either vegetated or non-vegetated treatments. The consistency of sociality was significantly greater than 0 in the vegetated treatment only (figure 15). The difference in consistency between vegetated and non-vegetated treatments was significant for sociality; sociality was significantly more consistent in the vegetated treatment (table 5). Examining only the pooled, low predation individuals, all three traits exhibited behavioral consistency for one of the two treatments. Sociality and boldness were significantly consistent in the non-vegetated treatments, and activity was significantly consistent in the vegetated treatments (figure 15). Despite the differences in consistency, only sociality was significantly different between treatments, with consistency in the non-vegetated treatment being significantly higher than in the vegetated treatments (table 5).

When we compared the intraclass correlation coefficients *between* the pooled predation regimes, we found that only the alpha values for sociality were significantly different from each other, with consistency of sociality being significantly higher for high predation individuals in the vegetated treatments and significantly higher for the low predation individuals in the non-vegetated treatments (table 6).

We calculated intraclass correlation coefficients for each blue hole combination for all three traits (table 8). The individuals from Cousteau (high predation) exhibited significant behavioral consistency for sociality in the vegetated treatments, but not for any of the other traits. The individuals from West Twin (high predation) exhibited high consistency for sociality (vegetated treatments only) and activity level (non-vegetated treatments only. East Twin (low predation) individuals only exhibited significant consistency for sociality in the non-vegetated treatments. Hubcap (low predation) individuals exhibited significant consistency for two traits, sociality (non-vegetated treatment only) and activity (vegetated treatment only). We compared the Cronbach's alpha values for all blue holes between the treatments, and found no significant differences among them (table 6).

Finally we examined the variance components (among-individual (I) and error (e)) for both the individuals pooled by predation (table 4) and by blue hole (table 8). Among individual (I) variance increased for sociality in the vegetated treatments for all pooled high predation individuals. Among-individual (I) variance increased and error (e) variance decreased in the non-vegetated treatment for sociality for the pooled low predation individuals. Both (I) and (e) variance increased for boldness in the non-vegetated treatments for low predation individuals. Finally, (I) increased in the vegetated treatment for activity in the low predation individuals. When examining variance components by blue hole, we found that (I) increased in the vegetated treatment for sociality for individuals from Cousteau. For individuals from West Twin, (e) was smaller in the vegetated treatments. For activity level, (I) increased in the non-vegetated treatments. When examining the East Twin individuals, (e) variance increased and (I) variance decreased in the non-vegetated treatments for sociality. Examining the variance components of the Hubcap individuals revealed that for sociality, (I) increased and error (e) decreased in the non-vegetated treatment. Finally, for activity level (I) was higher in the vegetated treatments.

Discussion

We found overall behavioral consistency to be low when we pooled all high predation individuals together, with only one trait exhibiting significant consistency (sociality). Consistency was higher for sociality in general, but, contrary to our predictions, consistency was not higher across both treatments for the high predation populations compared to the low predation populations. When we examined behavioral consistency for each high predation blue hole, individuals from West Twin exhibited relatively more consistency than Cousteau, but consistency was still not high *across* the treatments, as we had predicted. For both the pooled data, as well as the data from both high predation blue holes, sociality was only significantly consistent in the vegetated treatments, which was contrary to other work in the lab which determined that behavioral consistency was higher in non-vegetated treatments (Lindstedt and Hamilton, chapter 4).

Surprisingly, behavioral consistency overall was relatively higher for the low predation individuals when we pooled all low predation populations together. All three traits (sociality, boldness, activity) exhibited high consistency in one of the two experimental treatments. Additionally, for two of the three traits (sociality and boldness), consistency was significantly higher in the non-vegetated treatments than the vegetated treatments. This supports earlier work on our lab in which we demonstrated that behavioral consistency increased in high risk (non-vegetated) treatments in *Gambusia affinis* (Lindstedt, chapter 5). Furthermore, examination of the relative variance components revealed that a lower error term (e) contributed to higher consistency in sociality. This also supports earlier work in which we suggested that individuals have lower variance due to error (e) in high risk environments if the expression of high variance is costly (chapter 4). It is not clear why we observed this pattern only in the low predation populations (in fact, the high predation individuals exhibited the opposite effect: consistency was higher in the vegetated treatments).

We found no evidence for behavioral canalization for any of the traits that we measured. It could be that there has not been strong selection on these traits, however this seems unlikely since there is strong evidence for phenotypic divergence of other behavioral traits based on predation in this species (Heinen et al 2013). It seems more likely that selection may not favor the canalization of these particular traits. Perhaps flexibility in behavior is favored in high predation environments since it may make individual behavior harder to predict by predators (Humphries and Driver 1970). This pattern has been documented in variety of different taxa including insects (Domenici et al

2008), birds (Witherby et al 1947, Kruuk 1964) and fish (Godin 1997). Additionally, canalization may be more likely to evolve in traits directly involved in predator avoidance. Indeed, Edgall et al (2009) observed behavioral canalization when they measured soft tissue withdrawal which is a well-documented anti-predator behavior. Furthermore, there has been some increasing evidence that anti-predator traits exhibit high behavioral consistency (Briffa et al 2008, David et al 2014). Another possibility is that the traits we measured are under directional or disruptive selection. This would disallow the evolution of behavioral canalization, since the result of evolution is an ever-moving target, in which case canalization would be selectively disadvantageous. Furthermore, examination of the variance components revealed that the low consistency for high predation individuals (both pooled and blue hole populations) may be at least in part due to a decrease in among-individual variation, in other words, individuals from high predation populations tended to become more *similar* to each other when exposed to non-vegetated treatments. This supports some earlier work in *G. affinis*, in which individuals expressed more similar behavioral trait values once exposed to predation risk (Cote 2013). It is possible that selection favors low among-individual variation in high predation environments. Additionally, if shoaling is an important predator defense, then selection may favor conformity within the group in order to minimize coordination errors (Herbert-Read et al 2013).

Although we did not find evidence of canalization in these populations, we found strong evidence that behavioral consistency is context-dependent. This was true when we pooled individuals from the high and low predation regimes, as well as when we examined blue holes separately. Since the patterns regarding risk and consistency were different among low and high predation individuals, it suggests that different mechanisms may be acting on traits depending on the predation regime. Indeed, earlier work by Schjolden et al (2005), demonstrated that rainbow trout (*Oncorhynchus mykiss*) which were selected for a high cortisol response behaved more consistently with their movements over a 12 minute trial than those selected for low cortisol response. Therefore cortisol may have a large behavioral impact on low predation fish. Likewise, a decrease in among-individual variation in high risk environments may be what largely drives the differences in consistency observed for the high predation individuals. Taken together, these results suggest that selection may favor divergence in consistency related to a different evolutionary history of predation (figure 16).

Chapter 7: Conclusion

For my dissertation work, I examined how changes in ecological context related to use of social and private information, behavioral consistency, and the relationship between these in two poeciliid species in the *Gambusia* genus. I examined this at two timescales: short term differences in predation risk for individuals from the same population, and long term differences resulting from different evolutionary histories of predation among different populations.

In Chapter 2, I found that western mosquitofish (*Gambusia affinis*) use available public information differently depending on their physical state (specifically mass) (Lindstedt and Hamilton 2013). After being exposed to public information demonstrating the location of food, and which was in conflict with previously acquired private information, large individuals shifted foraging behavior to use the demonstrated patch. Smaller individuals did not. Smaller individuals may differ in their ability to acquire public information, or they may use public information to avoid costly competition.

In Chapter 3, I determined that *G. affinis* weighed public information more to make foraging decisions when predation risk was high. This was significant because contrary to the predications of the "costly information hypothesis" (Boyd and Richerson 1988), individuals in my population used public information more in a high risk environment even though the costs of gathering private information were minimal (or similar) among all treatments. This was preliminary support for the “concurrent information hypothesis” in which I suggest that individuals may gather information about multiple aspects of their environment, simply as a result of increased attention to conspecifics. In the case of my experiment, individuals may have gathered information about the presence of food because they were paying more attention to conspecifics in order to gather information about predators (in the high risk treatments).

In Chapter 4, I determined that inherent, consistent among-individual differences in behavior (so called “animal personalities”) in *G. affinis* did not influence public information use. Despite the fact that individuals in my population varied in both sociality (propensity to spend time near conspecifics) and exploration (time to explore a novel environment), these traits did not seem to influence whether the individuals used public (or private) information more to make decisions. It is possible in my population that sociality does not accurately measure how much attention to conspecifics such that ‘asocial’ individuals are still paying attention to, and using the public information provided by, conspecifics. It is also possible that smaller individuals are less likely to use public information (chapter 2) but they are more likely to be social. Therefore there may not be a clear relationship between sociality and public information use. Likewise, exploration may not be related to attention to conspecifics so exploratory individuals may still value and use public information as much as less exploratory individuals.

