BEHAVIORAL TYPE - ENVIRONMENT CORRELATIONS IN THREE-SPINED STICKLEBACK

BY

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DISSERTATION

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ABSTRACT

The aim of the research included in this dissertation is to contribute to our understanding of how consistent individual differences in behavior, so called animal personality, influences differences in how individuals within populations interact with their environment.

The specific aim of the work reported herein was to understand how non-random associations between the behavioral phenotype of individuals and the environment in which they occurred in the wild, hereafter referred to as behavioral type-environment correlations, affected important evolutionary and ecological processes in the three-spined stickleback (*Gasterosteus aculeatus*). In the first chapter I documented two such behavioral type-environment correlations, one between boldness and social environment and the other between exploratory behavior and habitat type. In the second chapter I test the hypothesis that behavioral type-environment correlations are generated via natural selection. My goal in the third chapter was to compare the predictive power of different factors that might explain why certain individual three-spined stickleback disperse greater distances than others, including exploratory behavior, social environment, habitat type, and physiological well-being. In the final chapter, I present an improved methodological approach for quantifying consistent individual differences in schooling behavior that utilizes a model 'school' in place of live conspecifics.

This work advances our understanding of how the ecological niche is shaped by the behavior of individuals. Through my own empirical evidence and a review of literature, I argue that behavioral type-environment correlations are likely to be prevalent in nature. I provide a framework for future research by describing various mechanisms that might generate behavioral type-environment correlations. Much of the fieldwork presented is aimed at elucidating the

causes and consequences of behavioral type-environment correlations in nature. For example in chapter two, I provide evidence that natural selection might generate a correlation between boldness and social environment. Chapter three provides a cautionary tale about jumping to conclusions concerning the ecological implications of consistent individual differences in behavior by showing that exploratory behavior is a relatively poor predictor of dispersal distance, a result that defies several previous field studies. Instead, the habitat in which the fish occured and its physiological well-being were better predictors of dispersal distance.

This dissertation is a step forward in research at the intersection of animal personality and ecological niche. The data, collected mostly in the field, fills an empirical gap in our understanding of animal personality, which has been based heavily on laboratory-based research. This collection demonstrates the important evolutionary and ecological implications of behavioral type-environment correlations. My hope is to inspire future work that teases apart the causes and consequences of behavioral type-environment correlations through fieldwork and carefully planned experimentation.

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CHAPTER 1: BEHAVIORAL TYPE - ENVIRONMENT CORRELATIONS IN THE FIELD: A STUDY OF THREE-SPINED STICKLEBACK

ABSTRACT

Behavioral type - environment correlations occur when specific behavioral types of individuals are more common in certain environments. Behavioral type - environment correlations can be generated by several different mechanisms that are probably very common such as niche-construction and phenotypic plasticity. Moreover, behavioral type - environment correlations have important ecological and evolutionary implications. However, few studies have examined behavioral type - environment correlations in natural populations. In this study, we asked whether some behavioral types of three-spined stickleback were more likely to occur in certain social environments (alone or in a shoal with other stickleback) or in certain microhabitats in a river (in the open or under cover). We found that individuals that were in shoals with other stickleback at the time of collection from the field emerged from a refuge more quickly compared to individuals that were found alone. In addition, fish that were alone in an open microhabitat explored more of a pool compared to fish that were alone in cover but this difference did not occur among fish that were in shoals at the time of collection. Subsequent analyses of gut contents suggested that differences in microhabitat use were consistent over time. Our study provides some of the first evidence for behavioral type - environment correlations in a natural population of non-human animals.

INTRODUCTION

Populations of animals are often comprised of individuals with different behavioral types (Bell et al. 2009). That is, individuals behave consistently across time and differently from one another (Sih and Bell 2008). In this study we adopt a statistical definition of behavioral type, namely that an individual's behavioral type is represented by their mean behavior and behavioral types exist when a statistically significant proportion of the total variance in a behavior can be attributed to differences among individuals (individual variance; Dingemanse et al. 2010). There is accumulating evidence that certain behavioral types are more likely to disperse (Dingemanse et al. 2003; Cote and Clobert 2007; Duckworth and Badyaev 2007; Chapman et al. 2011) or utilize a larger area of the habitat (Boon et al. 2008; Kobler et al. 2009). However, we know surprisingly little about whether certain behavioral types use particular niches within natural populations of non-human animals. A simple expectation, for example, is that timid individuals are more likely to occur in relatively safe habitats where predation risk is lower. Some authors have also suggested that certain behavioral types might occur more often in certain social environments (Bergmüller and Taborsky 2010).

When particular behavioral types occur more frequently in certain environments, there is a behavioral type - environment correlation (aka individual by environment correlation; Dingemanse et al. 2010; Stamps and Groothuis 2010b). Behavioral type - environment correlations can occur via numerous mechanisms, some of which are very common (Plomin et al. 1977; Stamps and Groothuis 2010a). Between-individual differences in behavior can lead to behavioral type - environment correlations. For example, individuals of a particular behavioral type might actively seek out certain environments (niche-picking; Stamps and Groothuis

2010a,b), potentially leading to an increase in fitness (Bateson 1988; Via 1999; Edelaar et al. 2008). Alternatively, an individual might influence its environment via niche construction (Odling-Smee et al. 1996; Donohue 2005). At the same time, within-individual plasticity might be a factor if the environment influences an individual's behavior. For example, being in a safe environment can encourage individuals to be bolder (Tuttle and Ryan 1982; Sharpe and Van Horne 1998; López et al. 2005; Webster et al. 2007; Peluc et al. 2008). Habitat-specific mortality can also lead to behavioral type - environment correlations if different behavioral types are more likely to survive in different environments (Jaenike and Holt 1991). Pleiotropic genetic variation for habitat choice and behavioral type could generate a behavioral type - environment correlation. The different mechanisms that can produce nonrandom associations between behavioral type and the environment are nonexclusive and might interact with each other. For example, an individual might select a certain environment according to its behavioral type and that environment might in turn influence the individual's behavior, potentially leading to positive feedback that reinforces the strength of the behavioral type - environment correlation (Stamps and Groothuis 2010a).

Behavioral type - environment correlations have important ecological and evolutionary implications. For example, if different behavioral types experience different environments, then the strength of selection will be unequal among members of the population. If individuals select environments for which they are particularly well suited (matching habitat choice; Edelaar et al. 2008; phenotype-matching habitat selection; Holt and Barfield 2008), then selection will be relatively weak compared to a situation where behavioral types are randomly distributed in the environment. Behavioral type - environment correlations generated by adaptive matching habitat choice provide a mechanism by which variation in behavioral type could be maintained within a population (Ravigné et al. 2003, 2009). Moreover, models of indirect genetic effects suggest that correlations between behavioral type and the social environment that have a heritable basis can lead to complex patterns of selection (Wolf et al. 1999; McGlothlin et al. 2010; Saltz and Foley 2011). Finally, behavioral type - environment correlations could potentially lead to divergent selection and ultimately reproductive isolation if certain behavioral types consistently select and experience selection in different environments (Rice 1987; Via 1999).

Although there are good examples of personality-environment correlations in humans (Rutter et al. 1997) and growing interest in behavioral types in animals (Réale et al. 2010), we are just beginning to learn about behavioral type - environment correlations in natural populations of non-human animals (Hensley et al. 2012). Perhaps the best example is a study showing that different behavioral types of pumpkinseed sunfish inhabited different parts of a lake, consumed different prey and were afflicted by different parasite communities (Wilson et al. 1993). This study suggested that an individual's behavioral type was part of a much larger, ecologically-relevant package of characteristics that were related to habitat use. A related literature is showing that intraspecific niche variation is widespread (Skulason and Smith 1995; Bolnick et al. 2003) and differences in niche use are often accompanied by differences in morphology, life history and/or foraging tactics that form an integrated suite of traits that are adaptive (Bentzen and McPhail 1984; Ehlinger 1990; Schluter and McPhail 1992; Skúlason et al. 1993). Individuals that differ in morphology often preferentially assort into different social environments (Ranta and Lindström 1990; Griffiths and Magurran 1999; Brown and Brown 2000; Ward et al. 2002, 2005) but grouping according to behavioral type has received less attention (but see Sih and Watters 2005). Recently, laboratory studies on *Drosophila melanogaster* (Saltz 2011; Saltz and Foley 2011) provided empirical evidence for a heritable basis to a behavior - social environment correlation. Altogether, these studies suggest that behavioral type - environment correlations might be common but they have rarely been explicitly examined in non-human populations in nature.

Therefore, in this study we asked whether certain behavioral types were more likely to occur in certain microhabitats and in certain social environments in three-spined stickleback (*Gasterosteus aculeatus*). Three-spined stickleback (stickleback hereafter) are small teleost fish that are especially well suited to the study of behavioral type - environment correlations because they exhibit pronounced within-population variation in niche use (Bentzen and McPhail 1984) and behavioral type (Bakker 1986). Moreover, juvenile stickleback are of particular interest because differences in the early environment can have strong effects on phenotypic development (Peuhkuri et al. 1995; Day and McPhail 1996; Bell et al. 2011) and therefore fitness later in life (Wootton 1973). We collected juvenile stickleback in different microhabitats and social environments in the field and measured their behavior in a standardized behavioral assay in the lab. In this behavioral assay, we recorded two behaviors; latency to emerge from a refuge (*latency to emerge*) and the number of sections of the test pool an individual explored (*number of sections*). Our rationale for focusing on these variables was that we expected that 1) remaining in a refuge or 2) restricting movement to new sections of the environment are two separate strategies that can decrease the likelihood of encountering predators (Sih 1987). We also recorded the number of *transitions* from one section of the pool to another as a measure of general activity so that we could avoid confounding differences in refuge use or exploration of the environment with differences in general activity. We tested a subset of these individuals repeatedly in the behavioral assay so that we could test for the statistical signature of behavioral types, repeatability, in both *latency to emerge* and *number of sections*. We then used these data to ask whether particular behavioral types (i.e. individuals that emerged more quickly or explored more sections) were more common in certain microhabitats or certain social environments in the field. We also analyzed the diet of a sample of these individuals in order to assess the stability of microhabitat use in nature and to determine if *latency to emerge* or *number of* sections were correlated with gut fullness or prey type.

METHODS

Use of environment in the field

Juvenile three-spined stickleback from the Navarro River, CA were the focus of this study (less than two months post-hatch, standard length = 21.2 ± 4.2 SD mm, N = 58). During preliminary snorkeling surveys of an approximately 100 m section of the river, we noticed that juvenile stickleback occurred in different social environments: some individuals moved through the habitat close to conspecifics in groups known as shoals (within four body lengths of at least 2 conspecifics, < 12 cm; Pitcher and Parrish 1993), while others were alone, i.e. no other stickleback within 50 cm. We also observed differences in microhabitat use. Much of the Navarro River is moderately shallow (< 1 m deep) and can be classified into either open microhabitat (shallow, gravel substrate, free of vegetation) or cover microhabitat (dense cover from grasses and submerged tree branches). While there were a variety of other microhabitats in the river (e.g. riffles and deep pools), juvenile three-spined stickleback rarely occurred outside of open or cover microhabitats. Therefore, after preliminary observations of juveniles in the field, we decided to collect individuals from four different categories: open alone (n = 14), open shoal (n = 13), cover alone (n = 14) and cover shoal (n = 17). Animals were collected with a trout landing net while snorkeling. Specifically, a focal individual was observed from a distance of approximately 2 m for at least 30 seconds to assign its social environment and microhabitat. We assume that the fish were not reacting to the observer during field observations because they maintained a constant level of foraging and did not orient toward or move away from the observer. One fish that began alone moved to a shoal during this observation period and was therefore excluded from the study (< 2% of the sample). No fish that was initially in a shoal moved away from the shoal during field observations. Collections alternated between open and cover microhabitats which were positioned on opposite banks of the river throughout a 100 m stretch (< 2 m separation between cover and open at any point) and the same location was not sampled more than once per day to avoid collecting multiple individuals from one shoal. Sampling was not biased towards more 'catchable' individuals as no focal individual escaped after observation (Biro and Dingemanse 2009).

Behavioral type

To quantify behavioral types of individuals, we first moved the fish to standardized holding chambers to minimize differences in the environment that could affect behavior. We transported the fish 14 km from the river by car to an outdoor area where observations would take place. Fish were visually isolated from each other in separate 0.5 L holding chambers within a larger 50 x 80 cm pool located outside and exposed to natural fluctuations in ambient light. We did not feed the fish in order to standardize hunger levels. Analysis of gut contents of a subset of these individuals showed that total number of prey in the gut at the time of collection was not related to behavioral type, microhabitat, or social environment (see *'Diet analysis')*. The first behavioral assay trial began at least one hour after the individual was transferred to the holding chamber, and was carried out in the afternoon. We euthanized a subset of these individuals after trial one to allow for analysis of gut contents (open alone n = 6, open shoal n = 7, cover alone n = 7, cover shoal n = 6; see ‘Diet analysis’). The remaining individuals were run through the behavioral assay two additional times so we could calculate the repeatability of these behaviors. Trials two and three were carried out on the following day 15-20 h and 18-26 h after trial one, respectively, and were separated by at least one hour. All trials were completed within 28 h of collection from the river.

We employed a standardized behavioral assay to quantify the behavior of individuals. This behavioral assay combines elements of the open field test (Walsh and Cummins 1976; modified in Verbeek et al 1994) as well as a refuge emergence test, which is frequently used in studies of animal personality (Hedrick 2000; Brown et al 2005; Wilson et al. 2010; Cote et al. 2011). An individual was gently poured into an opaque cylindrical refuge (10 cm diameter, 10 cm height) where it was allowed to settle for three minutes. The refuge was in the center of a circular, plastic pool (150 cm diameter, 10 cm water depth) marked into nine equally-sized sections (one circular section in the middle surrounded by eight identical sections). Each of the outer sections contained a small pile of rocks the fish could explore but to move between any two sections the fish had to cross an area with no substrate where it was highly conspicuous. Data was recorded via direct observation by one researcher (SP) who was hidden behind a blind with a small opening. After three minutes, we opened the side of the refuge remotely and recorded the time it took the individual to emerge completely (*latency to emerge*), which we interpret as willingness to trade the safety of the refuge for the opportunity to locate resources. If a fish did not emerge within ten minutes (n = 1 individual), then the individual was gently poured out of the shelter into the pool and assigned the maximum *latency to emerge* value of ten minutes. For three minutes after the fish emerged from the refuge, we recorded the total number of sections visited (*number of sections*) as a measure of how thoroughly the individual explored the environment. To control for differences in general activity, we also recorded the number of times the fish transitioned from one section to another regardless of whether they had been there before (*transitions*). Activity has been considered as an important axis of behavioral variation in studies of animal personality but interpreting both number of *transitions* (activity) and *number of sections* (exploratory behavior) would be inappropriate as they were measured simultaneously (Réale et al. 2007). We recorded the time of day at the beginning of each trial and length from tip of the mouth to the base of the caudal fin (standard length) after the last trial. Fish from different environments did not differ in size (standard length in mm ± SE: alone = 21.5 ± 0.7, shoal = 20.9 ± 0.9, cover = 21.3 ± 0.8, open = 21.1 ± 0.8). The order of testing of individuals was haphazard. Approximately equal numbers of fish from each environment category were observed each day. All field observations and behavioral assays were carried out between July 10-19, 2010.

