Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*

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In recent years, evidence for interindividual variation in "personality" within animal populations has been accumulating. Personality is defined as consistency in an individual's behavioral responses over time and/or across situations. One personality trait that has potentially far-reaching implications for behavioral ecology, and may provide insight into the mechanisms by which consistent behavioral correlations arise, is that of boldness. Boldness is defined as the tendency of an individual to take risks and be exploratory in novel contexts. Using the framework of behavioral syndromes, we tested for individual differences in boldness in the laboratory among field-caught juvenile bluegill sunfish (*Lepomis macrochirus*) within and across the contexts of exploratory behavior, activity, and risk taking (e.g., antipredator) behavior. After such testing, individuals were tagged and returned to their lake of origin as part of a mark–recapture study testing for the repeatability of individual differences in boldness. Here, we report strong and consistent individual differences in boldness within and across all 3 behavioral contexts. Additionally, we observed that at least some boldness behaviors were repeatable after a 1–3 month recapture period. This study provides novel evidence for a boldness syndrome in sunfish, as well as insight into how behavioral types (e.g., shy/bold) may evolve and be maintained in natural populations. *Key words:* activity, boldness, exploration, personality, risk taking, temperament. *[Behav Ecol 20:231–237 (2009)]*

The behavior of animals is known to vary widely within and between populations (Wilson 1998; Foster and Endler 1999). Understanding the origin and maintenance of such phenotypic variation has been of considerable importance to evolutionary and behavioral ecologists for many years. More recently, evidence from a number of taxa has shown that individual animals exhibit behaviors that are consistent over time and/or situations and, as such, have distinct "temperaments" or "personalities" (Réale et al. 2007). Personality traits are likely involved in many aspects of an animal's behavioral repertoire (e.g., foraging, habitat use, and antipredator behavior) (Réale et al. 2007; Martin and Réale 2008). Therefore, understanding the proximate and evolutionary mechanisms underlying these traits is of interest to behavioral ecologists (Dingemanse and Réale 2005; Réale et al. 2007). Although recent studies have begun to provide insight into the proximate bases of personality traits, the fitness consequences of different behavioral types (e.g., bold/shy) and the manner by which they evolve and are maintained in natural populations remains poorly understood.

One personality axis that has potentially far-reaching implications for behavioral ecology is that of "shyness" and "boldness." Boldness may loosely be defined as the tendency of an individual to take risks and be exploratory in novel contexts (Wilson et al. 1994; Wilson and Stevens 2005). Within the shy–bold continuum, individuals vary from being extremely bold (reacting to novel stimulus by either behaving normally or becoming actively exploratory) to extremely shy (either retreating or becoming vigilant when confronted with a novel stimulus) (Wilson et al. 1993). Individual variation in boldness has been documented for a variety of taxa, including birds (Carere and van Oers 2004; Both et al. 2005), mammals (Réale and Festa-Bianchet 2003; Svartberg et al. 2005;

Dochtermann and Jenkins 2007), reptiles (Lopez et al. 2005), fishes (Ward et al. 2004; Wilson and Stevens 2005; Bell and Sih 2007), insects (Kortet et al. 2007), and cephalopods (Sinn et al. 2006). Boldness has been shown to be associated with general activity and space use (Wilson and McLaughlin 2007), antipredator behavior (Brown and Braithwaite 2004; Brown et al. 2005; Lopez et al. 2005), aggression (Johnson and Sih 2005), mate selection (Godin and Dugatkin 1996), learning (Dugatkin and Alfieri 2003), dispersal (Fraser et al. 2001), invasiveness (Rehage and Sih 2004), and foraging and exploration (Wilson and Stevens 2005). Furthermore, several studies have provided evidence that boldness is correlated with fitness-related traits (Dingemanse et al. 2004; Sinn et al. 2006; Brown, Jones, and Braithwaite 2007), heritable (Dingemanse et al. 2002; Drent et al. 2003; Brown, Burgess, and Braithwaite 2007), and subject to natural selection (Réale and Festa-Bianchet 2003).