In chapter 5 I found that behavioral consistency in *G. affinis* was highly context-dependent, and increased under high risk conditions for two of the three traits I examined (i.e. increased for sociality and exploration but not boldness). Within a behavioral reaction norm (BRN) framework, I determined that this high consistency was the result of both increased among-individual variance (I) (both sociality and exploration) and decreased variance due to error (e). Among-individual variance may increase if selection favors highly variable behavioral phenotypes in order to reduce the chances of predators being able to predict behavior, or if there is an increase in social-niche construction under high risk. I suggest that error (e) may decrease if within-individual behavioral variation is decreased in order to minimize costly behavioral mistakes in a high risk context.

In chapter 6 I found no evidence of behavioral canalization in any of three traits (sociality, boldness, activity) among four blue hole populations of *Gambusia hubbsi* that varied in their history of predation. Despite differences in selection for a prolonged amount of time (15,000 years), I did not find that behavioral consistency was higher across different environmental contexts for the high predation populations, despite my predictions. I suggest that this could result if selection on the traits is not stabilizing but rather is directional or disruptive. Likewise, selection may favor a decrease in among-individual variation in behavioral traits in high predation populations leading to decreased behavioral constancy. I found further evidence for the context-dependence of behavioral consistency as all three traits showed context-dependence according to predation risk and this pattern was observed for high and low predation individuals.

There are several implications of these findings. First, the same source of information may mean different things to different individuals (chapter 2), and this could result in size segregation. Second, individuals that pay more attention to conspecifics, may be better informed about their environment (chapter 3). Additionally and importantly, predation risk matters for public information use. Taken together this suggests that there may be within-population differences in how informed individuals are based on their mass. More importantly, this could potentially lead to differences in cultural transmission based on differences in environmental risk, with cultural transmission being more likely in high risk environments. Additionally, if public information is involved with mate selection (e.g. Witte and Massmann, 2002), it may be possible that risk may influence speciation among individuals based on their size, sociality and propensity to use public information.

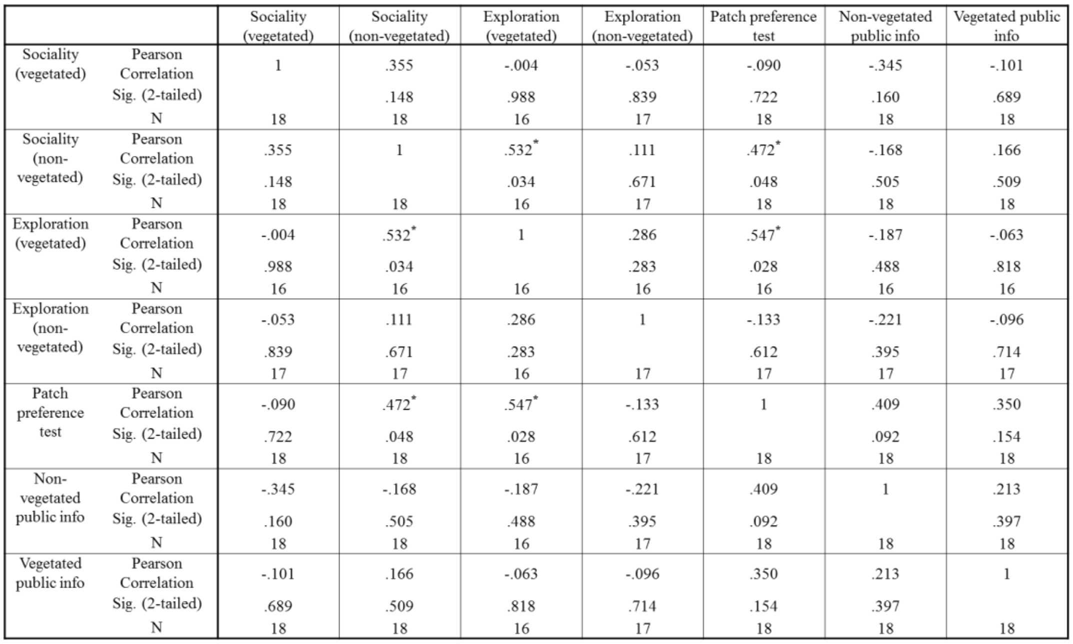
In two of my chapters (chapter 5 and chapter 6) I found strong evidence that behavioral consistency changed in response to changes in predation risk, in both the laboratory (chapter 5) and in wild populations of a closely related species, *G hubbsi* (chapter 6). These are among the first studies to demonstrate a relationship between risk and consistency. Interestingly, I found that differences in consistency were in part due to differences in among-individual variation (niche construction, selection for differences, etc). Another important implication for future work is to examine the proximate mechanisms that drive lower within-individual variation in high risk environments. Hormonal changes associated with risk, particularly cortisol, may increase behavioral consistency by lowering within-individual variance (e), as some previous work has found higher behavioral consistency in a "high cortisol" selection line of rainbow trout (*Oncorhynchus mykiss*) (Schjolden et al 2005). I would like to manipulate cortisol levels and examining how this affects behavioral consistency.

I suggest that *within*-individual modification of behavioral consistency as I observed, could be an evolutionary ‘first step’ towards behavioral canalization. However, I found no evidence of differences in behavioral canalization based on predation regime for the traits examined in *G hubbsi*. In the future, I would like to further examine behavioral canalization in the blue hole populations by directly examining traits that are associated with predator avoidance (e.g. locomotor performance). Even though I was unable to find evidence for differences in canalization in my experiments, earlier work suggests that canalization may be more likely in anti-predator behavioral traits (Edgell et al 2009). Additionally, I would like to include more blue hole populations beyond the 4 that I used here to better explore between population differences within predation regimes

How the environment influences behavior in both ecological and evolutionary timescales are central questions to behavioral ecologists. Here, I demonstrate that environmental change on an ecological scale (alteration of predation risk) affects both public information use as well as behavioral consistency in a group-living fish. This within-individual behavioral plasticity may allow individuals to behave in adaptive ways by altering their behavior in a context-appropriate ways. Surprisingly, when I examined environmental differences on an evolutionary scale (i.e. differences in predation regime) I did not find evidence for differences in trait canalization, however I did find evidence for overall behavioral divergence in boldness and activity (chapter 6). These results suggest that at either scale, environment can have large, potentially significant implications for various aspects of behavior.

This work is among the first to directly examine how the environment influences behavioral consistency. The importance of this environmental effect on consistency may lead to changes in how behavioral ecologists approach behavioral responses to environmental change, particularly in light of the recent surge of animal personality research. In the future, I suggest that behavioral consistency should be considered when examining how environment influences behavior, and an environment by error term (E x e) should be included in all models aimed at teasing apart the various sources of behavioral variance. It is increasingly clear that individual phenotypes are modified through processes like transmission of information and plasticity, and these processes interact with natural selection. Here, I demonstrated that these important processes are themselves strongly dependent on environmental context at different time scales.

Appendix A: Tables and Figures



		Sociality (vegetated)	Sociality (non-vegetated)	Exploration (vegetated)	Exploration (non-vegetated)	Patch preference test	Non-vegetated public info	Vegetated public info
Sociality (vegetated)	Pearson Correlation	1	.355	-.004	-.053	-.090	-.345	-.101
	Sig. (2-tailed)		.148	.988	.839	.722	.160	.689
	N	18	18	16	17	18	18	18
Sociality (non-vegetated)	Pearson Correlation	.355	1	.532*	.111	.472*	-.168	.166
	Sig. (2-tailed)	.148		.034	.671	.048	.505	.509
	N	18	18	16	17	18	18	18
Exploration (vegetated)	Pearson Correlation	-.004	.532*	1	.286	.547*	-.187	-.063
	Sig. (2-tailed)	.988	.034		.283	.028	.488	.818
	N	16	16	16	16	16	16	16
Exploration (non-vegetated)	Pearson Correlation	-.053	.111	.286	1	-.133	-.221	-.096
	Sig. (2-tailed)	.839	.671	.283		.612	.395	.714
	N	17	17	16	17	17	17	17
Patch preference test	Pearson Correlation	-.090	.472*	.547*	-.133	1	.409	.350
	Sig. (2-tailed)	.722	.048	.028	.612		.092	.154
	N	18	18	16	17	18	18	18
Non-vegetated public info	Pearson Correlation	-.345	-.168	-.187	-.221	.409	1	.213
	Sig. (2-tailed)	.160	.505	.488	.395	.092		.397
	N	18	18	16	17	18	18	18
Vegetated public info	Pearson Correlation	-.101	.166	-.063	-.096	.350	.213	1
	Sig. (2-tailed)	.689	.509	.818	.714	.154	.397	
	N	18	18	16	17	18	18	18

Table 1. Pearson correlation coefficients for all comparisons (all personality trait and public information trial combinations). Significant correlations are indicated with an asterisk (*).