We calculated the repeatability of *latency to emerge* and *number of sections* to test whether a significant amount of the variation in these variables could be attributed to differences between individuals. Repeatability is a dimensionless statistic that compares between-individual variance to total variance and therefore shows the amount of overlap in the behavior of different behavioral types. We calculated the repeatability of *latency to emerge* and *number of sections* from restricted maximum likelihood mixed models with individual as a random effect and the population intercept as a fixed effect. We used parametric bootstrapping (1000 bootstraps) to estimate 95% confidence intervals (Nakagawa and Schielzeth 2010). All N = 58 individuals including those that had only one trial of data were used in this calculation to improve the power of our repeatability estimate (Martin et al. 2011).

We tested whether *latency to emerge* or *number of sections* differed between individuals that occurred in different social environments or microhabitats using linear mixed models. We examined the normality of the data through visual inspection of the residuals. *Latency to emerge* data were right skewed and thus were +1 log transformed to improve normality before analysis. *Number of sections* were normally distributed. We used repeated measures models with individual as a random effect. We included microhabitat, social environment and their interaction as fixed effects. Standard length was included as a covariate. We included mean number of *transitions* of each individual and the deviation from the individual's mean of each trial as covariates to control for between- and within-individual differences in activity, respectively (within-subject centering; van de Pol and Wright 2009; Dingemanse and Dochtermann 2013). Degrees of freedom were determined by Satterthwaite approximation.

Diet analysis

Stability of microhabitat use. We analyzed the gut contents of a subset of our sample to gain insight into the consistency of microhabitat use (open alone n = 6, open shoal n = 7, cover alone n = 7, cover shoal n = 6). Our rationale for measuring gut contents is that we expected that fish foraging in different habitats were eating different prey types. Therefore, if there was a difference between the whole gut contents of fish from open vs. cover microhabitat that would suggest that microhabitat use is at least as enduring as the time to evacuate the gut (at least 6 hours; Svanbäck and Bolnick 2007). Furthermore, Bolnick et al. (2008) found that within-population differences in gut contents in stickleback were related to differences in stable isotopes and therefore reflect long-term differences in habitat use.

As stated above, 26 individuals were euthanized after the first behavioral assay within two hours of collection to cease the digestion of gut contents. Standard length was taken immediately following euthanasia. We identified gut contents to the lowest feasible taxonomic level (Chironomidae, Ephemeroptera, Trichoptera, Plecoptera, Arachnida, Bivalvia). We focused our analyses on two taxa, Chironomidae and Ephemeroptera, which comprised 95% of the 446 prey items identified (SP, unpublished data).

We examined the normality of gut contents data through visual inspection of the residuals. Counts of Chironomidae were square root transformed to improve normality. Counts of Ephemeroptera and total number of prey items were normally distributed. We tested for differences in total number of prey items, number of Chironomidae, and number of Ephemeroptera between fish collected in open and cover microhabitats using general linear models that included microhabitat as a fixed effect and standard length as a covariate.

We conducted an invertebrate survey on the last day of the study to determine the composition of prey types in each microhabitat. Invertebrate samples were taken with a kicknet and a 10cm diameter stovepipe sampler. Kicknet samples were taken by disturbing the bottom substrate 1m upstream of the net. The stovepipe sampler was used to take a 10cm diameter column of the substrate that was transferred to a tray for invertebrate sorting. Six samples were taken with each sampling method in each microhabitat.

Diet of behavioral types. We tested whether differences in behavior type were related to diet or hunger (Godin and Crossman 1994) by calculating Kendall's tau-b rank correlations between number of Chironomidae, number of Ephemeroptera, total number of prey items and behavior (*latency to emerge, number of sections*). This correlation coefficient was used to correct for frequent rank ties. Correlations were analyzed separately for each microhabitat type to control for differences in prey availability between microhabitats. Calculations of repeatability and bootstrapping were performed with the rptR package in R (Nakagawa and Schielzeth 2010). All other statistical analyses were conducted using SPSSv19.0.0.1. Procedures were carried out in accordance with Institutional Animal Care and Use Committee at the University of Illinois, IACUC protocol #09204.

RESULTS

Behavioral type

We found consistent individual differences in behavior in a standardized assay. While some individuals emerged from the refuge within four seconds, others took up to 10 minutes (mean *latency to emerge* = 65.5 ± 13.0 SE s). After emergence, some individuals moved through all nine sections of the pool, while others explored only two (mean *number of sections* = 5.9 ± 0.2 SE). The variation among individuals in their behavior was consistent over time. The repeatability of *latency to emerge* was $R = 0.38$ (95% CI: 0.14-0.58, $p < 0.001$) and the repeatability of *number of sections* was $R = 0.26$ (95% CI: 0.02-0.46, $p = 0.019$).

Behavioral type - environment correlations

Fish found in shoals with other stickleback emerged from the refuge faster compared to fish that were captured while alone (social environment, $F_{1,43.2} = 10.3$, $p = 0.003$, Table 1.1, Figure 1.1). Smaller fish emerged faster compared to larger fish (Table 1.1). Between- and within-individual variation in number of *transitions* were both related to *latency to emerge* in the negative direction (Table 1.1). *Latency to emerge* did not differ between fish from different microhabitats.

In general, fish from open microhabitats explored more sections than fish from cover microhabitats (microhabitat, $F_{1,\,50.4} = 5.8$, $p = 0.019$, Table 1.1). This effect was driven by an interaction between microhabitat and social environment (microhabitat * social environment, $F_{1,\,48.5} = 9.4$, $p = 0.003$, Table 1.1, Figure 1.2). Examination of the estimated marginal means showed that, among fish that were alone, those from an open microhabitat explored more sections of the pool compared to fish from a cover microhabitat while there was no difference across microhabitats among fish that occurred in shoals (estimated marginal means 95% CI: open alone 6.0-6.8, cover alone 4.8-5.7, open shoal 5.3-6.2, cover shoal 5.5-6.3*). Number of sections* was positively related to between- and within-individual variation in number of *transitions* (Table 1.1). Size was not a significant factor (Table 1.1).

Diet analysis

Stability of microhabitat use. Diet was related to microhabitat use: gut contents of individuals from a cover microhabitat contained fewer Ephemeroptera ($F_{1,23} = 13.6$, $p = 0.001$) and trended towards a greater number of Chironomidae ($F_{1,23} = 4.1$, $p = 0.053$, N.S.) compared to individuals from open microhabitats (Figure 1.3). These data were consistent with the relative abundance of prey found via invertebrate sampling in each microhabitat (number of Chironomidae in open = 51, cover = 84; number of Ephemeroptera in open = 40, cover = 4). We did not detect a difference in the average number of total prey items between fish from the cover vs. open microhabitats ($p = 0.28$; cover = 18.1 ± 3.0 SE; open = 13 ± 3.1 SE). Diet was not related to standard length (all $p > 0.18$).

Diet of behavioral types. We tested whether diet was quantitatively related to behavioral type. Among fish from cover microhabitats, individuals that explored a greater *number of sections* had more Ephemeroptera in their guts (tau = 0.69, $p = 0.007$, n = 13). We interpret this correlation with caution, as it did not achieve significance at the Bonferroni corrected level ($p \leq 0.004$). Behavioral type was otherwise unrelated to diet (Table 1.2).

DISCUSSION

This study provides evidence for behavioral type - environment correlations in a natural population. We found evidence that differences in *latency to emerge* from a refuge and *number of sections* of a pool explored were repeatable over time, which indicates that, while there is overlap in the behavior of different behavioral types, a significant proportion of variation is due to differences between individuals. Behavioral types that emerged from the refuge relatively quickly were more likely to occur in shoals in the field. In addition, fish that were alone in an open microhabitat explored more sections of the pool compared to fish that were alone in cover but this difference did not occur among fish that were in shoals at the time of collection.

An issue with studies of individual differences in behavior in the field is that, if behavioral observations are conducted in the animal's natural habitat, we cannot determine whether differences between individuals are due to differences in the environment, i.e. plasticity (Martin & Réale 2008). Indeed, phenotypic plasticity is widespread (West-Eberhardt 2003) and future studies should utilize the behavioral reaction norm framework to tease apart the role of the environment in generating inter-population differences in behavior (Dingemanse et al. 2010). However, in this study we sought to control for differences in the environment by standardizing the holding environment and behavioral tests of all individuals. Furthermore, differences in behavior between individuals that occurred in different environments in the river did not decrease over time and were not related to size or overall number of prey items in the gut, which suggests that these differences did not reflect a carryover of environmental effects. Instead, our findings suggest that individuals with enduring differences in behavior occurred in different environments in nature.

We detected a behavioral-type environment correlation between social environment in the field and *latency to emerge* from a refuge: individuals that were in shoals at the time of collection were relatively fast to emerge from a refuge compared to individuals that were alone. There are mixed results in the literature about the relationship between ‘boldness’ and social environment (Budaev 1997; Ward et al. 2004; Rödel et al. 2006; Pike et al. 2008; Cote et al. 2010, 2011). One possible explanation for the pattern observed in this study is that individuals that do not join shoals are willing to accept the predation risk of being alone (Krause and Ruxton 2002), but compensate for their increased vulnerability by relying more heavily on other anti-predator strategies, such as hiding in refuges. There are important evolutionary implications of this type of phenotypic plasticity: if individuals that occur in environments that put them at a heightened risk of predation compensate by behaving cautiously, that could influence patterns of selection because it can decrease variance in fitness between individuals (Dewitt et al. 1999; Hedrick 2000; Fowler-Finn and Hebets 2011).

Larger and less active (lower mean number of *transitions*) individuals remained in the refuge longer compared to smaller and more active individuals. Our results are consistent with theory, which states that individuals that maintain larger energy reserves can afford to remain in a refuge for longer to reduce their exposure to predators (Dill 1987). Ours is not the first study to support the hypothesis that refuge use is state-dependent. Krause et al. (1998) found the same positive relationship between body size and refuge use. Killen et al. (2011) recently showed that individuals with higher metabolism that depleted their energy reserves faster spent less time in a refuge compared to low-metabolism individuals. Accelerated depletion of reserves might also explain why individuals that are more active in general emerged earlier in our study. This explanation does not, however, account for the significant effect of within-individual variation in *transitions* on refuge use; when an individual's *latency to emerge* from the refuge decreased, that individual increased its activity. Inactivity was not required for the juvenile stickleback to remain in the shelter (i.e. emergence did not appear to be inadvertent). Instead, this within-individual correlation provides evidence that an underlying mechanism links plasticity in refuge use and activity.

We detected a relationship between microhabitat and the *number of sections* of a pool explored among fish that were alone: individuals from open microhabitats that were alone consistently explored more of the pool compared to fish that were alone in cover at the time of collection. Fish that were in shoals at the time of collection did not show this difference across microhabitats and instead explored an intermediate *number of sections*. This correlation between habitat use and exploratory behavior might reflect niche-picking; perhaps intermediate behavioral types move to shoals while particular extreme behavioral types preferentially move to different microhabitats. This complex behavioral type - environment association might be adaptive if individuals are moving to environments that increase their fitness. Adaptive niche-picking has ecological and evolutionary implications that are just beginning to be appreciated (Edelaar et al. 2008). For example, phenotype-dependent habitat selection can maintain genetic diversity (Levene 1953; Van Valen 1965) and increase the rate of local adaptation and adaptive speciation (Via 1999; Bolnick et al. 2003). Distinguishing between different mechanisms that generate behavioral type - environment correlations is an important task for future work (Edelaar et al. 2008; Stamps and Groothuis 2010a,b).

In our interpretation of behavioral type - environment correlations, we hypothesize that the behaviors we quantified, refuge use and exploration of the environment, have fitness consequences. However, experiments that explicitly test whether these behaviors affect differences in predation risk in nature are a necessary step toward developing hypotheses about the evolutionary consequences of behavioral type - environment correlations (Adriaenssens and Johnsson 2011).

We provide indirect evidence that the conditions under which we collected individuals in the field reflect stable habitat use. Fish from open microhabitats had more Ephemeroptera in their guts, which is consistent with the higher abundance of Ephemeroptera in open microhabitats. Likewise, Chironomidae were more abundant in cover microhabitats and fish found in cover had consumed more Chironomidae. These data suggest that stickleback had been in their respective microhabitats at least as long as it takes to digest gut contents (at least 6 hours, Svanbäck and Bolnick 2007) and potentially over much longer periods (Bolnick et al. 2008). Moreover, other populations of sticklebacks exhibit consistent intraspecific variation in diet and microhabitat use (benthic-limnetic; Larson 1976; Bentzen and McPhail 1984), lending plausibility to the claim that the variation in microhabitat use that we observed in this study is relatively long-lasting. Interestingly, we also detected evidence that individuals that explored a greater *number of sections* might be more likely to move between microhabitats: among the individuals collected in cover microhabitats, those that explored more sections had more Ephemeroptera in their guts, suggesting that they might recently have been in the open microhabitat where Ephemeroptera are more abundant. Systematic studies that track the microhabitat use of different behavioral types of individuals in the field are a priority for future work and may provide interesting insights into ecological effects of different behavioral types.

We assumed that our brief observations adequately characterized differences in social environment among individuals. More rigorous field studies that follow individuals over time are required to validate this assumption. However, a recent study showed that there is a heritable basis to shoaling behavior in three-spined stickleback (Wark et al. 2011). Therefore some of the observed behavioral variation in shoaling behavior might reflect genetic variation that predisposes certain individuals to be more likely to occur in certain social environments. We leave it to future studies to determine whether particular individuals do indeed occur in certain social environments more than expected by chance.

The observed behavioral type - environment correlations represent a departure from the simple assumption that behavioral types are randomly distributed in the environment. Although this study was conducted on a short temporal scale, we do not view our results as trivial. Even a short-term deviation from a random distribution could be ecologically very important. For example, important selective events often happen instantaneously, e.g. being depredated or parasitized, and are probably more likely to occur in certain environments. Therefore if certain behavioral types (e.g. fast emergers) occur more often in a particular environment (e.g. a shoal) because they make short forays to that environment, selection will be different for different behavioral types. For example, a stickleback that is more likely to join a shoal for any length of time might be more likely to be afflicted by a parasitic copepod that is transmitted via close contact between individuals (Poulin 1999).