Until recently, behavioral ecologists have primarily relied on natural selection and optimality approaches to examine individual-level differences in behavior, including boldness. Optimality models generally assume that animals are selected to adopt behaviors that yield the highest fitness benefit–cost ratio. However, the typical optimality approach frequently fails to recognize the importance of individual-level differences in behavior and tends to consider such variation as nonadaptive "noise" around an adaptive population mean (or single optimal phenotype) (Dall et al. 2004). Alternatively, the recent concept of behavioral syndromes (Sih et al. 2004; Sih and Bell 2008) emphasizes individual-level responses and, at times, limited behavioral plasticity as a constraint to the evolution of optimality even when favored by selection.

A behavioral syndrome is defined as a suite of correlated behaviors across multiple (2 or more) observations (Sih et al. 2004; Sih and Bell 2008). The concept of behavioral syndromes has increasingly been applied to wild populations (e.g., Wilson and McLaughlin 2007) and has already been extensively developed by psychologists for humans and a few domesticated animal species (Gosling 2001; Sih et al. 2004). One of the primary investigative advantages of using the concept of syndromes in behavioral research is that it advocates

considering an organism's behavior holistically, over both short and long timescales and across different behavioral contexts or situations. Owing to the fact that boldness is associated with many behaviors (e.g., Fraser et al. 2001; Rehage and Sih 2004; Wilson and Stevens 2005), it seems appropriate to consider a syndrome approach that encompasses a range of ecologically relevant behaviors across and within contexts, as opposed to considering behaviors independently and solely within contexts. This is particularly true as the contextual nature of boldness remains a subject of some contention, with arguments both for an adaptive domain-specific view (Coleman and Wilson 1998; Wilson and Stevens 2005; Webster et al. 2007) and a more constraint based domain-general (Johnson and Sih 2005; Dochtermann and Jenkins 2007) evolutionary view for the origin of risk-taking behaviors. Thus, the experimental framework of behavioral syndromes may provide important insight into the ecological and evolutionary implications of boldness and associated behaviors, as well as their underlying mechanistic and contextual bases (Sih and Bell 2008).

Here, we use the framework of behavioral syndromes to ascertain whether wild-caught juvenile bluegill sunfish (*Lepomis macrochirus*) exhibit individual variation in boldness and whether boldness behavior is correlated across foraging, activity, space use, and exploratory and antipredator contexts. Sunfish are particularly good candidates for studying boldness as they occur naturally in many lake systems across southern Ontario, Canada, are amenable to a variety of capture methods (e.g., trap, seine, and line) and acclimatize readily to laboratory conditions. Furthermore, sunfish exhibit distinct morphological and behavioral variation in relation to individual differences in habitat and resource use (Robinson et al. 1993; Robinson and Wilson 1994; Skulason and Smith 1995; McCairns and Fox 2004), and at least one species, the pumpkinseed sunfish (*Lepomis gibbosus*), has previously been used as a model system to examine the context dependency of boldness behavior (Wilson et al. 1993; Coleman and Wilson 1998).

To quantitatively assess the existence of a boldness syndrome in the bluegill sunfish, we captured juvenile individuals from a small north-temperate lake and exposed them to behavioral tests quantifying activity, exploration, and antipredatory behaviors over a 3-day period in the laboratory. Should a boldness syndrome incorporating activity, exploration, and risk taking (e.g., antipredator) behaviors exist, we predicted that boldness behaviors would be consistent and repeatable across contexts. After laboratory trials, all fish were individually marked (where possible) and returned to their point of capture in the lake as part of a mark–recapture project assessing repeatability of behavior over a 3-month period. Repeatability is an important aspect of animal personality, as it provides insights into the consistency of individual differences in behavior under changing environmental conditions and thus the underlying proximate (e.g., physiological, genetic) basis for the behavior being observed. Based on the definition of personality given above, understanding the repeatability of variation in personality is key to understanding its role and importance in the ecology of wild populations (Martin and Réale 2008).