	Cronbach’s alpha (α) for vegetated treatments	**Cronbach’s alpha (α) for non-vegetated treatments**
Sociality	0.516	0.768*
Exploration	0.267	0.712*
Boldness	0	0.463

Table 2. Cronbach’s alpha (α) values for vegetated and non-vegetated treatments for all behavioral traits. Significant α values are indicated with an asterisk (*).

	Among-individual variance (I)	**Variance due to error (e)**
Sociality	Veg: 5.302 Non: 9.580	Veg: 9.934 Non: 5.776
Exploration	Veg: 0.06781 Non: 0.6796	Veg: 0.37223 Non: 0.5503
Boldness	Veg: 0.00 Non: 0.5517	Veg: 1.086 Non: 1.2799

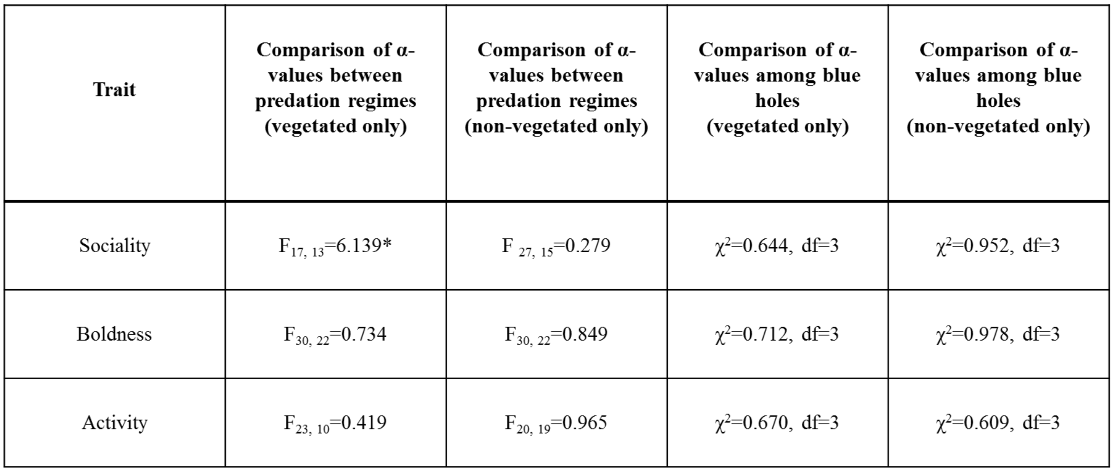
Table 3. Among-individual (I) and error (e) variance estimates for all three behavioral traits for the vegetated and non-vegetated treatments.

Behavioral trait	**RM ANOVA within subjects effect of vegetation**	**RM ANOVA between subjects effect of predation regime**	**RM ANOVA between-subjects effect of predation(blue hole) interaction**
Sociality	$F_{1,23}=0$, $p=0.51$	$F_{1,23}=1.142$, $p=0.296$	$F_{2,23}=0.606$, $p=0.554$
Boldness	$F_{1,50}=20$, $p<0.001$*	$F_{1,50}=12.251$, $p=0.001$*	$F_{2,50}=7.392$, $p=0.002$*
Activity	$F_{1,40}=270$, $p<0.001$*	$F_{1,40}=6.119$, $p<0.018$*	$F_{2,40}=2.862$, $p=0.069$

Table 4. Results from RM ANOVA analyses. Significant effects are individuated with an asterisk (*)

Predation Regime	**Trait**	**Cronbach alpha (α) values for vegetated treatments**	**Cronbach alpha (α) values for non-vegetated treatments**	**Comparison between α values**
High	Sociality	0.878*	0	t=-4.61*, df=7
	Boldness	0.184	0.417	t=0.854, df=22
	Activity	0	0.226	t=0.351, df=6
Low	Sociality	0.251	0.721*	t=2.64*, df=16
	Boldness	0.401	0.505*	t=0.585, df=29
	Activity	0.581*	0.253	t=-1.34, df=21

Table 5. Intraclass correlation (Cronbach alpha) values for pooled high and low predation populations for the three traits we measured (sociality, boldness, exploration). Also the T-test results from comparisons of alpha values within each predation regime. Significant results ($p<0.05$) are indicated with an asterisk (*).



Trait	Comparison of α-values between predation regimes (vegetated only)	Comparison of α-values between predation regimes (non-vegetated only)	Comparison of α-values among blue holes (vegetated only)	Comparison of α-values among blue holes (non-vegetated only)
Sociality	$F_{17, 13}$=6.139*	$F_{27, 15}$=0.279	χ^2=0.644, df=3	χ^2=0.952, df=3
Boldness	$F_{30, 22}$=0.734	$F_{30, 22}$=0.849	χ^2=0.712, df=3	χ^2=0.978, df=3
Activity	$F_{23, 10}$=0.419	$F_{20, 19}$=0.965	χ^2=0.670, df=3	χ^2=0.609, df=3

Table 6. Comparisons of intraclass correlation coefficients (Cronbach's alpha, α) between pooled predation regimes (high predation and low predation) for the different treatments (vegetated and non-vegetated) and among all individual blue holes (Cousteau, East Twin, West Twin, Hubcap) for the different treatments (vegetated and non-vegetated). All significant values (p<0.05) are indicated with an asterisk (*).

Predation Regime	**Trait**	**Among-individual variance (I)**	**Variance due to error (e)**	**Cronbach's alpha (α)**
High	Sociality	Veg: 0.423 Non: 0	Veg: 0.99 Non: 0.143	Veg: 0.878* Non: 0
	Boldness	Veg: 0.043 Non: 0.298	Veg: 0.012 Non: 0.004	Veg: 0.184 Non: 0.417
	Activity	Veg: <0.01 Non: 0.037	Veg: <0.01 Non: 0.266	Veg: 0 Non: 0.226
Low	Sociality	Veg: 0.068 Non: 0.201	Veg: 0.298 Non: 0.156	Veg: 0.251 Non: 0.721*
	Boldness	Veg: <0.01 Non: 0.013	Veg: <0.01 Non: 0.022	Veg: 0.401 Non: 0.505*
	Activity	Veg: 0.122 Non: 0.030	Veg: 0.176 Non: 0.178	Veg: 0.581* Non: 0.253

Table 7. Variance components (among-individual (I) and error (e)) for all traits for each of the pooled predation regimes (high and low).

Blue hole Identity	Trait	Among-individual variance (I)	Variance due to error (e)	Cronbach's alpha (α)
Cousteau	Sociality	Veg:0.564 Non: 0	Veg: 0.193 Non: 0.075	Veg: 0.86* Non: 0
	Boldness	Veg: 0.046 Non: 0	Veg: 0.028 Non: 0.004	Veg: 0.35 Non: 0.588
	Activity	Veg: 0 Non: 0	Veg: 0.147 Non: 0.329	Veg: 0 Non: 0
West Twin	Sociality	Veg: 0.290 Non: 0.362	Veg: 0.017 Non: 0.021	Veg: 0.954* Non: 0.549
	Boldness	Veg: 0.032 Non: 0.079	Veg: 0.004 Non: 0.001	Veg: 0.49 Non: 0
	Activity	Veg: <0.01 Non: 0.154	Veg: <0.01 Non: 0.146	Veg: 0 Non: 0.679*
East Twin	Sociality	Veg: 0.326 Non: 0.211	Veg: 0.103 Non: 0.198	Veg: 0 Non: 0.691*
	Boldness	Veg: 0.068 Non: 0.004	Veg: 0.006 Non: 0.032	Veg: 0 Non: 0.365
	Activity	Veg: 0.010 Non: 0.065	Veg: 0.204 Non: 0.202	Veg: 0.398 Non: 0.39
Hubcap	Sociality	Veg: 0.091 Non: 0.200	Veg: 0.267 Non: 0.126	Veg: 0.4 Non: 0.761*
	Boldness	Veg: 0 Non: 0.022	Veg: 0.026 Non: 0.016	Veg: 0.361 Non: 0.524
	Activity	Veg: 0.190 Non: 0	Veg: 0.173 Non: 0.160	Veg: 0.699* Non: 0

Table 8. Variance components (among-individual (I) and error (e)) for all traits for each of the blue hole populations