In conclusion, despite the fact that the mechanisms that generate behavioral type - environment correlations are probably widespread (West-Eberhard 2003; Edelaar et al. 2008; Stamps and Groothuis 2010a) and growing evidence for consistent individual differences in behavior (Bell et al. 2009), we know little about whether particular behavioral types are more likely to occur in certain environments in nature. This study provides evidence for behavioral type - environment correlations in a snapshot of time in one location. By presenting these data, we hope to stimulate work that will improve on our study by 1) increasing sample sizes at the individual and population level, 2) tracking the use of different environments and changes in behavior across development and 3) teasing apart the mechanisms that generate behavioral type - environment correlations to determine whether they affect ecological or evolutionary processes.

FIGURES AND TABLES

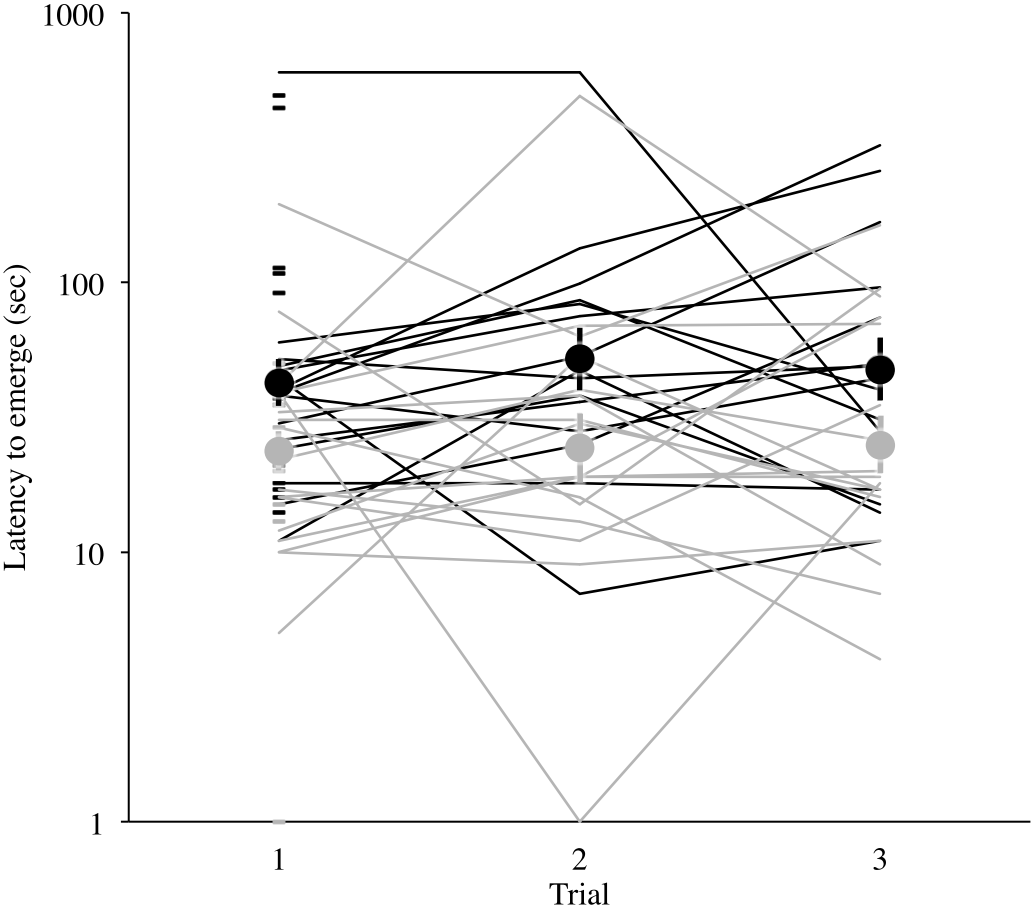

Figure 1.1. Latency to emerge. Fish that were in a shoal at the time of collection emerged from the refuge faster than fish that were collected while alone. *Latency to emerge* is plotted on the Y-axis on a logarithmic scale as analysis was on +1 log transformed data. Black symbols represent fish that were alone at the time of collection, gray symbols represent fish that were in shoals at the time of collection. Dashes represent individuals that were assayed once. Lines show individuals that were assayed three times. Means ± one standard error are represented by circles with vertical bars. Means and standard errors were calculated with log transformed data and back transformed to the raw data scale. N = 1 test of 26 individuals, 13 shoal, 13 alone. N = 3 tests of 32 individuals, 17 shoal, 15 alone

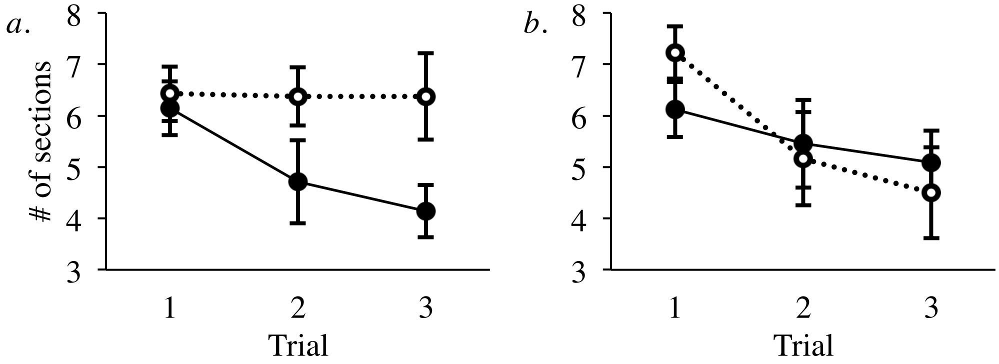

Figure 1.2. Number of sections. Among fish that were collected while alone, those in an open microhabitat explored more sections of the pool compared to fish found alone in a cover microhabitat. Data are separated into *a*. fish that occurred alone in the field (open alone n = 14, cover alone n = 14) and *b*. fish that occurred in shoals (open shoal n = 13, cover shoal n = 17). Dotted lines with open circles represent means for fish from open microhabitats. Solid lines with filled circles indicate individuals from cover microhabitats. Error bars are ± one standard error

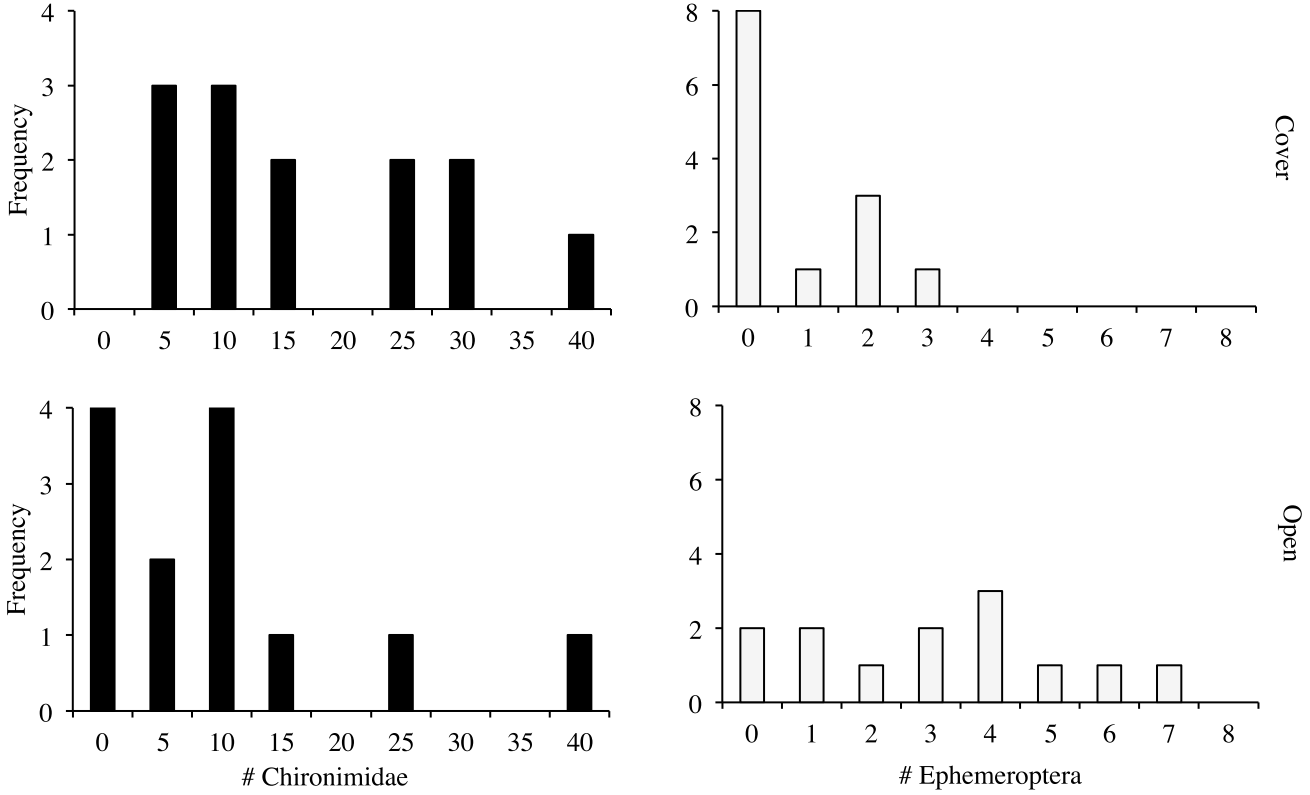

Figure 1.3. Gut contents. Comparison of the distributions of numbers of Chironomidae and Ephemeroptera in the guts of individuals from the open and cover microhabitats. Note that the scale of the X and Y axes differ between prey types. Open n = 13. Cover n = 13

Behavior	Factor	Estimate	Estimate SE	d.f.	Statistic	*p*-value
Latency to emerge	Microhabitat	0.09	0.10	1, 44.2	1.6	0.207
	Social environment	0.23	0.11	1, 43.2	10.3	**0.003***
	Microhabitat * Social environment	0.02	0.15	1, 42.4	0.0	0.902
	Transitions (between-individual)	-0.04	0.01	1, 48.4	33.3	**<0.001***
	Transitions (within-individual)	-0.02	0.01	1, 67.7	4.7	**0.034**
	Standard Length	0.04	0.01	1, 39.9	18.8	**<0.001***
	Individual (between-individual)	0.02	0.02	NA	1.2	0.227
	Individual (within-individual)	0.11	0.02	NA	5.8	**<0.001***
Number of sections	Microhabitat	0.14	0.30	1, 50.4	5.8	**0.019***
	Social environment	0.69	0.32	1, 49.4	0.0	0.902
	Microhabitat * Social environment	-1.32	0.43	1, 48.5	9.4	**0.003***
	Transitions (between-individual)	0.28	0.02	1, 55.1	177.2	**<0.001***
	Transitions (within-individual)	0.27	0.02	1, 76.7	139.4	**<0.001***
	Standard Length	-0.05	0.03	1, 45.8	3.4	0.072
	Individual (between-individual)	0.09	0.13	NA	0.7	0.458
	Individual (within-individual)	1.11	0.18	NA	6.2	**<0.001***

Table 1.1. Linear mixed models for *latency to emerge* and *number of sections*. Models were run with microhabitat and social environment as fixed effects, transitions and standard length as covariates, and individual as a random effect. The statistic listed for the random effect of individual is a Wald Z test. F ratios are listed for all other factors. N = 3 tests of 32 individuals. N = 1 test of 26 individuals

	# Chironomidae		# Ephemeroptera		Total # prey items	
	tau	*p*-value	tau	*p*-value	tau	*p*-value
Open microhabitat						
Latency to emerge	0.08	0.71	-0.03	0.90	0.09	0.67
Number of sections	-0.29	0.19	0.08	0.71	-0.35	0.11
Cover microhabitat						
Latency to emerge	0.21	0.33	-0.25	0.29	0.18	0.39
Number of sections	-0.01	0.95	**0.69**	**0.007**	0.04	0.85

Table 1.2. Correlations between gut contents and behavioral type variables. Correlation coefficients are Kendall's tau-b. Significant correlation is in bold. Open n = 13. Cover n = 13

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CHAPTER 2: EVIDENCE THAT SELECTION FAVORS ASSOCIATIONS BETWEEN BEHAVIORAL TYPES AND CERTAIN SOCIAL ENVIRONMENTS IN THE FIELD

ABSTRACT

Although there is growing evidence that populations of animals often comprise distinct behavioral types of individuals, we know little about whether different behavioral types are equally distributed across different social environments in natural populations, and the prevalence of mechanisms that can generate such associations. Here, we report evidence from a mark-recapture experiment that different behaviors were favored in different social environments within a wild population of threespined sticklebacks. Among individuals that occurred in shoals, relatively 'bold' behavioral types were more likely to be recaptured. In contrast, among individuals that occurred by themselves, relatively 'shy', inactive behavioral types were more likely to be recaptured. These findings suggest that natural selection on behavioral types can create nonrandom associations between behavioral types and social environments within natural populations.

INTRODUCTION

Populations are often composed of individuals that differ consistently in behavior relative to one another, and thus individuals can be categorized based on differing behavioral types (Bell et al. 2009). When behavioral types within a population are non-randomly distributed across environments, a behavioral type - environment correlation exists (rBTE). Non-random associations between personality traits and environments have been studied for decades in humans (Rutter et al. 1997), but behavioral ecologists are just beginning to appreciate that rBTE's occur in a wide array of nonhuman animals as well (Birds, (Duckworth and Badyaev 2007); Flies, (Saltz 2011); Cnidaria, (Hensley et al. 2012); Lizards, (Cote and Clobert 2007); Fish, (Pearish et al. 2013)), and that they have important ecological and evolutionary implications (Stamps and Groothuis 2010).

rBTE's can be generated by a number of mechanisms that are not necessarily mutually exclusive. For example, relatively shy individuals might be more likely to occur in safer environments because they actively seek out such environments (niche picking, (Stamps and Groothuis 2010)). rBTEs can also emerge when there are consistent individual differences in behavior, and an individual's behavior influences their environment (niche construction (Odling-Smee et al. 1996)). Finally, selection is another mechanism that can generate behavioral type - environment correlations if certain behavioral types suffer higher mortality depending on the environment in which they occur (Jaenike and Holt 1991). A first step in determining what mechanisms or combinations of mechanisms generate rBTE's is to determine the prevalence of these different mechanisms within natural populations of animals. In this study we focused on the latter mechanism, selection, but an important goal for the future is to tease apart the roles of these and other mechanisms that might contribute to the occurrence of rBTE's in nature.