METHODS

Field collection

Between June 25 and August 7, 2007, we used a 10-m beach seine to capture 60 juvenile (Yr. 1+) bluegill sunfish (fork length: 6.2–8.3 cm; weight: 3.55–9.17 g) from the littoral zone of a small bay in Patterson Lake, Ontario, Canada. Captured individuals were placed in a cooler containing lake water (mean temperature = 23.1 °C) and transported to our labora-

tory at Carleton University (transit time = 1.5 h). Ten individuals were captured during each collection period, once per week. Patterson Lake is a high-predation environment, as it contains many piscivorous fishes (e.g., smallmouth and largemouth bass, northern pike, and pickerel) and birds (e.g., belted kingfisher, northern loon, and great blue heron).

Experimental holding conditions and general experimental apparatus

On arrival at the laboratory, each wild-caught fish was placed singly into the "refuge" area of 1 of 10 glass aquaria (82 l, 92 × 30 × 30 cm, Figure 1). Each aquarium contained aerated and filtered dechlorinated tap water maintained at 23 ± 1 °C. All aquaria were exposed to overhead fluorescent lighting on a 12:12 h light:dark cycle. Each aquarium was divided into thirds horizontally as well as vertically with lines drawn on the front and back walls. In doing so, each aquarium was divided into 9 distinct zones to facilitate the recording of fish water column use. The refuge area was located at one end of the aquarium, contained a corner filter and a plastic aquarium plant for cover, and was separated from the remainder of the aquarium by an opaque white plastic partition equipped with a sliding door. This gated partition was located 25 cm from the left side of the aquarium. All aquaria were covered externally with tan cardboard at both ends and the back wall to prevent interaction between subjects in other aquaria. In addition, all aquaria contained a 2.5-cm layer of natural colored aquarium gravel as a substratum.

During the holding period, each fish was fed 10 brine shrimp (*Artemia* sp.) per day, except on Day 2 of holding (Day 1 of experimentation) as the behavioral experiment the following day involved a foraging component. Modifications to the experimental aquaria for each behavioral test are described in detail in the appropriate section below. To eliminate the risk of confounding our results to the novel stimulus on Days 2 and 3 of experimentation, we chose to use the same general experimental setup as that for Day 1, except for the addition of a novel stimulus (e.g., food/object or predator model). This allowed us to both obtain a measure of behavioral consistency across days and behavioral contexts in the same arena as well as insure that the behavior being observed was attributable to the novel within-aquarium stimulus and not the novel aquarium itself. In addition to these modifications, all aquaria were placed behind an observation blind to minimize external disturbance and to facilitate the recording of behavioral trials.
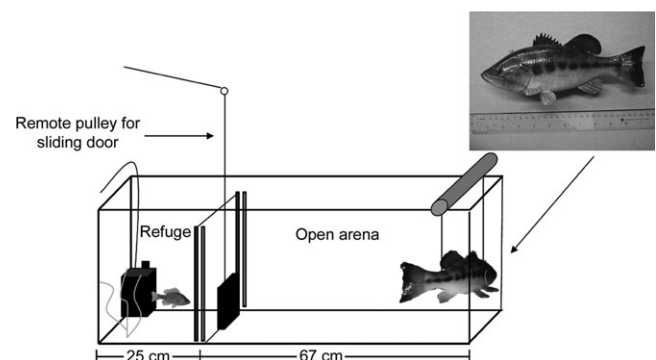

Figure 1
Schematic representation of the experimental aquarium used on Day 3 of initial experimentation to quantify the risk-taking tendencies of individual sunfish in the presence of a realistic 3-dimensional model of a largemouth bass (*Micropterus salmoides*). The same experimental setup was used in experiments 1 (Day 1) and 2 (Day 2), except that the predator model was excluded.

All trials were recorded using a Digital8 video camera (Sony DCR-TRV280, Song of Canada, Ltd., Toronto), and fish behaviors were quantified from the tapes using the behavioral analysis software JWatcher (v1.0).