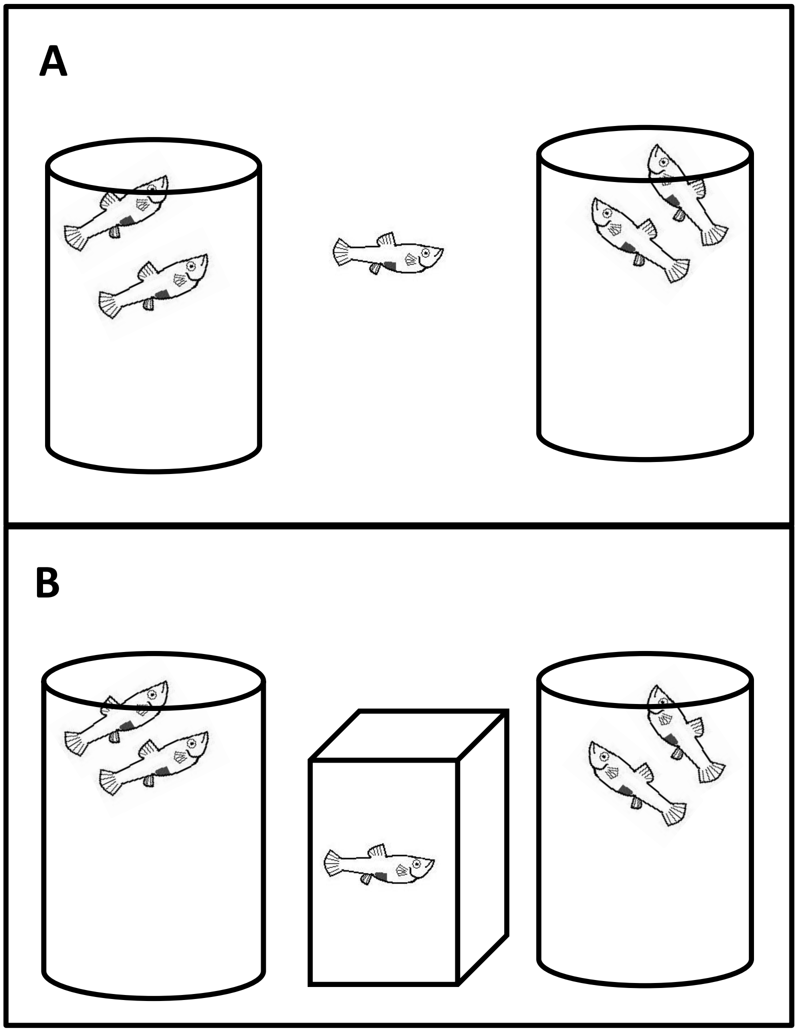


Figure 1. Individuals were either allowed to forage in the tank while the social group was fed as in the "Current " treatment (A) or individuals were confined to a transparent holding compartment and were only allowed to forage in the tank after the social groups had finished eating as in the "Older" treatment (B).

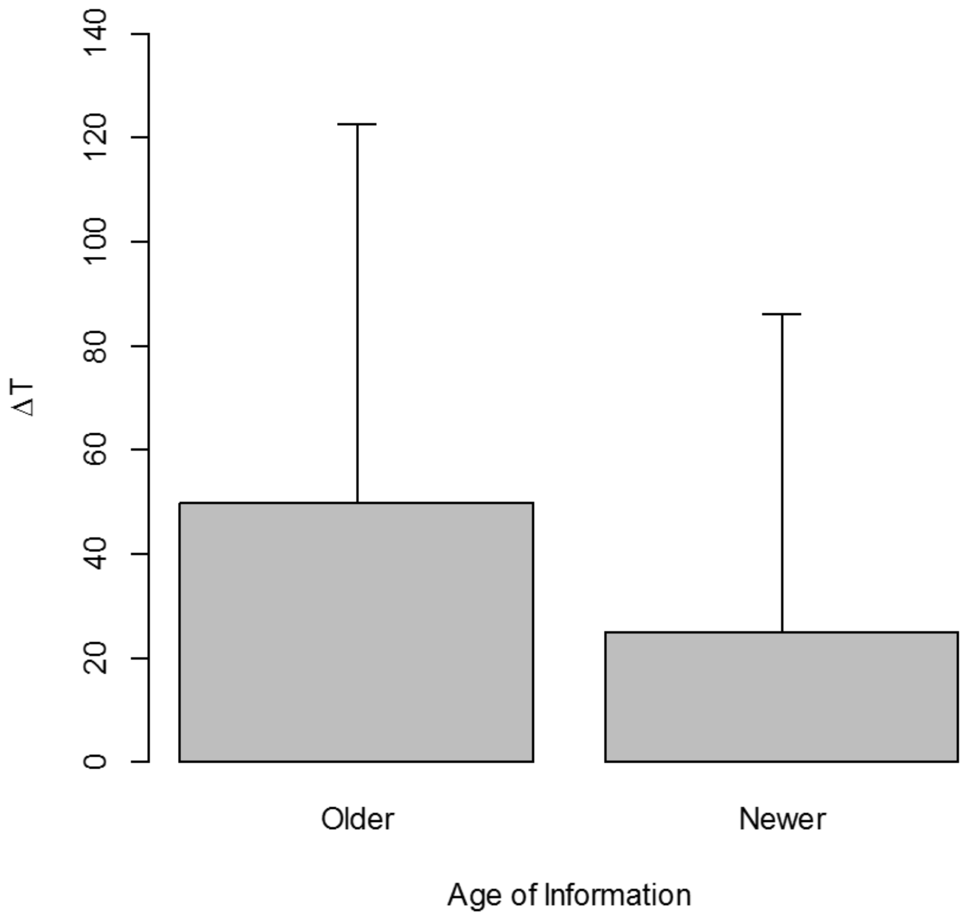


Figure 2. Data shown are mean differences in patch use for individuals for new and old social information + SE. Δ T = P-S (where P is the proportion of time an individual spends over the substrate type it was trained on when no social information is available, and S is the proportion of time an individual spends over the substrate type it was trained over when conflicting social information was present) such that a larger ΔT indicates a larger switch in substrate type preference.

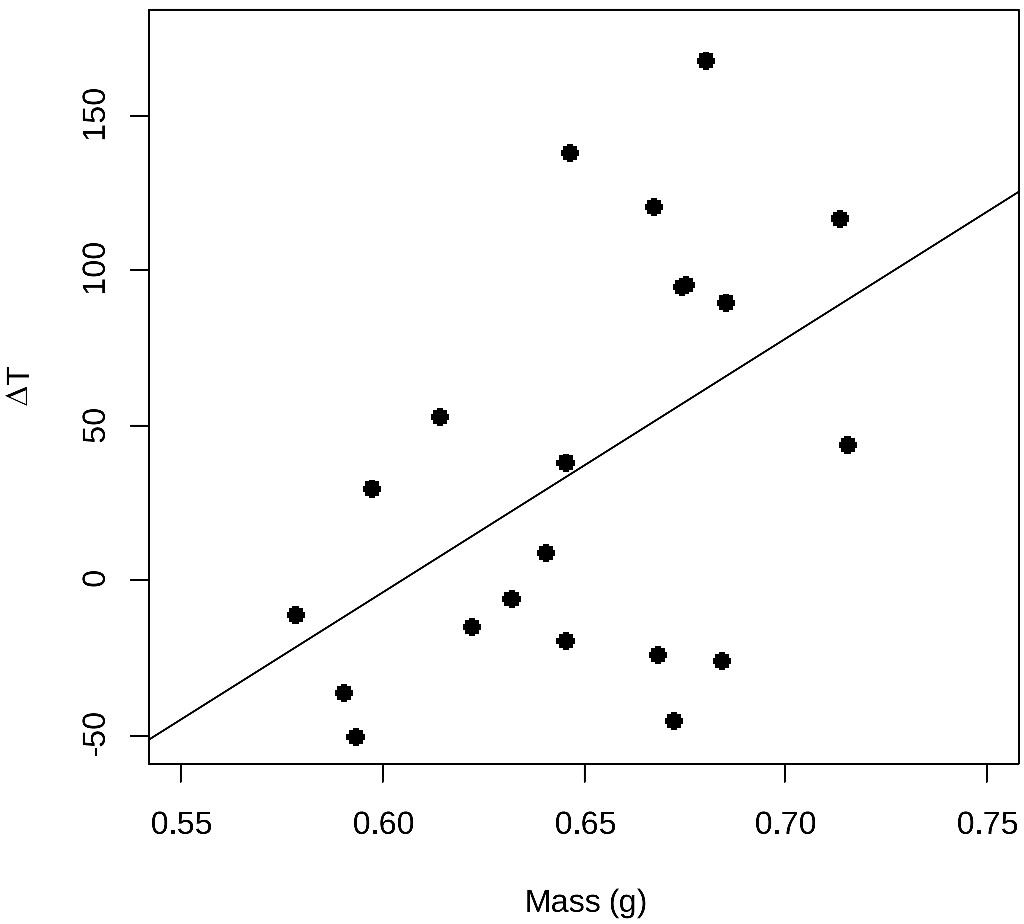


Figure 3. Changes in substrate use plotted against mass. Larger individuals changed their substrate preference more (indicated by a larger ΔT) than smaller individuals, indicating they used social information more to make foraging decisions.