We tested the hypothesis that environment-dependent mortality (selection) plays a role in generating rBTE's in three-spined sticklebacks. Sticklebacks are well-known for their intraspecific variation in ecologically relevant behaviors such as latency to emerge from a refuge, exploration of a novel environment (Webster et al. 2009; Pearish et al. 2013), recovery time after predator attack (Ward et al. 2004), microhabitat use (Bentzen and McPhail 1984; Pearish et al.

2013)and shoaling behavior (Ward et al. 2004; Pearish et al. Chapter 4). In a previous study, we found two rBTE's within a natural population of juvenile three-spined stickleback. First, bold behavioral types were more likely to occur in shoals than by themselves. Second, exploratory behavioral types were more common in open habitats that lacked vegetation (Pearish et al. 2013). Altogether, these findings show that stickleback are an excellent model for studying rBTE's.

We collected juvenile sticklebacks from a riverine population in the field, noting their social environment (alone or in a shoal) and habitat type (open or cover) at the time of collection, and assessed individual levels of boldness and exploratory behavior in a standardized behavioral assay. Individuals were assigned a unique mark and returned to the stretch of river where they were captured. Approximately sixteen days later later, we swept the river via seining, electrofishing, and snorkeling in order to recapture the survivors. We assumed that recapture rate was a good proxy for mortality because impassible riffles up and downstream of the site prevented dispersal. To test if selection is responsible for nonrandom associations between behavioral types and social environment and/or habitat, we compared the recapture rate of fish with different behavioral phenotypes in different environments.

METHODS

The study was conducted in a 240 meter stretch of the Navarro River near Philo, CA. We created 16 transects by placing flags 15 meters apart along each bank. Each transect included a shallow bank that lacked vegetation (open habitat) and a relatively deep bank that was covered by low hanging tree branches and grasses (cover habitat). The Navarro River is not dammed and experiences regular seasonal variation in flow and depth. Our study took place in July and

August, 2013, during the summer low point. We assumed that dispersal outside of the study site was minimal because riffles up and downstream of the site were dried up and rendered impassible. Common predators in the Navarro River include sculpin (*Cottus* species), salmonids, aquatic invertebrates (Odonata species) and avian predators (*Ardea alba, Ardea herodias, Mergus merganser, Phalacrocorax auritus*).

Juveniles (25.1 mm +/- 0.2 SE standard length) were captured during snorkeling surveys. Starting at the edge of a randomly selected transect, we collected a single individual with a trout landing net, alternating between habitats (open n=215 vs. cover n=216). We recorded whether the focal fish was alone (n = 145) or in a shoal (n = 286, less 4 body lengths from another fish, (Pitcher 1993)) at the time of collection. No focal fish escaped capture. Each individual was placed into a separate 500 mL opaque container of river water and held overnight.

The following day, individuals were observed in a standardized behavioral assay similar to that used in Pearish et al. (Pearish et al. 2013). The testing arena was a circular plastic pool (150 cm diameter) filled with 10 cm of water with an opaque refuge (10 cm diameter, 10 cm height) in the center. Lines on the bottom divided the pool into nine equally-sized sections (one centered, eight around the perimeter, Figure 2.1). Perimeter sections contained rocks, which were often investigated by focal fish as they moved around the arena.

At the start of an assay, a randomly selected individual was gently poured from its holding chamber into the refuge where it was allowed to settle. Behavioral observations were recorded by an observer positioned behind a blind. After three minutes, the observer opened the side of the refuge remotely and recorded the amount of time it took for the fish to emerge completely (*latency to emerge*) which we interpret as an individual's willingness to trade the safety of the refuge with the opportunity to locate resources. If a fish did not emerge within ten minutes (n=10 of 310) the individual was gently poured out of the shelter into the pool and assigned the maximum latency to emerge value of ten minutes. Following emergence, we recorded the number of times the individual moved into a different section of the pool (*transitions*) as a measure of activity in a novel environment. After three minutes, we simulated a predator attack by moving a clay predator model (sculpin, 20 cm length) from a hidden location into the section of the pool that contained the focal fish. We recorded the interval from the attack until the fish resumed movement in the presence of the predator model (*time frozen*). Fish that did not resume movement after six minutes (n=12 of 310) were assigned a time frozen of 360 sec. As remaining motionless is an effective predator defenses for stickleback (McGhee et al. 2012), we interpret fast emergence from a refuge, frequent transitions, and short freeze times to boldness (Réale et al. 2007). A subset of individuals was not tested in this assay so that we could assess whether behavioral phenotyping affected the likelihood of recapture (n=130).

Following behavioral observations, fish were weighed, measured for standard length (tip of nose to base of caudal fin), and given unique markings using fluorescent visible implant elastomer tags (Northwest Marine Technology). Fish were released back into the river at the transect where they were collected. The river was swept for survivors between August 14-18, 2013 (16.6 days ± 0.5 SE after initial capture). To improve the likelihood of recapturing all individuals, we started at the end of the study site that was furthest downstream and moved methodically up the river using block nets to isolate fifteen meter sections. This prevented fish from moving up or downstream. We used three methods for recapturing marked individuals (seining, snorkeling, and electrofishing) in an effort to avoid biasing capture toward particular behavioral types (Wilson et al. 1993). We are especially confident that we were able to avoid gear bias since snorkeling was used for all initial captures and the majority (>95%) of recaptures were via seining. We spent three hours in each fifteen-meter section. By noting habitat during recapture, we could statistically test (Chi-square) whether habitat use (open vs. cover) was consistent between initial capture and recapture. We were unable to measure social environment use (alone or in a group) with the seining and electrofishing methods and therefore could not test whether social environment use was consistent between initial capture and recapture. However, lab studies suggest that individual differences in social environment use are consistent in stickleback (Ward et al. 2004; Pearish et al. Chapter 4).

We used logistic regression to determine whether different behaviors were favored in different environments. We performed three separate analyses, one for each behavior (latency to emerge, transitions, or time frozen). We tested for the effect of habitat (open vs. cover), social environment (alone vs. in shoal), behavior and all interactions on recapture. Behavior variables were log+1 transformed for all analyses. To aid in interpretation of significant interactions we calculated Cohen's D, a metric of effect size. Cohen's D values less than 0.2, less than 0.5, or less than 0.8 are considered to be small, medium, or large effects respectively (Cohen 1988). Finally, in cases where we detected effects of behavior on survival, we calculated one-way ANOVAs to determine if the distribution of behavioral phenotypes present in different environments had shifted between the initial capture and recapture. We used R 3.0.2 (R Core Team 2014) for all analyses.

RESULTS

We recaptured 118 of the 431 individuals we initially captured, which is on par with typical recapture rates for stickleback (Hagen 1967; Ingram et al. 2012). Recapture rate was comparable across habitats (open = 31%, cover = 24%, $Chi^2 = 3.09$, df = 1, $p = 0.08$). Similarly, fish in shoals or by themselves were equally likely to be recaptured (alone = 30%, in shoal = 26%, $Chi^2 = 0.57$, df = 1, $p = 0.45$). We did not detect an effect of body size (standard length, recaptured 25.1 ± 0.3 SE mm, not recaptured 25.1 ± 0.2 SE mm) on the probability of recapture. Our behavioral assay did not affect probability of recapture (tested vs. control, $Chi^2 = 0.75$, df = 1, $p = 0.39$).

One of the first striking patterns in the mark-recapture data is that fish captured in cover were more likely to be recaptured in cover and fish found in open habitats were likely to be recaptured in the open ($Chi^2 = 23.1$, df = 1, $p < 0.001$, Figure 2.2). Of 67 fish initially captured in the open, 47 of them were in the open at recapture while 38 out of 51 fish initially captured in cover remained in cover at recapture. This result suggests that differences in habitat use were consistent over time.

The probability of recapture depended on the fit between an individual's activity and their social environment (social environment*transitions interaction, $p = 0.02$, N = 310, Figure 2.3b, Table 2.1). For example, individuals that were less active (fewer transitions) were more likely to be recaptured, but only if they were alone when first spotted in the river (Effect size of difference in mean behavior, recaptured loners vs. loners that were not recatpured, Cohen's D = 0.43, Table 2.2). In contrast, among fish that were initially in shoals, probability of recapture did not depend on how active they were (Cohen's D = 0.04).

The probability of recapture was also affected by an individual's freezing behavior following a simulated predator attack, but the direction of the effect of freezing on recapture depended on the individual's social environment (social environment*time frozen interaction, $p = 0.04$, N = 310, Figure 2.3c, Table 2.1). Among fish that were initially captured by themselves, individuals with long freeze times were more likely to be recaptured (Cohen's D = 0.29, Table

2.2). In contrast, among individuals that were initially captured in a shoal, individuals with short freeze times were more likely to be recaptured (Cohen's D = 0.22). Recapture was not related to latency to emerge from a refuge (Figure 2.3a) and habitat was non-significant in all models (Table 2.1). We retained habitat as a factor because removing it did not qualitatively change the results.

Our aim was to test whether selection could generate behavioral type-environment correlations previously reported in this population (Pearish et al. 2013). Adding to the results above that show that selection on behavior was social environment-dependent, we found that the distribution of behavioral phenotypes present in different social environments had shifted between the initial capture and recapture. Mean transitions and time frozen did not differ across social environments at initial capture (Table 2.3). However among the subset of fish that were recaptured, those that were initially found in shoal were more active in a novel environment and spent less time frozen after a simulated predator attack compared to the survivors initially found alone (transitions: $F_{1,79} = 4.08$, $p = 0.047$; time frozen: $F_{1,79} = 4.93$, $p = 0.029$, Table 2.3).

DISCUSSION

The results of this study provide evidence that social environment-dependent mortality can generate rBTE's in nature. In a previous study, we found that relatively shy individuals were more likely to occur by themselves while bold individuals were more common in shoals (Pearish et al. 2013). Here, we provide evidence that selection can generate this association. Among the individuals that occurred by themselves, relatively shy individuals that remained frozen longer following a simulated predator attack were more likely to survive. Relatively bold individuals that froze for shorter durations were more likely to survive among fish that occurred in shoals. Furthermore, the mean boldness score of fish that were alone and fish that were in shoals did not differ at the outset of our study but had shifted by recapture; recaptured shoalers were bolder (had shorter freeze times) compared to recaptured loners. The difference in boldness between fish that occurred alone and fish that occurred in shoals we detected among the recaptured fish in this study mimics the difference between shoalers and loners we found in Pearish et al. (Pearish et al. 2013) and provides strong evidence that selection in the form of social environment-dependent mortality generated the rBTE between boldness and social environment.

Although we do not know the cause of mortality, regular observations of avian and aquatic predators actively consuming stickleback at the field site suggest that mortality due to predation was high. One possible reason why fish that were alone and that showed high levels of freezing behavior had relatively high fitness is because reduced activity and increased freezing behavior can be an effective antipredator strategy when sticklebacks are by themselves (McGhee et al. 2012). However, inactive, timid behavioral types might not do as well in a group setting where the best antipredator strategies, such as dilution (Foster and Treherne 1981) or predator confusion (Pulliam and Caraco 1984), depend on coordinated movement. Indeed, our data support the hypothesis that freezing is actually disadvantageous for individuals that occur in groups. An alternative cause of death that might explain why boldness is favored among fish found in shoals is starvation. Bold stickleback outcompete shy stickleback in direct competition for food items (Ward et al. 2004), thus shy individuals that occur in shoals might be more likely to die of starvation.

We provide evidence that inidividuals consistently occur in the same habitat type (open vs. cover), but we did not find evidence that selection drove the other rBTE reported in Pearish et al. (Pearish et al. 2013) between exploratory behavior and habitat type. A plausible explanation for this is that other mechanisms such as niche picking or plasticity contribute to that association. For example, three-spined and nine-spined stickleback that were more active in a novel environment also spent a small amount of time in vegetation relative to less active individuals (Webster et al. 2009) which indicates that less active fish might occur in cover habitats more often due to niche picking.

rBTE's generated by selection have interesting implications for evolution. One expectation is that selection should favor mechanisms that make adaptive rBTE's more likely, a genetic correlation between preference for social environment and boldness for example (Ravigné et al. 2009). Another interesting possibility is that indirect genetic effects might accelerate the evolution of bold behavior among individuals that tend to occur in groups (Moore et al. 1997). If bold behavior is favored to overcome the challenges of the emergent environment that is a shoal, i.e. competition, the fact that shoals tend to be composed of bolder than average individuals will increase the strength of selection and potentially accelerate the evolution of bold behavior.

Although we present evidence that selection played a role in generating rBTE's, other mechanisms should not be overlooked (Stamps and Groothuis 2010). For example, niche-picking might work in parallel with selection, i.e. if shy behavioral types have difficulty acquiring enough food in shoals they might starve or alternatively learn to avoid shoals. Either mechanism can cause shy individuals to occur alone more often than in shoals.

In summary, we found evidence that selection in the form of environment-dependent mortality can generate rBTE's between boldness and particular social environments in three-spined stickleback. rBTE's generated by selection are interesting because of the potential to generate complex patterns of selection (indirect genetic effects, genetic correlations between boldness and social environment preference). Our suggestion for the future is that attention should be given to other mechanisms that could generate rBTE's (niche picking, niche construction, etc…) to lay the groundwork for experiments that can tell us which mechanisms or combination of mechanisms generate the non-random associations between behavioral types of individuals and the ecological niches in which they occur.

FIGURES AND TABLES

Figure 2.1. Experimental arena including the acclimation chamber and model Sculpin.

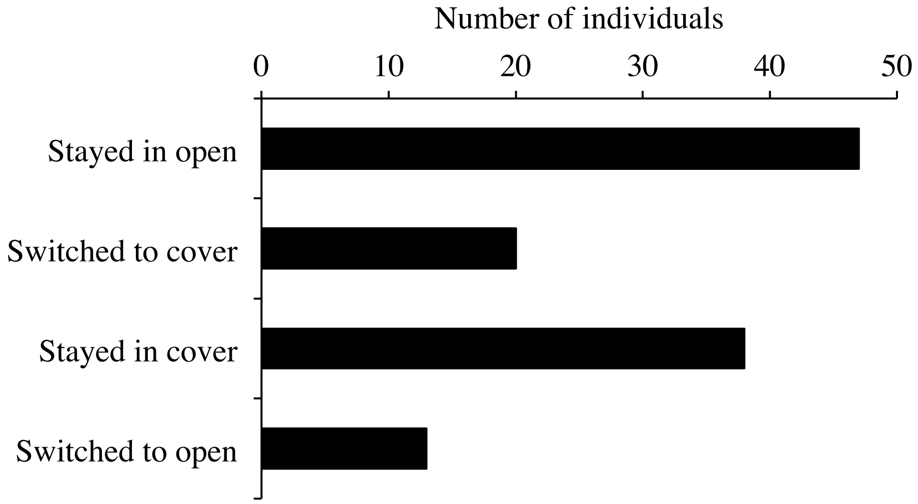

Figure 2.2. Consistency of habitat use. Categories represent location at recapture relative to initial capture.