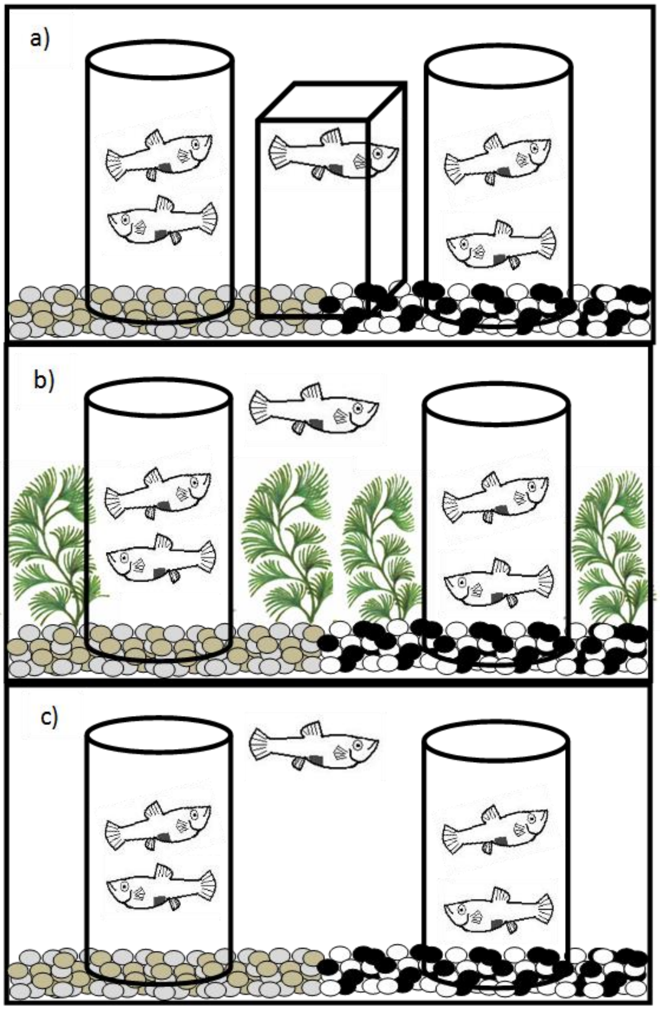


Figure 4. Experimental setup for all trials. Individuals were first confined to a transparent holding compartment for three minutes prior to testing in which they were able to observe the behavior of the social groups (a). After the three-minute acclimation period the compartment was lifted and the focal individual was allowed to freely forage. The proportion of time spent over the trained side was calculated for both vegetation (b) and no vegetation (c) treatments. Only one social group was fed (the one positioned over the opposite patch than the one the focal individual was trained over) in all trials except "equivalent public information".

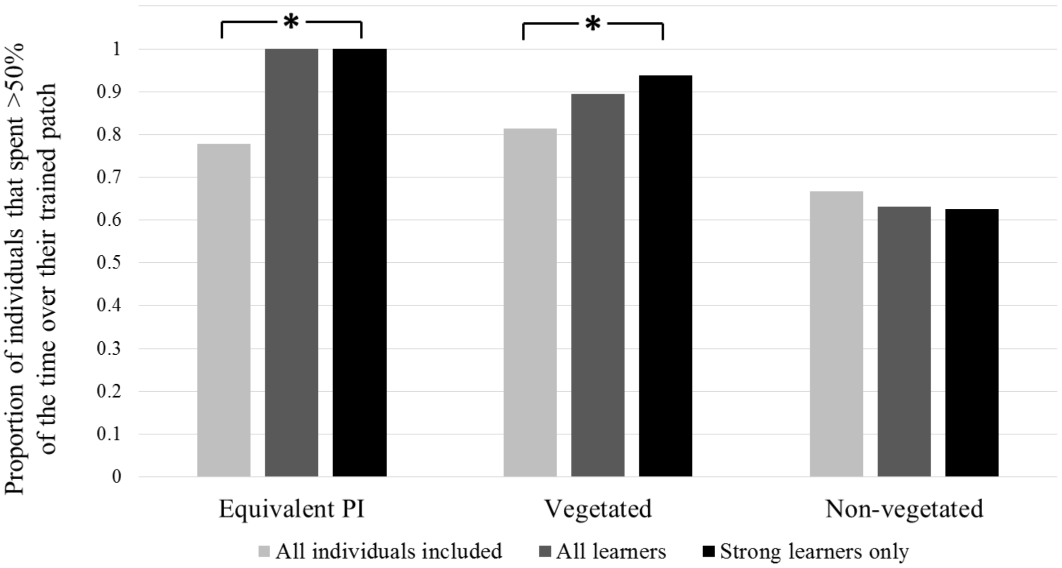


Figure 5. Bar graphs of the proportion of individuals in the population that preferred their trained patch for all experimental individuals, all learners (55% cutoff subset) and strong learners (65% cutoff). Asterisks (*) mark proportions of individuals that preferred their trained patch that is significantly different from 50%. (Note: all learners and strong learners are a proportion of 1 for the ‘equivalent public information’ trials because we selected them as having to prefer their trained patch in this treatment.)

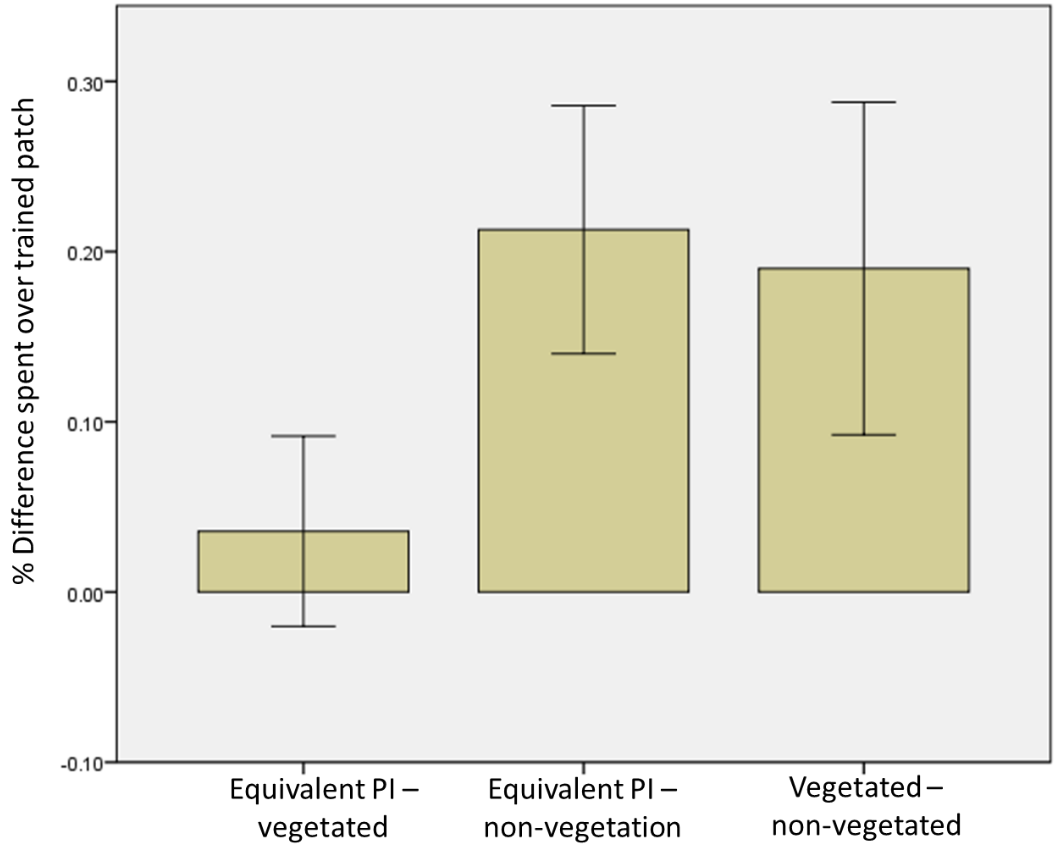


Figure 6. The difference between the % time spent over the trained side for all three combination of treatments (±SE). Individuals spent relatively more time over their trained side during the vegetation treatments than during the non-vegetated treatments. (Note: PI is public information)