(a)

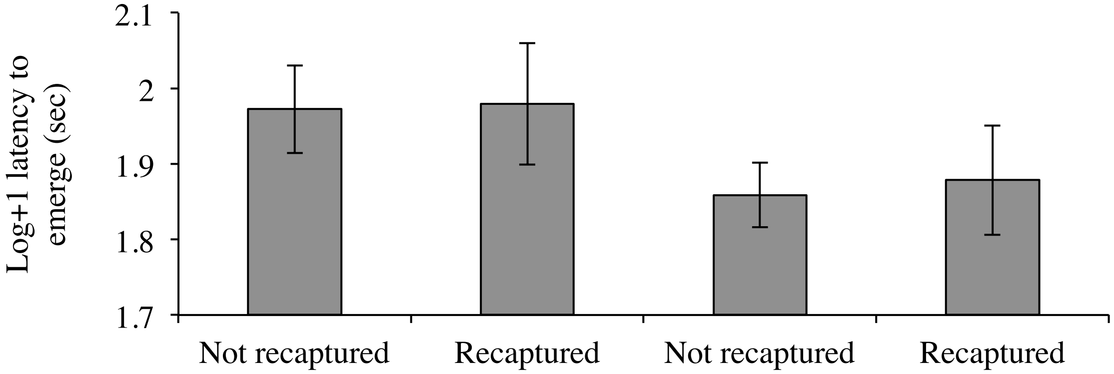

Figure 2.3. The behavior (y-axes, mean ± 1 se) of fish that were not recaptured vs. fish that were recaptured. The x-axes are divided into fish that were found alone vs. fish that were found in shoals. (a) Latency to emerge did not differ between fish that were recaptured and fish that were not. This is true among loners and fish found in shoals. (b) Among loners, fish that were less active (low transitions) were more likely to be recaptured. This was not true among fish found in shoals. (c) The relationship between recapture likelihood and time frozen depended on social environment. Among loners, long freeze times were favored (recaptured more often). Conversely, short freeze times were favored among fish in shoals. N = 310.

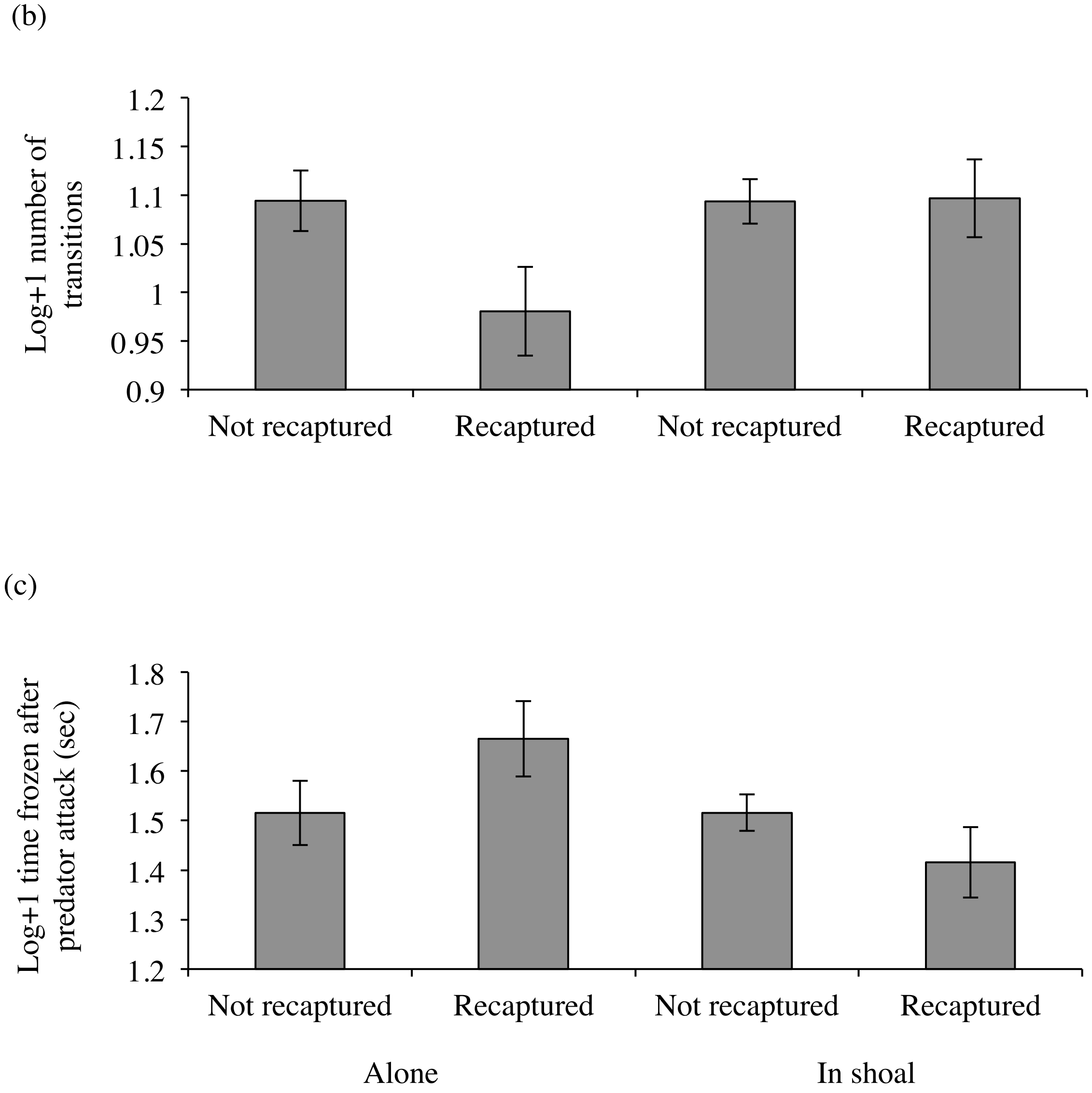

Figure 2.3 continued.

	Factor	Estimate	Estimate SE	p value
a)	Habitat (open vs. cover)	-1.2	1.9	0.54
	Social environment (alone vs. in shoal)	-0.6	1.7	0.74
	Latency to emerge	-0.1	0.7	0.93
	Habitat*social environment	0.9	2.3	0.71
	Habitat*latency to emerge	0.3	0.9	0.76
	Social environment*latency to emerge	0.1	0.8	0.88
	Habitat*social environment*latency to emerge	-0.4	1.2	0.76
b)	Habitat (open vs. cover)	-2.6	1.8	0.15
	Social environment (alone vs. in shoal)	-4.0	1.6	0.02*
	Transitions	-1.2	0.5	0.02*
	Habitat*social environment	3.3	2.3	0.15
	Habitat*transitions	0.8	0.7	0.28
	Social environment*transitions	1.5	0.7	0.02*
	Habitat*social environment*transitions	-1.3	0.9	0.17
c)	Habitat (open vs. cover)	1.6	1.4	0.26
	Social environment (alone vs. in shoal)	2.4	1.4	0.08
	Time frozen	1.2	0.6	0.05
	Habitat*social environment	-2.2	1.8	0.23
	Habitat*time frozen	-1.4	0.9	0.11
	Social environment*time frozen	-1.7	0.8	0.04*
	Habitat*social environment*time frozen	1.5	1.2	0.20

Table 2.1. Logistic regressions testing for the effect of habitat, social environment and a) latency to emerge; b) transitions; c) time frozen on recapture. N = 310.

Log+1 latency to emerge from refuge

	Alone		In shoal	
	Not recaptured	Recaptured	Not recaptured	Recaptured
Mean	1.97	1.98	1.86	1.88
SD	0.55	0.42	0.45	0.50
N	72	31	147	49
Effect Size	0.02		0.04	

Log+1 number of transitions

	Alone		In shoal	
	Not recaptured	Recaptured	Not recaptured	Recaptured
Mean	1.09	0.98	1.09	1.10
SD	0.26	0.25	0.28	0.28
N	72	31	147	49
Effect Size	0.43		0.04	

Log+1 time frozen after simulated predator attack

	Alone		In shoal	
	Not recaptured	Recaptured	Not recaptured	Recaptured
Mean	1.52	1.67	1.52	1.42
SD	0.55	0.42	0.45	0.50
N	72	31	147	49
Effect Size	0.29		0.22	

Table 2.2. Effect sizes of mean difference in behavior between fish that were not recaptured vs. fish that were recaptured. The effect sizes reported are Cohen's D.

	All fish				Recaptured fish			
	Alone	In shoal	$F_{1,309}$	p value	Alone	In shoal	$F_{1,79}$	p value
Log transitions	1.06 +/- 0.03	1.09 +/- 0.02	0.89	0.35	0.97 +/- 0.05	1.10 +/- 0.04	4.08	0.047*
Log time frozen	1.55 +/- 0.05	1.48 +/- 0.03	1.19	0.28	1.65 +/- 0.07	1.42 +/- 0.07	4.93	0.029*

Table 2.3. Behavioral differences between fish that occurred alone vs. fish that were in shoals at the time of collection. The left side of the table shows mean +/- SE for each category among all fish that were tested. The right side shows the mean +/- SE for each category but includes only the fish that were recaptured. Mean number of transitions and time frozen did not differ among all fish. In the subset of fish that were recaptured, shoalers were more active (more transitions) and stayed frozen for a shorter duration compared to loners.

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CHAPTER 3: EFFECT OF BODY CONDITION ON DISPERSAL DISTANCE IS HABITAT-DEPENDENT IN A RIVERINE POPULATION OF THREE-SPINED STICKLEBACK

ABSTRACT

Dispersal influences important processes including gene flow, local adaptation, and range expansion. Individuals within populations differ in how far they disperse. Several factors that are not necessarily mutually exclusive can influence dispersal distance. Here, we present the results of a mark-recapture study of juvenile three-spined stickleback in which we examine how dispersal is affected by internal (behavioral phenotype and body condition) and external (habitat type and social environment) factors. We detected a positive relationship between body condition and dispersal distance that was driven almost completely by fish that occurred in cover habitat that was relatively more structurally complex and patchier. Our results suggest that habitat type and body condition act in concert to influence the dispersal behavior of individual three-spined stickleback.

INTRODUCTION

Dispersal can mediate important processes in evolution and ecology such as gene flow, local adaptation, and species interactions (Clobert et al. 2001). Individuals within a population disperse different distances, which can affect home range size (Bowman et al. 2002) and determine which animals will be first to colonize and potentially monopolize new or ephemeral patches (Bowman et al. 2001; Johst et al. 2002). Therefore, understanding the phenotypic and environmental factors that make some individuals disperse further than others might provide important insights about how the ecology of individuals will affect range expansion (Duckworth and Badyaev 2007; Lindström et al. 2013) or the genetic structuring of metapopulations (Whitlock 2001).

Three-spined stickleback (*Gasterosteus aculeatus*) are attractive models for studying the dispersal behavior of individuals because we have several examples of limited gene flow between neighboring populations (Snyder 1991; Moore and Hendry 2005; Jones et al. 2006). Ward et al. (Ward et al. 2013) documented homing behavior, showing that individuals from a riverine population of three-spined stickleback returned to familiar territory after being displaced. However, some of the animals in Homing behavior might generate population structuring if individuals are philopatric from the time they hatch. For example, Bolnick et al. (2009) indicated phenotype-dependent habitat preference as the mechanism that limited the dispersal of lake fish into a river and river fish into a lake, thus limiting gene flow between populations that are separated by less than 100 meters. However, Hendry et al. (2002) presented a case where limited gene flow allowed adaptive divergence between a lacustrine population of three-spined stickleback and the riverine population upstream of the lake but found that gene flow was not limited between the lacustrine population and the downstream riverine population and therefore adaptive divergence was less complete in the downstream fish. Elucidating factors that inhibit or stimulate individuals to disperse further might help us understand why some populations have considerable gene flow while others do not.

Several biotic and abiotic environmental factors (habitat, conspecific or heterospecific density) as well as internal characteristics (age, size, sex, phenotype) are known to drive dispersal (Clobert et al. 2001; Bowler and Benton 2005; Ronce 2007). In the present study, our aim was to determine the relative importance of several key factors in predicting dispersal distance in a natural population of three-spined stickleback. We used an information criteria approach in which we compared 6 different a priori models formulated based on the literature.

Model 1: Body condition

Body condition is often related to dispersal distance. Body condition is an index that is frequently used to approximate physiological wellness, especially in fishes (Pope and Kruse 2007). The general trend in most animals is for high condition individuals to disperse further (Bonte et al. 2012) but the causal relationship is difficult to disentangle. On one hand, individuals in good condition might be able to disperse further because they have surplus energy to invest in locomotion (Bowler and Benton 2005). Conversely, dispersing further might improve an individual's body condition if they are able to learn about the location of high quality food patches. Though less common, body condition can be negatively correlated with dispersal distance, i.e. low condition individuals disperse away from an unfavorable local environment (Lawrence 1987). For example, hatchling western whip snakes that were in low body condition dispersed further from the nest than snakes with high body condition (Rugiero et al. 2012). Body condition-dependent dispersal in this species appeared to be adaptive, as the relationship between distance from nest and condition had disappeared after one year.

Model 2: Habitat type

The amount of cover provided by biotic and abiotic features of the habitat might affect how far individuals disperse. For example, structural complexity in the habitat can enhance movement, presumably because it provides protection from predators (Doncaster et al. 2001; Gilliam and Fraser 2001). On the other hand, an individual that is utilizing a structurally complex habitat for protection from predators might be reluctant to move between patches and risk detection. For example, three-spined stickleback increased the time they spent hiding in vegetation in the presence of a predator and overall movement was lower when the fish occupied vegetation (Candolin and Selin 2012). Differences in distance between suitable patches (patch isolation, (Bowler and Benton 2005)) between alternate habitat types might also affect dispersal distance. The cost of dispersing to a new patch, i.e. energy expenditure for locomotion, lost foraging time, and increased risk of mortality, is proportional to the distance between patches (Hanski 1998). By this logic, we would expect dispersal to be inhibited in habitat types that have relatively high patch isolation.

Model 3: Body condition and habitat type

Body condition might drive dispersal more or less depending on the habitat type in which the individual occurs. Krause et al. (1998) showed that three-spined stickleback that are deprived of food are more willing to leave the safety of a refuge. However, stickleback occur in a variety of habitat types, many of which lack structural complexity that could provide physical refuges from predators (gravel beds in rivers, pelagic zones of lakes or oceans). Thus, body condition-dependent refuge use might lead low condition individuals to disperse further than high condition individuals but this would only have relevance in structurally complex habitat with abundant refuges. Another possibility is that body condition-dependent dispersal might be affected by the patchiness of resources. For example in a heterogeneous habitat where food is concentrated in patches, individuals might develop a foraging pattern that they would only break if the patches become depleted. Whereas in a more homogeneous habitat where food patches are less distinct, individuals might be less likely fixate on a set home range (Mikheev et al. 2010).

Model 4: Social environment

The social environment in which an individual occurs might influence dispersal distance. Juvenile stickleback can occur alone or in loose social groups with conspecifics, i.e. shoals. Individuals that occur in groups might exhibit different dispersal behavior compared to individuals that occur alone because they might be constrained by their reluctance to leave the safety of their social group. For example, three-spined stickleback that were displaced downstream ‘homed’ more quickly if they were released alone rather than with a group of conspecifics (Ward et al. 2013). On the other hand, safety in numbers might allow group members to move more freely than if they were traveling alone (Magnhagen and Bunnefeld 2009).

Model 5: Body condition and social environment

The effect of body condition on dispersal distance might depend on the social environment. Fish that are in poor condition have an urgent need to locate food to avoid starvation and the method they use to achieve this goal might differ depending on the social environment in which they occur. For example, one reason animals disperse is so that they can sample information about the location of high-quality food patches (Valone 1991). However, animals that occur in social groups get additional information about patch quality by observing the sampling behavior of conspecifics (public information, Valone 1989), which might allow them to make decisions about where to forage without dispersing as far (Templeton and Giraldeau 1996; Valone and

Templeton 2002). Therefore we might predict a negative relationship between body condition and dispersal in fish that occur alone but not in fish that occur in shoals.

Model 6: Exploratory behavior

There is evidence that consistent individual differences in exploratory behavior drive within species variation in dispersal in fish (Fraser et al. 2001; Cote et al. 2010b; Chapman et al. 2011; Rasmussen and Belk 2012), birds (Dingemanse et al. 2003), and amphibians (Lindström et al. 2013). Personality-dependent dispersal is interesting because, if certain behavioral types are more likely to move, the individuals that arrive at new resources or habitats will be a particular subset of the population, the most exploratory individuals for example (Cote et al. 2010a). This phenomenon has recently been implicated in the spread of invasive species (Phillips and Suarez 2012). An example of the ecological consequences of personality-dependent dispersal is seen in the displacement of mountain bluebirds by western bluebirds. The western bluebirds that dispersed to the invasion front were a particularly aggressive subset of the population and their aggressive behavior allowed them to outcompete the native mountain bluebirds for breeding territories (Duckworth and Badyaev 2007).

METHODS

The study was conducted in a 240 meter stretch of the Navarro River near Philo, CA. We created sixteen transects by placing flags fifteen meters apart along each bank. Each transect included a shallow bank that lacked vegetation (open habitat) and a relatively deep bank that was covered by patches of low hanging tree branches and grasses (cover habitat). The open habitat was a bed of sand and fine gravel that stretched uninterrupted from the furthest upstream transect to the furthest downstream transect. Juvenile stickleback were evenly distributed throughout this habitat type. The cover habitat was dominated by submerged branches of the trees that lined the deep bank. Juvenile stickleback tended to aggregate at tree branches that were separated by as much as 10 meters, i.e. the cover habitat was patchier. Thus, stickleback distribution was less evenly distributed across space in the cover habitat compared to the open habitat. The Navarro River is not dammed and experiences regular seasonal variation in flow and depth. Our study took place in July and August, 2013, during the summer low point. We assume that dispersal outside of the study site was negligible because riffles up and downstream of the site were impassible.

Between July 21 and August 8, 2013, age-0 juveniles were captured during snorkeling surveys (25.1 mm +/- 0.2 SE standard length, N = 431). Starting at the edge of a randomly selected transect, we collected one, randomly selected individual at a time with a trout landing net, alternating between habitats (open vs. cover). Before approaching, we noted the focal individual's social environment, recording whether the focal fish was alone or in a shoal (< 10 cm or 4 body lengths from another fish, Pitcher 1993). No fish escaped capture so we assume our sample was not biased toward more 'catchable' individuals (Biro and Dingemanse 2009). Each individual was placed into a separate 500 mL opaque container of river water and held overnight.

The following day, individuals were observed in a standardized behavioral assay similar to the open field test classically used in animal psychology (Walsh and Cummins 1976). The testing arena was a circular plastic pool (150 cm diameter) filled with 10 cm of water with an opaque refuge (10 cm diameter, 10 cm height) in the center. Lines on the bottom divided the pool into nine equally-sized sections (one centered, eight around the perimeter, Figure 3.1). Each perimeter section contained a rock.

At the start of an assay, a randomly selected individual was gently poured from its holding container into the refuge where it was allowed to settle. Behavioral observations were recorded by an observer positioned behind a blind. After three minutes, the observer opened the side of the refuge remotely to allow the fish to emerge. If a fish did not emerge within ten minutes (n = 10 of 310) it was gently poured out of the shelter into the pool. We recorded the number of unique sections of the pool the fish entered during the three minutes immediately following emergence as a measure of exploratory behavior (ranging from 0-9). We interpret movement in a novel environment as exploratory behavior following the definition of Réale et al. (2007). In a previous study at this site, we found this behavior to be repeatable (R = 0.26, Pearish et al. 2013) suggesting that individuals differ consistently in exploratory behavior in this population. A subset of the fish was not tested so that we could test whether behavioral phenotyping affected dispersal (N = 130).

Following behavioral observations, fish were weighed, measured for standard length (tip of nose to base of caudal fin), and given unique markings using fluorescent visible implant elastomer tags (Northwest Marine Technology). Each fish was released back into the river at the transect from which it was collected. We recaptured between August 14-18, 2013 (16.6 days ± 0.5 SE after initial capture). We started at the transect furthest downstream and moved methodically up the river using block nets to isolate each transect. This prevented fish from moving up or downstream in response to our activity. We used three methods for recapturing marked individuals (seining, snorkeling, and electrofishing) in an effort to avoid biasing recapture toward particular behavioral types (Wilson et al. 1993). We spent three hours in each transect and recaptured 120 fish (28% recapture rate).

Statistical analysis

Our analysis was conducted in two steps. First, we developed a set of a priori models corresponding to the predictions that were developed from our knowledge of the stickleback system and relevant literature (Introduction, models 1-6). The dependent variable for all models was the distance between the transect of initial capture and the transect where the fish was recaptured (*distance moved*). The amount of time between capture and recapture was not correlated with distance moved (Kendall's tau = -0.02, p = 0.75, n = 120). Distance moved was non-normal so we specified a Poisson error distribution (generalized models) and confirmed that this distribution was a good fit with Q-Q plots. We corrected for the anti-conservative nature of the Poisson distribution by using quasi-likelihood estimation of parameters (see below). Average movement was upstream (18.1 ± 6.0 SE m, Figure 3.2) and fish in upstream transects were limited in how far upstream they could travel. To account for this, we used generalized linear mixed models with transect included as a random factor in all models. Models were constructed using the lme4 package in R (R Core Team 2014).

We tested seven models corresponding to six a priori models and one model that contained only the random effect of transect (Table 3.1). Fixed factors included in models were body condition, habitat type, social environment, and exploratory behavior. We used Fulton's K calculated from body measurements taken during the initial capture as the body condition index. Fulton's K is calculated by

$$K = (W/L^3) * 100{,}000$$

where W is weight (g), L is length (mm), and values are multiplied by 100,000 to achieve an index with values close to 1 (Pope and Kruse 2007). Fulton's K is usually avoided in cases where comparison across age classes, populations, or species are desired but is appropriate for this application as we limited our study to juvenile three-spined stickleback of a single population. Models that contained two fixed factors also included the interaction (body condition and habitat, body condition and social environment)

The second step of our analysis was to use multi-model inferencing to compare our a priori models and calculated model-averaged estimates. This method is preferred over step-wise model selection because model selection uncertainty is accounted for in the model-averaged parameter estimates (Burnham et al. 2011). The ability of each model to predict dispersal was assessed using the second order quasi Akaike's information criteria (QAICc) to account for overdispersion in the data and small sample size (Burnham and Anderson 2002). For each model, we calculated the number of parameters estimated (K), QAICc, delta (Δ, difference in QAICc between the focal model and the model with the lowest QAICc), Akaike's weight (w), and conditional R^2. Models with deltas less than 2 are considered to have "substantial support" while models with deltas of 4 or more receive "considerably less support" (Burnham and Anderson 2002). Akaike's weight is the relative probabilities of each model given the data and sums to one over the set of models (Johnson and Omland 2004; Burnham et al. 2011). Conditional R^2 represents the variance explained by fixed and random factors (Nakagawa and Schielzeth 2013). When no single model can be specified as the best model, a 95% confidence set can be constructed by summing Akaike's weights from largest to smallest until the sum is ≥ 0.95. This set can be used to calculate model-averaged estimates and 95% confidence intervals for fixed effects (Burnham and Anderson 2002). We interpreted effects with confidence intervals that did not overlap zero as statistically significant. We used the MuMIn package in R for this analysis (R Core Team 2014).

RESULTS

We found considerable variation in distance moved (range = 0 - 180 m). Although movement tended to be in the upstream direction, visual inspection of the data did not suggest that fish were converging on particular transects (Figure 3.2). Behavioral phenotyping did not affect distance moved (distance moved (m) ± SE: tested = 35.3 ± 4.9, control = 38.7 ± 8.2, t test: $p = 0.73$).

Several of the a priori models were considered to have received substantial support (delta < 2), including models that contained main effects of body condition, social environment, and habitat type and the model that contained only the random effect of transect (Table 3.1). Only the 'body condition and social environment' model received substantially less support (delta ≥ 4). Since there was not a single "best" model, we created a 95% confidence set of models (which excluded the 'condition and social environment' model) from which we calculated model-averaged estimates and 95% confidence intervals.

Dispersal was affected by social environment with fish that occurred in shoals moving further than fish that occurred alone (model averaged estimate, effect of social environment = 0.21 [0.13, 0.29]). However, we interpret this result with caution as standard errors overlapped in a comparison of mean distance moved between shoalers and loners (mean ± SE, in shoal = 36.7 ± 6.7, alone = 33.2 ± 7.2, Figure 3.3). Fish in better condition moved further (effect of condition = 0.41 [0.12, 0.71]) but this effect was driven primarily by fish that occurred in cover habitat (effect of condition*habitat type interaction = 1.42 [1.16, 1.69], Figure 3.4). We detected a non-significant trend for fish in the open to move further than fish that occurred in cover (Table 3.2). Exploratory behavior had a relatively small and non-significant effect on dispersal (Table 3.2), which was evident by the lack of predictive power of the exploratory behavior model (Table 3.1).

DISCUSSION

We found evidence that the dispersal of juvenile three-spined stickleback is jointly influenced by an individual's body condition and its habitat. More robust individuals moved further but this relationship was driven by fish that occurred in the cover habitat that consisted of patches of submerged grasses and tree branches. Our results are consistent with other studies showing that dispersal is limited by the physiological wellness of the animal (Bonte et al. 2012), but show that this phenomenon might be limited to a subset of the habitat types that are utilized by juvenile three-spined stickleback. We also found that fish that occurred in groups with conspecifics moved further than fish that occurred alone and a non-significant trend for fish in the open to moved further than fish in cover. We found relatively little support for the hypothesis that dispersal is personality-dependent (Cote et al. 2010a).

Although we do not know the precise function of dispersal in this population, we can speculate about possible reasons for the patterns we observed. The cover habitat in this study consisted of distinct patches of submerged grass and tree branches separated by areas that were not inhabited by stickleback. The open habitat, consisting of relatively homogeneous gravel beds, was much more continuous and stickleback inhabiting this habitat were more evenly distributed. This difference in space between patches (patch isolation, Bowler and Benton 2005) in the open vs. cover habitats might explain why we only detected condition-dependent dispersal in the cover habitat. High condition individuals might have been better able than low condition individuals to afford the costs associated with moving between distant patches in the cover habitat (lost foraging time, energetic requirements of locomotion, Hanski 1998). Whereas the relatively small costs of movement between less distant patches in the open were probably less constraining for low condition individuals.

Our data support the hypothesis that group living encourages individuals to disperse further. Previous work has shown that fish in groups are less inhibited in the presence of a predator than fish that are alone (Magnhagen and Bunnefeld 2009), perhaps due to the added safety from predation provided by group living (Krause and Ruxton 2002). A plausible explanation for why shoaling fish in our study dispersed further is that their perception of the predation risk of dispersal was lower compared to fish that occurred alone. We interpret the effect of social environment on dispersal distance with caution as the error bars for mean distance moved by shoalers and loners overlapped.

The non-significant trend for fish in the open habitat to move further is counter to previous research (Doncaster et al. 2001; Gilliam and Fraser 2001). We assumed that more structurally complex ‘cover’ habitat would provide a refuge from visual predators, especially avian predators that were frequently observed foraging in the open habitat (*Ardea alba, Ardea herodias, Mergus merganser, Phalacrocorax auritus*), allowing fish to move more freely. This assumption may have been violated in two ways. First, the presence of avian predators in the open habitat might have actually motivated fish to disperse. Second, juvenile stickleback in this population are also preyed upon by sculpin (*Cottus* species), salmonids, and aquatic invertebrates (Odonata species) and we assumed that stickleback were equally likely to encounter these predators in either habitat type. If these predators preferred cover, the risk of dispersal within this habitat type might have actually been higher relative to the open.

Interestingly, we did not detect a relationship between exploratory behavior and dispersal in juvenile three-spined stickleback. Our results confirm similar results found in an independent study of adult three-spined stickleback (K. Laskowski, Unpublished manuscript). These results are counter to previous studies that found links between exploratory behavior and dispersal in a wide array of taxa, including fishes (Fraser et al. 2001; Dingemanse et al. 2003; Cote et al. 2010a,b; Chapman et al. 2011; Rasmussen and Belk 2012; Lindström et al. 2013). While it is too early to speculate, determining what, if any, ecological factors promote personality-dependent dispersal should be a goal for future work.

A goal of our study is to encourage researchers to continue to work towards an understanding of dispersal that considers intrinsic and extrinsic factors simultaneously (Rasmussen and Belk 2012). Recently, researchers have suggested that dispersal might be related to a correlated suites of physiological, morphological, and behavioral characteristics, a dispersal syndrome (Ronce and Clobert 2012). We see this as a step in the right direction but encourage researchers to also consider environmental factors such as habitat type or social environment in an integrative approach to understanding dispersal.

FIGURES AND TABLES

Figure 3.1. Arena used for exploratory behavior observations. Pool contained fresh river water and was exposed naturally to partial shade throughout all trials.