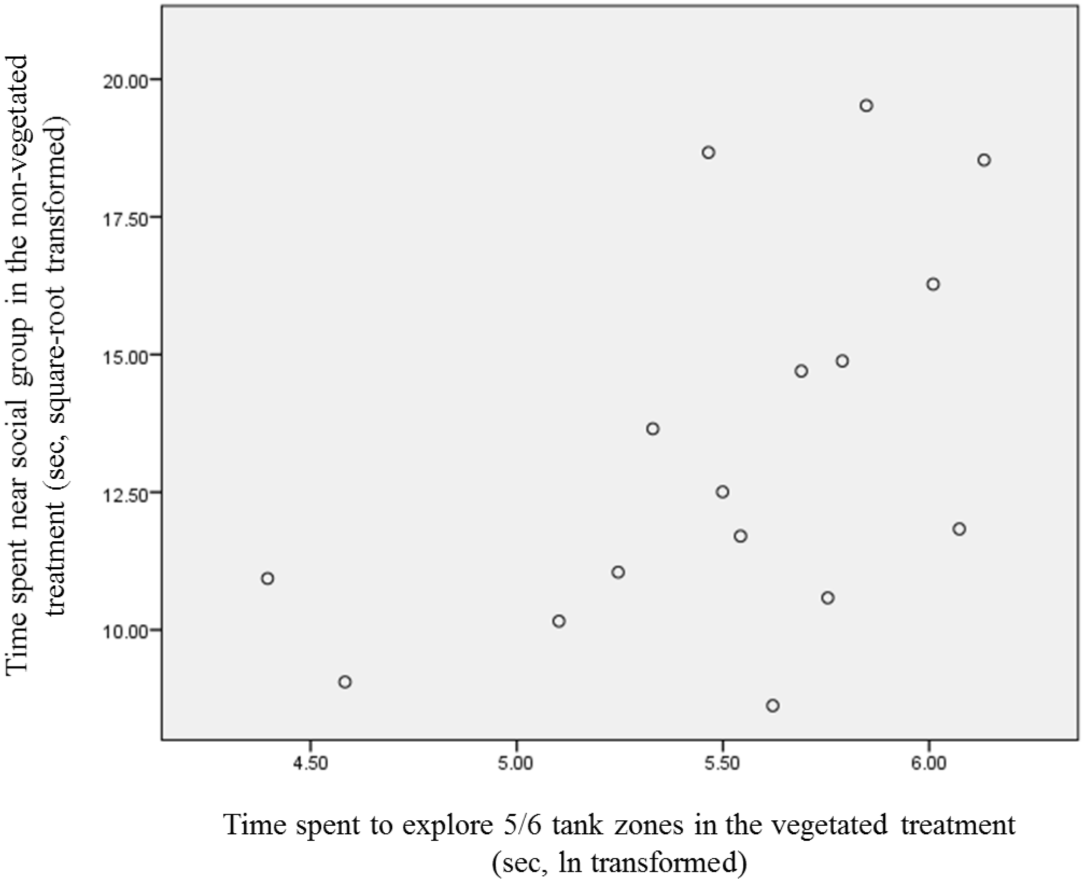


Figure 7. Scatterplot of exploration in the vegetated treatment and sociality in the non-vegetated treatment.

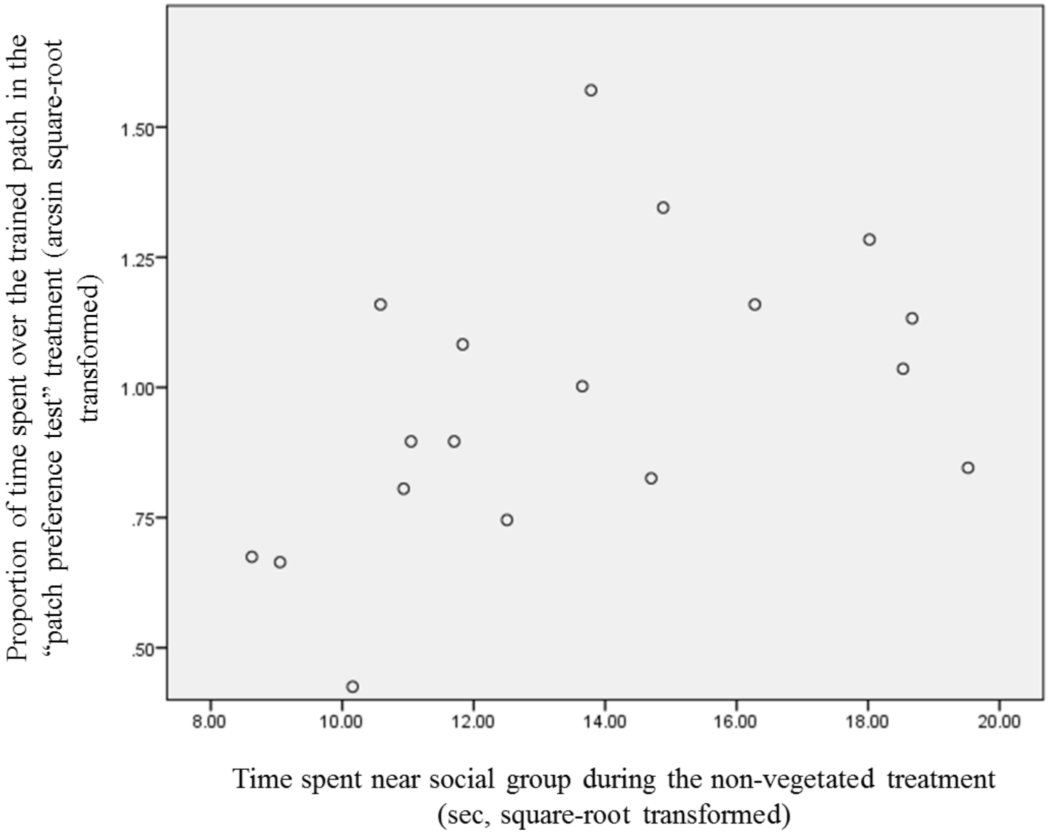


Figure 8. Scatterplot of sociality in the non-vegetated treatment and proportion of time spent over the trained patch in the "patch preference test" trial.

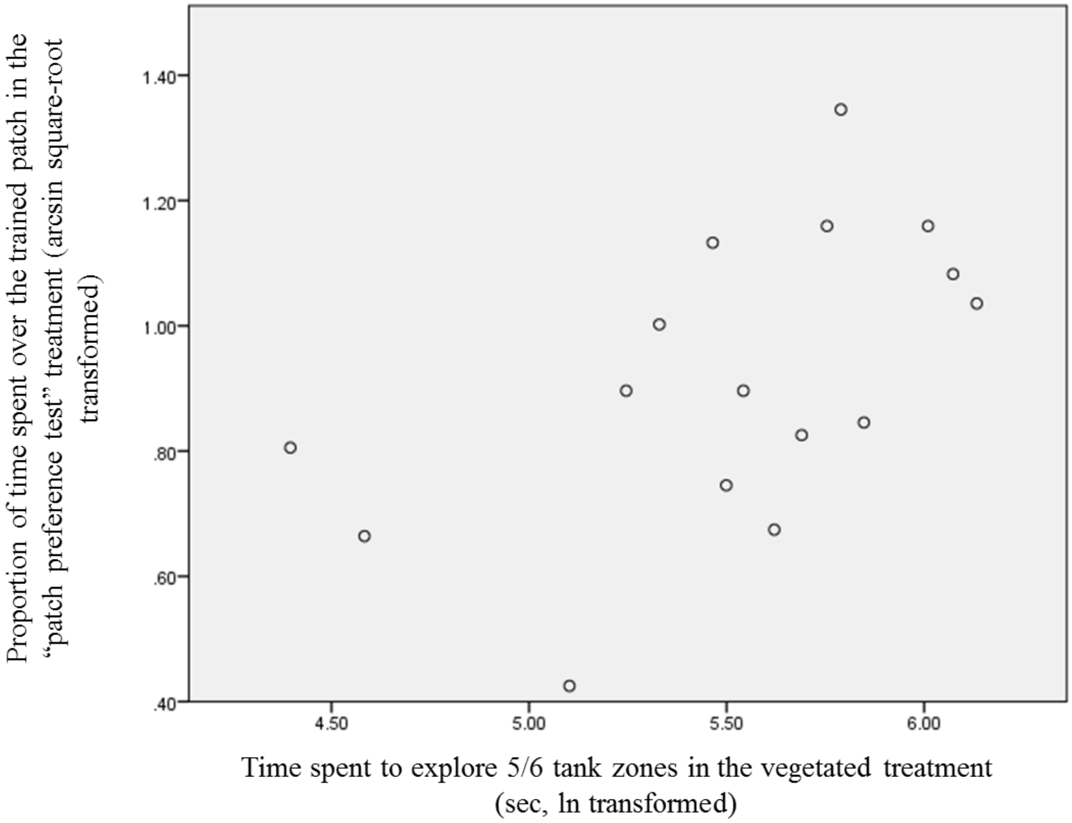


Figure 9. Scatterplot of exploration in the vegetated treatment and the proportion of time spent over the trained patch in the "patch preference test" trial.

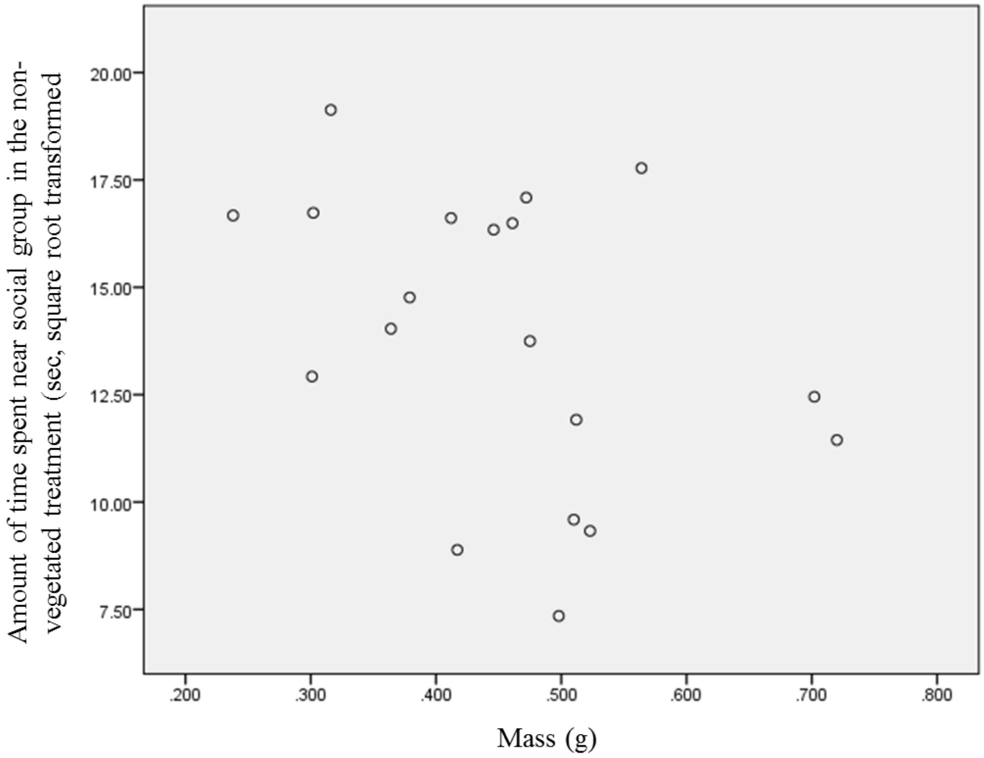


Figure 10. Scatterplot of mass and sociality in the non-vegetated treatments.

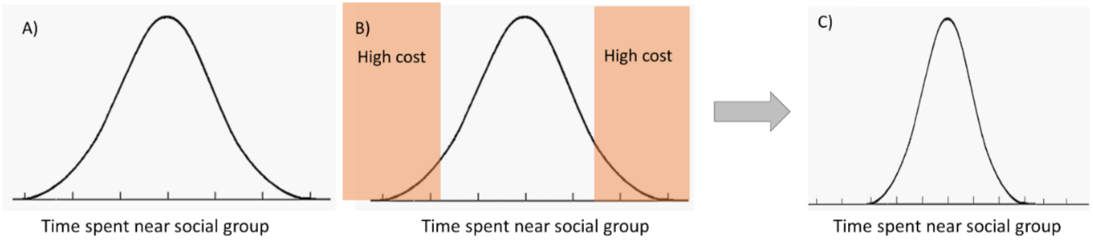


Figure 11. A). An individual may normally express a variety of behavioral trait values for a behavioral trait (e.g. sociality as pictured above) B). When risk is increased, the costs of expressing particular trait values may likewise increase. C) Selection therefore may favor a narrower distribution of behavioral trait values (e.g. not too social and not too asocial as pictured above), leading to a narrower distribution of trait values expressed in high risk. (Note: this is *not* representing stabilizing selection as these changes in behavioral variation occur within an individual's lifetime).

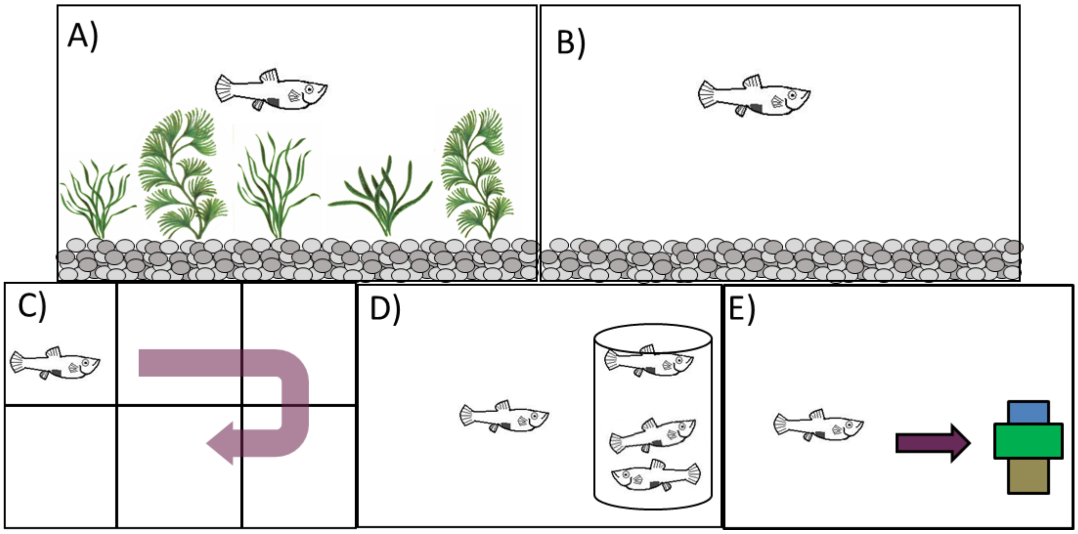


Figure 12. Experimental setup. All individuals were tested twice in A) vegetated (low risk) and B) non-vegetated (high risk) for all behavioral traits treatments. All individuals were measured for values of exploration C), sociality D) and boldness E) (protocol described above in text)

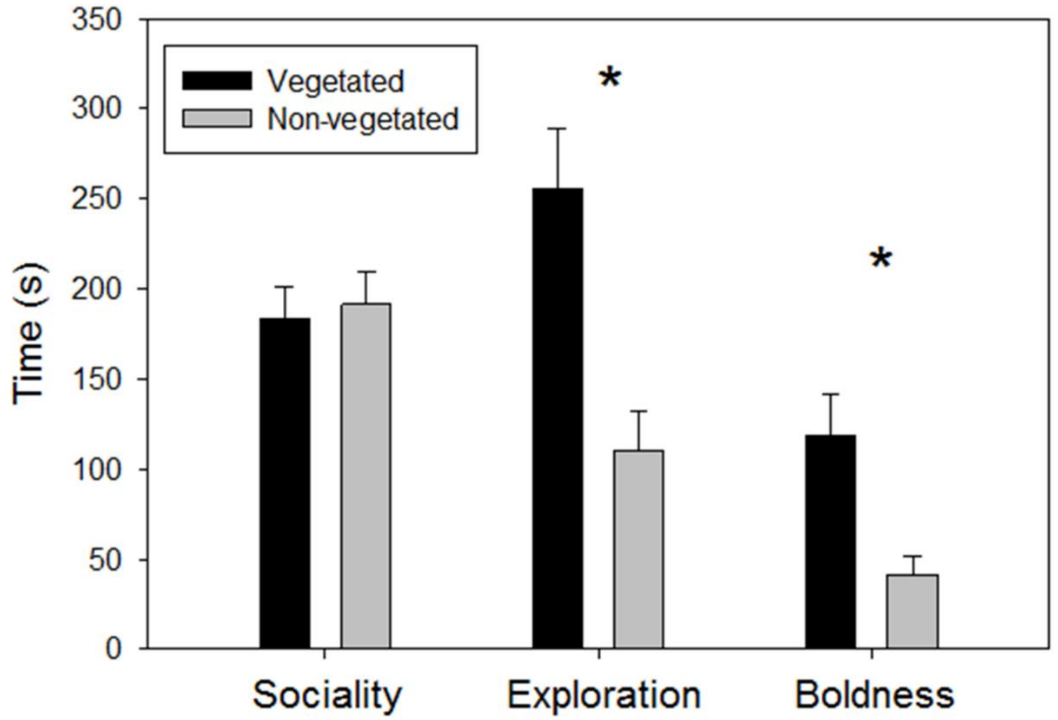


Figure 13. Bar graphs representing back transformed data for mean time (s) for all three behavioral traits + SE. Sociality: time spent near social group, exploration: time spent to explore 5 of 6 tank zones, boldness: time to approach a novel object within 1 body length