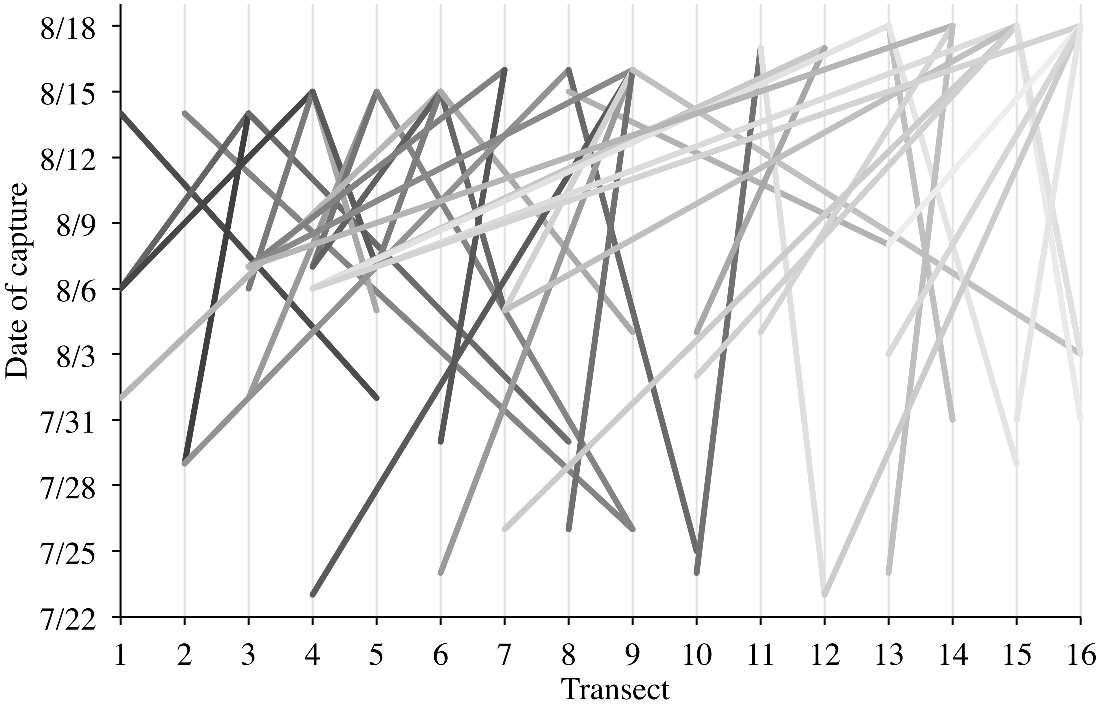

Figure 3.2. Movement across time. Each line represents an individual. N = 82.

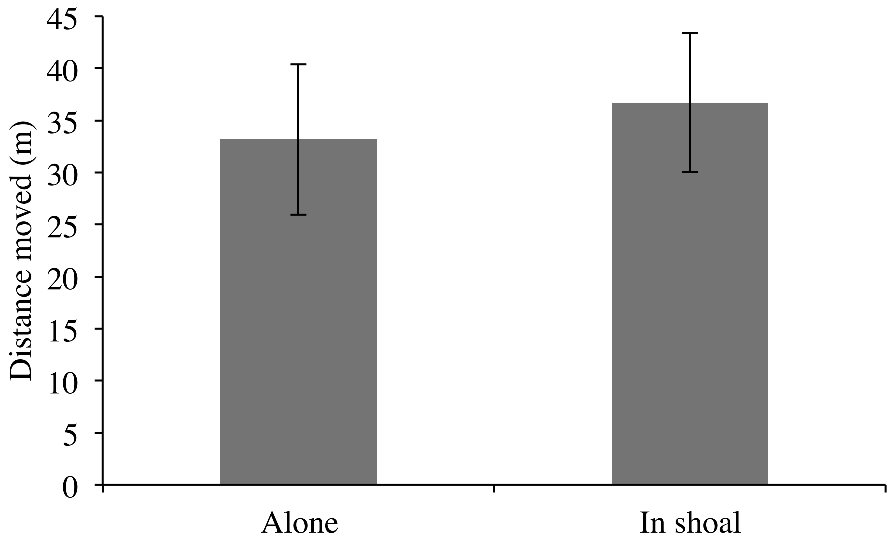

Figure 3.3. Distance moved between initial capture and recapture. Fish that occurred in shoals moved further than fish that occurred alone. Error bars are ± 1 SE. N, alone = 33, in shoal = 49.

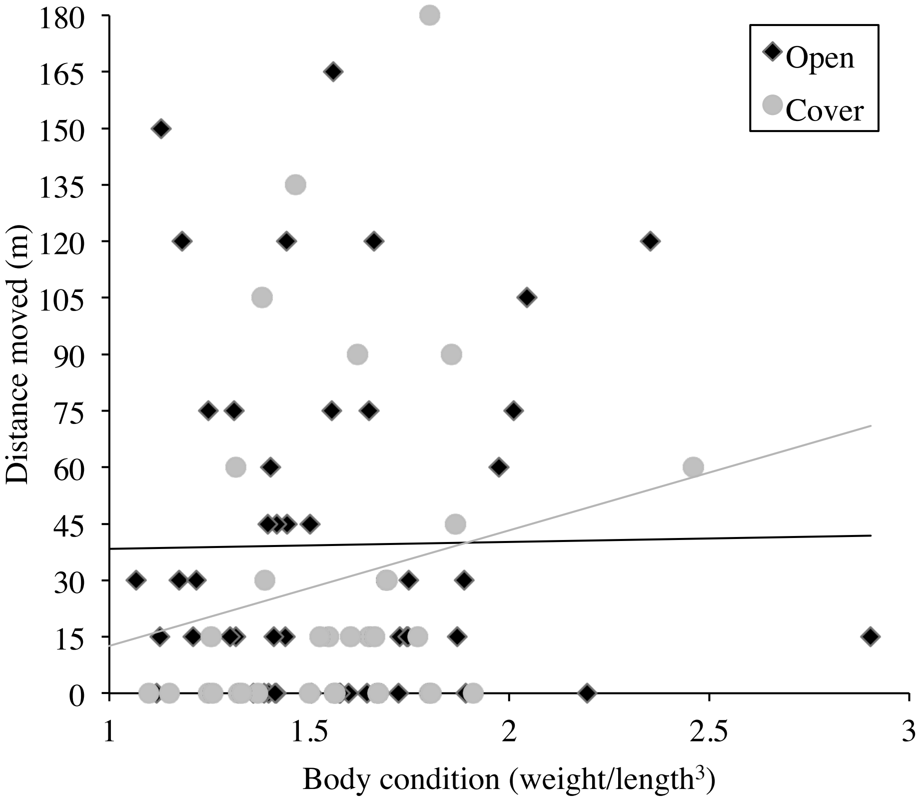

Figure 3.4. Scatterplot showing the relationship between body condition and movement. Black diamonds and line represent fish from open habitats. Gray circles and line represent fish from cover habitats. Among fish in cover habitats, individuals that were in better condition moved further. Condition was not related to distance moved among fish in the open. N, open = 48, cover = 34.

Model	**K**	**QAICc**	**Δ**	***w***	**R squared**
Random effect only (Transect)	2	93.8	0	0.29	0.10
Body condition	3	94.3	0.45	0.23	0.13
Social environment	3	95.3	1.51	0.13	0.11
Habitat type	3	95.7	1.85	0.11	0.11
Condition and Habitat type	5	95.9	2.07	0.10	0.18
Exploratory behavior	3	96.0	2.15	0.10	0.11
Condition and Social environment	5	97.8	4.00	0.04	0.13

Table 3.1. Results of information theoretic analysis in order from most to least informative. K is the number of parameters estimated. QAICc is a smaller-is-better measure of goodness of fit. Delta (Δ) is the difference between the "best" model with the lowest QAICc and all other models. Akaike's weight (*w*) is the relative probabilities of each model given the data. R squared is the conditional R squared that shows the variance explained by fixed and random effects.

Factor	**Estimate**	**95% CI**
Body condition	0.41*	0.12, 0.71
Social environment	0.21*	0.13, 0.29
Habitat type	-1.23	-3.46, 1.00
Exploratory behavior	0.02	-0.00, 0.03
Condition*Habitat type	1.42*	1.16, 1.69

Table 3.2. Model averaged estimates and 95% confidence intervals of factors predicting dispersal calculated from the 95% confidence set of models. Asterisks highlight significant factors where confidence intervals did not overlap zero. N = 82.

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CHAPTER 4: A STANDARDIZED METHOD FOR QUANTIFYING CONSISTENT INDIVIDUAL DIFFERENCES IN SCHOOLING BEHAVIOR

ABSTRACT

An increasing number of researchers in behavioral ecology are asking questions pertaining to consistent individual differences in several axes of behavioral variation, including social behaviors. The first step in studying animal personality is quantifying between-individual variation in behavior. Social behaviors are difficult to standardize because of the potential for interactions between the focal animal and the animals that serve as stimuli. As an alternative to live stimuli, researchers sometimes use models to elicit social behaviors. Here, we present a method developed for genetic studies that utilizes a model school to quantify consistent individual differences in schooling behavior in three-spined stickleback. We show that allowing fish to recover from netting for 24 hours before testing increases the likelihood that they will swim with the model school. We also show that individual differences in schooling are consistent across two weeks. Our study suggests that individual three-spined stickleback differ consistently in their tendency to join a school and that a model school can be used to quantify between-individual variation in schooling behavior.

INTRODUCTION

Growing interest in consistent individual differences in behavior within populations has led researchers to ask questions about the causes (Biro and Stamps 2008; Biro and Stamps 2010;

Stamps and Groothuis 2010a; Stamps and Groothuis 2010b) and consequences (Bolnick et al. 2003; Dingemanse and Réale 2005; Smith and Blumstein 2008; Bolnick et al. 2011; Wolf and Weissing 2012; Sih et al. 2012) associated with animal personality traits. A first step in addressing questions about consistent individual differences in behavior within populations is accurately quantifying between-individual variation in the trait in question (Dingemanse and Dochtermann 2013).

Between-individual variation in behavior is typically estimated by repeatedly measuring focal individuals during standardized exposures to an ecologically relevant stimulus or situation. This methodology is appropriate when the stimulus or situation can be standardized across all individuals. However, live predators (for measuring boldness) or conspecifics (for measuring sociality or aggression) are likely to change behavior in response to changes in their own internal states (hunger or acclimation to the experimental arena) or in response to external stimuli, such as the behavior of the focal animal. The latter case is especially troublesome because it means that the focal animal could influence the behavior of the stimulus animal, which can feed back to change the behavior of the focal fish (McGhee et al. 2013). Another drawback of using live stimuli is that it increases the number of animals that need to be collected and/or housed in captivity, which can conflict with animal welfare and conservation priorities to minimize the number of animals required to conduct research.

To overcome the difficulties of using live stimuli, researchers have utilized an array of artificial stimuli in behavioral experiments including mirror images, models, videos (Rowland 1999) and even robots (Faria et al. 2010). Models in particular have a long history in behavioral research dating back to studies in which Niko Tinbergen used models to elicit social behavior from three-spined stickleback (*Gasterosteus aculeatus*) (Pelkwijk and Tinbergen 1937).

Schooling behavior, when fish orient in the same direction and move in a coordinated fashion (Pitcher 1993), is particularly difficult to quantify because the problem of behavioral feedback is compounded by the necessity for multiple stimulus fish. Wark et al. (2011) recently introduced an experimental protocol in which they used a model school to quantify schooling behavior. Rather than live fish, the model school consisted of size-matched models as the stimulus, effectively controlling for feedback between the focal and stimulus fish. Models were arranged in the formation of a school and moved in a circle to simulate schooling behavior. Using this assay, Wark et al. (2011) showed that populations of sticklebacks differ in schooling behavior, and Greenwood et al. (2013) identified QTL associated with variation in schooling based on a cross between different stickleback populations. However, they did not repeatedly measure the behavior of the same individuals in order to determine whether individuals consistently differed in schooling behavior in this assay. The present study tests whether this methodology can be utilized to quantify consistent individual differences in schooling behavior among individuals within populations.

A first step in animal personality research is measuring the consistency of individual differences in behavior across time or contexts. The metric researchers typically used for this is repeatability (a.k.a. intra-class correlation) (Bell et al. 2009). Repeatability is the proportion of variation in a behavior that is explained by differences between individuals (Lessells and Boag 1987). Recently, researchers have noted significant difficulties that arise when calculating estimates of repeatability of behavioral data that are not normally distributed, require transformation or specification of error structure, and thus are difficult to compare to other repeatability estimates (Nakagawa and Schielzeth 2010). Furthermore, estimates of repeatability will be erroneous when certain individuals do not respond to stimuli in a measurable way (non-responders) and thus receive behavior scores of zero. For example, we would hesitate to interpret a dataset of responses to a model school as evidence of consistent individual differences in schooling behavior if a significant proportion of individuals tested did not actually school. Preliminary testing of the model school assay indicated that non-responders might cause our data to be non-normally distributed and zero-inflated so the first part of our study sought a remedy to this problem.

We evaluated the effect of recovery time after netting on performance with the model school in an effort to reduce the number of non-responders. Wark et al. (2011) netted fish from their home tanks, placed them in isolation chambers for two hours prior to testing, and then used netting to transfer fish to the testing arena immediately before testing. Although they did not report the prevalence of non-responders in their study, we suspect that netting was a source of stress that might have reduced the likelihood of schooling. Our goal was to decrease the amount of netting-related stress that carried over to testing with the model school. To this end, we eliminated the second netting and transferred fish to the testing arena by gently pouring them from the isolation chamber into the testing arena. We tested the hypothesis that netting-related stress decreased the likelihood of schooling by giving individuals different recovery times before testing, i.e. different amounts of time in isolation chambers to recover from netting.

Next, we tested whether individual differences in schooling were consistent across time. We measured the amount of time individual three-spined stickleback spent schooling with models on two different occasions separated by approximately two weeks. We tested for differences in schooling related to body size and sex so that we could control for these variables when calculating repeatability.

METHODS

Experimental subjects were sexually mature three-spined stickleback (*Gasterosteus aculeatus*) collected from the Navarro River, CA in June, 2010. Fish were housed in 9.5 L tanks in groups of 4-5. Clean water was provided via a recirculating flow-through system that consisted of a series of particulate, biological, and UV filters (Aquaneering, San Diego, USA). Ten percent of the water volume in the tanks was replaced each day. Fish were fed a mixed diet consisting of frozen bloodworms, brine shrimp, and Mysis shrimp in excess each day. Two weeks prior to testing, fish were individually tagged with a unique combination of colored marks using fluorescent visual implant elastomer (Northwest Marine Technology, Inc.).

The testing arena was similar to that used in Wark et al. (2011). A circular plastic pool (1 m diameter, 10 cm water depth) was filled with water from the flow-through system. The model school was composed of seven clay models that were shaped and colored to match stickleback from this population (Figure 4.1, but see Wark et al. (2011) for an alternative to clay models). Three-spined stickleback prefer to group with similar sized conspecifics (Ranta et al. 1992). We used models that spanned the size range of adult stickleback in this population (30-45 mm). To create the model school, models were arranged in a 5 cm radius hexagon with one model in the center (approximately one body length nearest neighbor distance, Figure 4.1). The model school was suspended with fishing line from a clear disc, which hung 30 cm above the surface of the water. The disc was connected to a microwave turntable motor (Timetech Inc., Catalog No. S430.1) and when the motor was activated, the models moved together in a circle at a velocity of 7.5 cm/sec (models were offset 15 cm from center, rotation direction was random). While still, the models were oriented haphazardly. When set in motion remotely, all models oriented in the forward direction.

Experiment 1: Effect of recovery time

In preliminary trials, we netted individuals from their home tanks and moved them directly to the arena for testing. Approximately half of all individuals tested did not swim with the model school within a ten minute observation (M. Grobis, unpublished data). Stickleback tend to aggregate almost immediately when placed in an aquarium so the lack of schooling we observed was uncharacteristic of the species. We suspected that carry-over stress from netting might have contributed to the failure to school. To test this idea, we manipulated the amount of time that fish were allowed to recover between netting and testing (*recovery time*). We created two recovery time treatments by netting individuals and then placing them individually into 500 mL opaque isolation chambers filled with tank water for either 1 or 24 hours (1 hour vs. 24 hour recovery time) prior to testing. Equal numbers of fish from each treatment were tested each day.

At the start of each schooling trial, a focal individual chosen randomly by coin flip was gently poured from the isolation chamber into the pool and allowed to acclimate for 5 minutes. Then we set the model school in motion for ten minutes and recorded whether or not the fish swam within one body length of the model school (success or failure to school).

Statistical analysis. To test whether recovery time affected the likelihood that fish would school, we compared the proportion of fish that schooled in the 1 hour (n=13 individuals) vs. 24 hour (n=14 individuals) treatments using a Z test.

Experiment 2: Consistent individual differences in schooling

To assess the consistency of schooling, we measured the amount of time three-spined stickleback spent swimming with the model school on two occasions. On May 10-12, 2011 we observed the schooling behavior of 35 individuals (body length range 30-45.5 mm, mean length ± SE = 39.2 ± 0.7 mm). Based on the results from experiment #1 (see Results), individuals were netted from their home tanks and given 24 hours to recover before testing. A focal individual was gently poured from the holding container into the pool and allowed to acclimate for 5 minutes. After this, we set the model school in motion for ten minutes and recorded how much time the focal fish spent moving within one body length of the models (*time schooling*). At the end of the trial, fish were returned to their home tanks after we recorded sex (indicated males that were visually identifiable by their nuptial coloration), ID (using elastomer tags), and body length. We repeated observations of time schooling with the same individuals on May 26 and 27, 2011.

Statistical analysis. Our goal was to assess the consistency of individual differences in schooling behavior. We inferred that there were statistically significant consistent individual differences in behavior if the 95% confidence intervals surrounding a repeatability estimate did not overlap zero (Nakagawa and Schielzeth 2010; Wolak et al. 2011). We wanted to control for other factors that could contribute to between individual variation and therefore might inflate repeatability estimates (Dingemanse and Dochtermann 2013). We used a linear mixed model to estimate the fixed effects of sex, body length, and trial on time schooling with fish ID included as a random effect. We verified that time schooling was approximately normally distributed through visual inspection of a QQ plot of the residuals. We used 95% confidence intervals of fixed effects to determine whether they needed to be controlled for when calculating repeatability. To test whether individuals consistently differed from one another in time schooling, repeatability and 95% confidence intervals were calculated using the R package ICC (Wolak et al. 2011). R was used for all statistical calculations (R Core Team 2014).

RESULTS

Experiment 1: Effect of recovery time

Recovery time had a significant effect on the likelihood on schooling ($Z = 2.57$, $p = 0.01$). All 14 of the fish in the 24 hour recovery treatment schooled while only 8 of the 13 fish in the 1 hour recovery time treatment schooled (proportion = 0.615).

Experiment 2: Consistent individual differences in schooling

Time spent schooling varied greatly, ranging from 1-293 out of 600 seconds. The mean time schooling was 124.6 ± 15.2 se seconds. We did not detect an effect of sex, body length, or trial on time schooling (effect and 95% CI, sex = 23.8 [-39.6, 87.0], body length = 0.47 [-7.02, 8.01], trial = 1.76 [-30.7, 35.2]) and therefore did not control for these factors when calculating repeatability. Between individual differences accounted for more than 50% of the variation in schooling behavior (repeatability = 0.57 [0.28, 0.76], N = 35, Figure 4.2). To put this in perspective, a meta-analysis of published repeatability estimates found that the average amount of variation explained by differences between individuals was 37% (R = 0.37 [0.36, 0.38], Bell et al. 2009).

DISCUSSION

We present a method that utilizes a model school to quantify consistent individual differences in schooling behavior between individuals of a single population of fish. In our first experiment, we found that allowing fish to recover for 24 hours prior to testing increases the likelihood of schooling compared to allowing just one hour to recover. In our second experiment, we show that individuals consistently differ in time schooling and differences in schooling are independent of sex and body size. These results suggest that the model school originally developed by Wark et al. (2011) might be a useful tool for quantifying consistent individual differences in schooling behavior within populations.

The first experiment showed that allowing the fish to recover for a full day before testing increased the likelihood of schooling. This was an important step as it allowed us to collect behavioral data for every individual. Behavioral datasets are often plagued by non-responsive individuals and the difficult question of how to deal with missing data is left to the experimenter. If they decide to exclude non-responders from analysis, their results are bias because they have ignored a particular population, i.e. the inactive or timid individuals. However if the experimenter assigns non-responders scores of zero and includes them, repeatability calculated from these data will give erroneous results since any individual that receives zero in multiple trials will appear to be extremely consistent (Stamps et al. 2012). By increasing the recovery time, we avoided the problem of non-responders presumably because of the added time the animals had to recover from the stress of netting. It is possible that the decrease of non-responders we observed in the 24 hour recovery time treatment group was population specific and researchers are encouraged to assess the need for long recovery in the population or species they study. The prevalence of non-responders was not reported in either of the studies that previously used the model school to assess schooling behavior so a population comparison is not possible at this time.

Another possibility is that longer social isolation increases the motivation to school. Researchers interested in observing foraging behavior often deprive animals of food prior to testing to increase the motivation to feed. Fish that are deprived of social interactions might similarly experience an increase in the urge to interact with conspecifics. Teasing apart the impact of netting stress vs. social isolation on schooling is an interesting direction for future research.

Time schooling was significantly repeatable suggesting that three-spined stickleback within a population exhibit consistent individual differences in schooling behavior. Importantly, differences between individuals were independent of sex and body size and instead appear to be a fundamental difference in behavioral phenotype that is consistent across time. The amount of variation in schooling behavior that was attributed to differences between individuals in this study was high relative to the average between-individual variation in behavior reported elsewhere in biology literature (Bell et al. 2009) lending confidence that the methodology we used successfully eliminated unwanted sources of variation.

There are two major drawbacks to using live stimuli when quantifying consistent individual differences in behavior. First, it is extremely difficult to standardize the experience of each focal animal during exposure to live animal stimuli, which introduces unwanted variation. At best, stimulus animals' behaviors will differ randomly from trial to trial such that the variation they introduce is random noise. However, it is possible that stimulus animal(s) will react to the focal animal that is being observed and vice versa creating a feedback loop such that the behavioral interaction between the focal animal and the stimulus animal(s) overshadows the underlying tendency of the focal animal (Rands et al. 2003; Conradt and Roper 2005). Eliminating the possibility of a back-and-forth interaction is especially important in a schooling context where the behavior of the majority typically outweighs the tendency of individuals (Ward et al. 2008). The second drawback to using live stimulus animals is the necessity to collect or rear a larger number of animals for an experiment. Minimizing the number of animals needed for experiments is a concern for animal welfare and conservation alike (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011). The experimental apparatus we present here meets the need for a suitable replacement for live stimuli in the measurement of schooling behavior.

In conclusion, in this study we present an experimental apparatus for quantifying consistent individual differences in schooling behavior. We used it to demonstrate that three-spined stickleback show consistent differences in time schooling that cannot be explained by size or sex. The model school can be used to elicit schooling behavior, avoiding the shortcomings of using live conspecifics as stimuli.

FIGURES

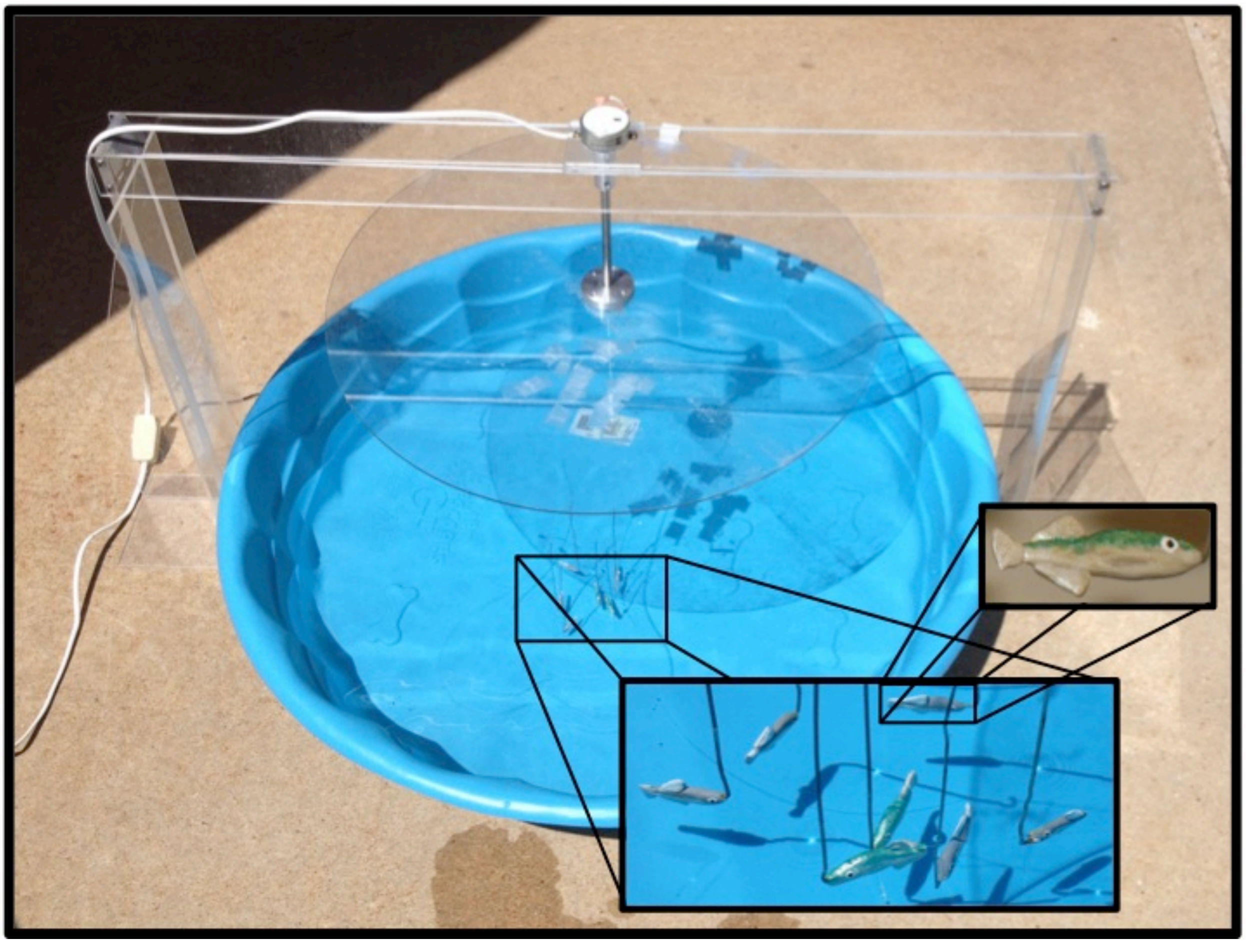

Figure 4.1. Behavioral arena containing model school. The first inset shows the model school at rest. When set in motion all models orient in the same direction. The second inset shows a clay model stickleback. Models ranged from 30-45 mm in length to match the range of length of adult stickleback in the Navarro River population.

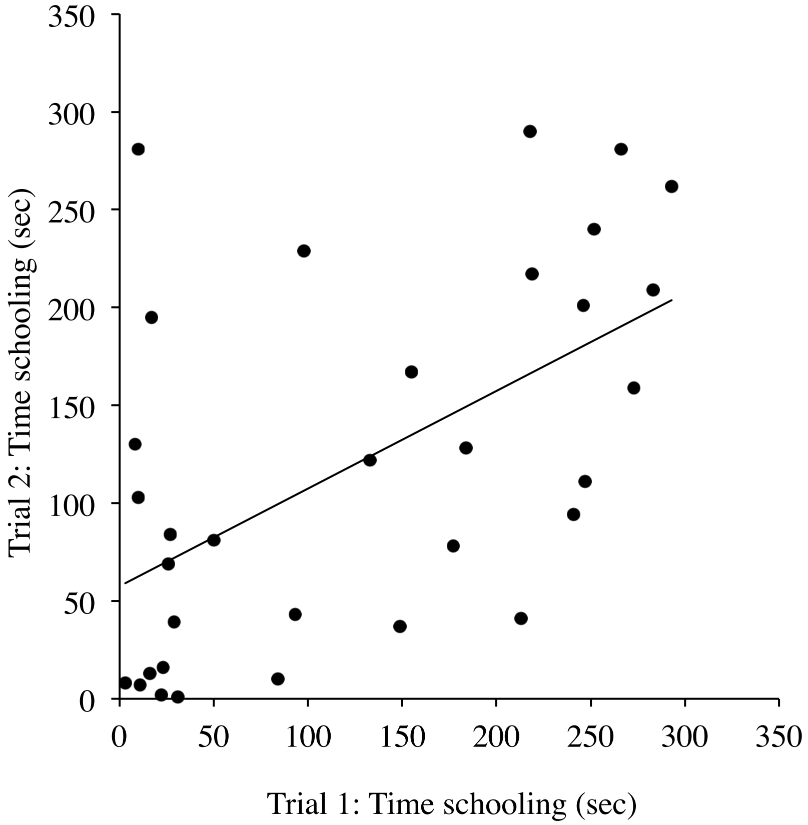

Figure 4.2. Experiment 2. Individuals consistently differed in time schooling across two weeks. Dots represent the amount of time individuals spent schooling with models over 600-second observations. N = 35.

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