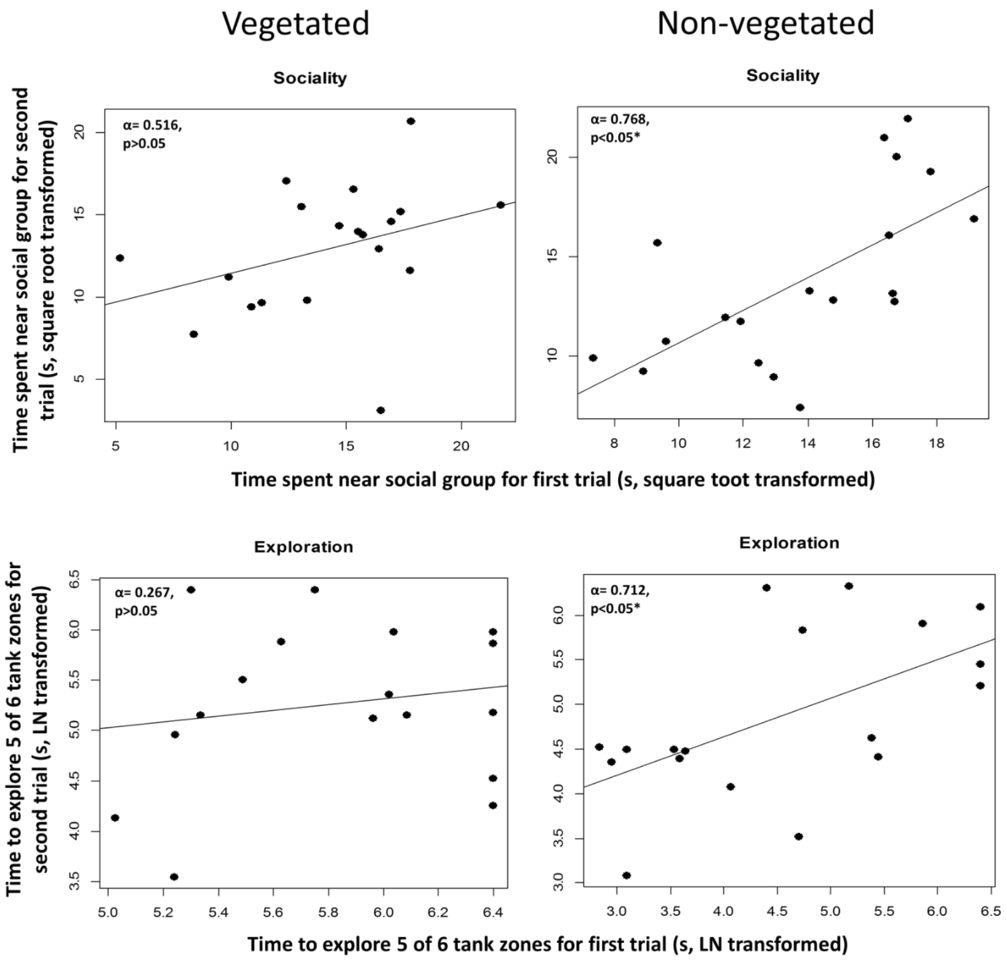


Figure 14. Times for sociality and exploration (seconds, transformed as mentioned above) for each trial for both the vegetated and non-vegetated treatments.

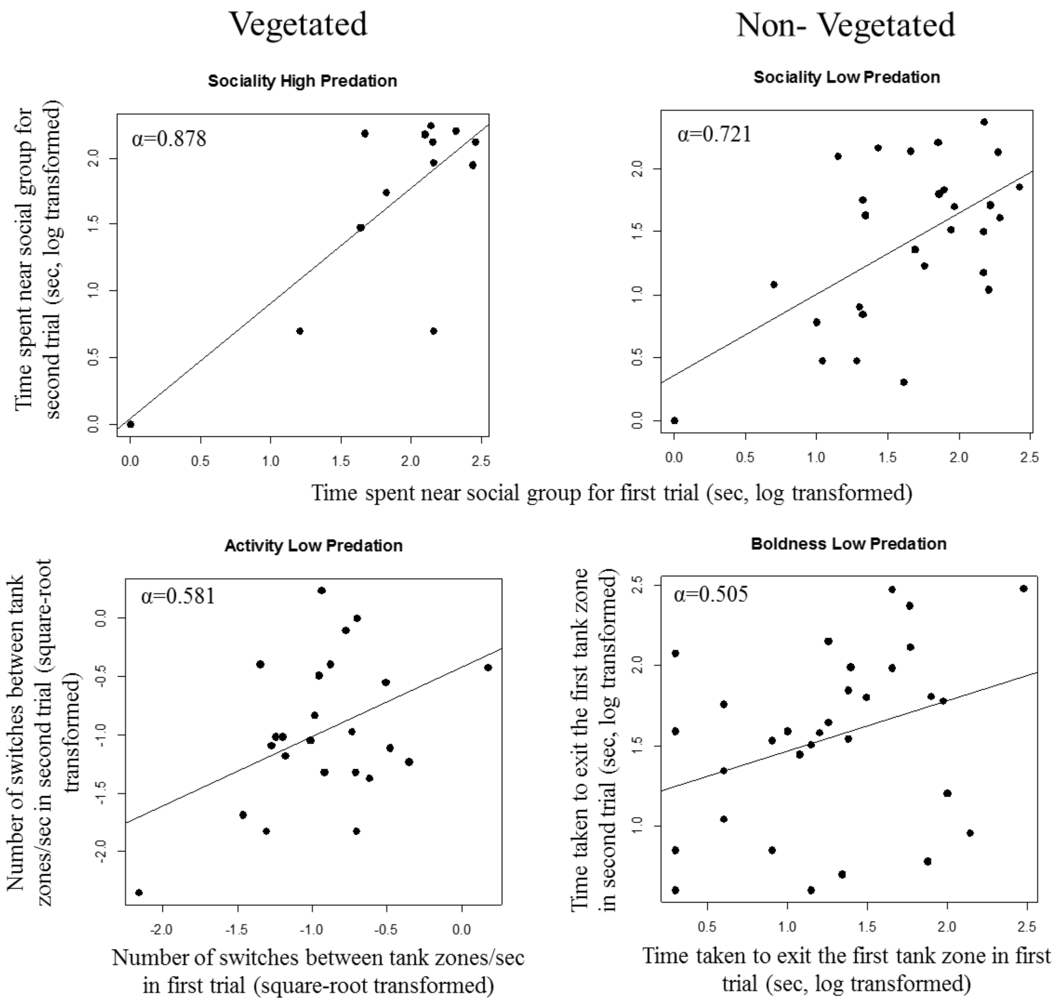


Figure 15. Scatterplots of all significant consistency values for the pooled data (high predation and low predation). Note: only sociality in the vegetated treatments showed significant consistency for high predation. All other graphs depict low predation behaviors.

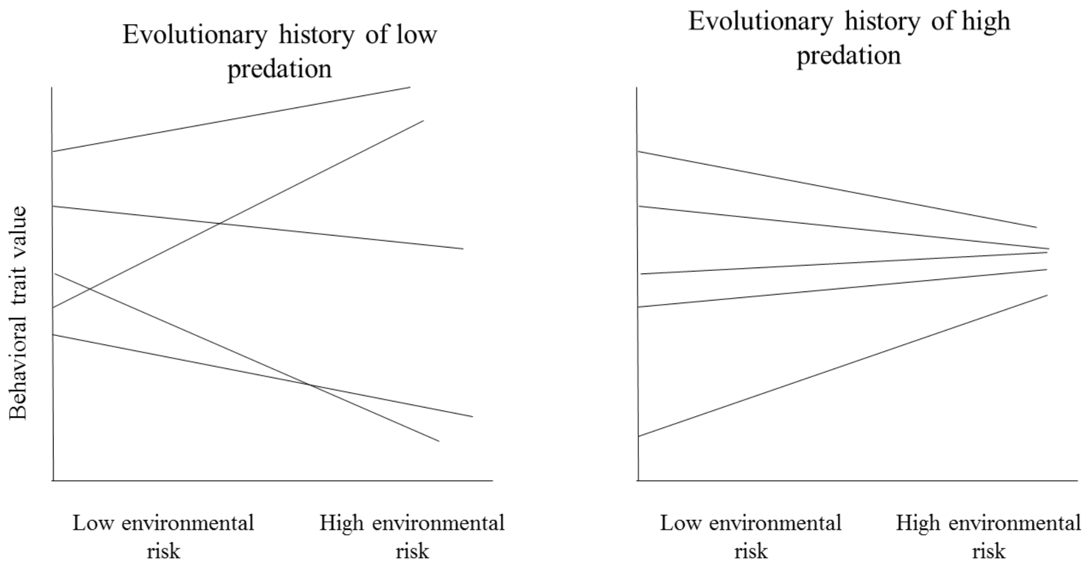


Figure 16. Theoretical relationship between among-individual variation in behavior (I) and environmental risk. It may be that selection favors differences in behavioral variation in high risk (high variation for low predation individuals and low variation for high predation individuals) based on their evolutionary history of predation. Note: this pattern may be caused by different mechanisms, for example cortisol may have large behavioral impacts for low predation individuals whereas group conformity may be driving the among-individual variance patterns seen in high predation populations.

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