Behavioral experiments

Day 1: latency to exit a refuge and explore a novel environment
Approximately 24 h after arrival in the laboratory, individual sunfish were tested in their respective aquarium for their willingness to explore a novel environment. The experimental aquaria being used for this measure were identical to those described in the general experimental apparatus section above. At the onset of each behavioral trial, the sliding door of the test aquarium was lifted manually via a remote pulley system operated from behind the blind, thereby allowing the fish to enter and swim freely throughout the remaining open area of the aquarium (hereafter the "arena"). Each fish was given 60 min to exit the refuge area and explore the arena. On exiting the refuge, several measures of individual boldness were quantified for a further 10 min. These boldness measures included individual differences in latency to emerge from the refuge area, total time spent active, and total time spent in the upper third zone of the water column of the arena. Latency to exit the refuge was defined as the total time required for the focal fish to completely exit the refuge area within the 60-min pretrial period. Activity was defined as the total time spent swimming (vs. holding position or resting on the bottom) in the arena during the 10-min observation period. Both activity and water column use are increasingly being recognized as ecologically relevant measures of risk taking and exploratory behavior among studies of animal personality (e.g., Sih et al. 2003; Bell 2005; Wilson and McLaughlin 2007). In addition, proximity to the water surface and higher levels of activity in fishes tend to increase an individual's exposure to fish and particularly avian predators (e.g., Lima and Dill 1990; Godin 1997; Sih et al. 2003). Because we are primarily interested in measures of risk taking, behaviors were only quantified while fish were in the open arena of the aquarium, not the refuge, though time spent in the refuge after initial exit was recorded.

After the 10-min trial period, the partition door was closed, and each fish was returned to the refuge area of the aquarium. Fish that did not exit during the 60-min pretrial period were assigned a latency-to-exit score of 60 min and were excluded from all further behavioral assays (Days 2 and 3). Fish were then left undisturbed until the onset of the next experiment on Day 2.

Day 2: latency to inspect a novel object/food item
Approximately 24 h after the conclusion of experiment 1 (Day 1), the sunfish were tested individually in their respective aquarium for their willingness to explore a novel object and/or food item in the arena. The experimental aquaria being used for this measure were identical to those described in experiment 1, except for the additional presentation of a novel object and food item. Approximately 5 min prior to the onset of behavioral testing, a piece of dried krill (1 cm long) was lowered into the experimental aquarium via a pulley system similar to that used for the partition door. The pulley system consisted of a small teardrop-shaped lead weight (novel object) attached to the most distal portion of a monofilament line, followed by the krill (novel food item) attached 15 cm above the weight. The food item was lowered slowly to minimize disturbance to the aquarium and suspended such that the weight just rested on the substratum, and the food item was directly in the center of the middle third of the aquarium.

Refuge door operation at the onset of each behavioral trial was identical to that described for the first experiment (Day 1). Each fish was given 60 min to exit the refuge area and explore

the novel object/food item in the open arena. Individuals that did not exit within the pretrial period were assigned the maximum latency-to-exit value of 60 min and held over for further experimentation on Day 3.

On exiting the refuge, the focal fish's latency times to touch the novel object or food item with its mouth were quantified over a 10-min trial period. After this trial, the door to the refuge partition was lowered, and the focal fish was returned to the refuge. Fish were then fed and left undisturbed until the onset of the third experiment on Day 3.

Day 3: simulated predation risk
Approximately 24 h after the completion of experiment 2, the fish were tested individually for their willingness to take risks when confronted with a simulated risk of predation. The experimental aquaria being used for this measure was identical to those described for experiment 1, except for the additional presentation of a realistic 3-dimensional model of a large (total length 25 cm) piscivorous predator (largemouth bass, *Micropterus salmoides*) (Figure 1). Five minutes prior to the onset of the pretrial period, the predator was placed 15 cm from the side of the experimental tank opposite the refuge. The model was positioned such that its lateral side faced the door of the refuge at the other end of the tank. Additionally, the model was suspended in midwater of the arena by monofilament line attached overhead to a clear Plexiglas rod. The rod extended from its position above the aquarium to outside of the blind such that the observer was able to produce slight lateral undulations in the model by small rotations of the rod at regular 30-s intervals. As with experiments 1 and 2, focal fish were given 60 min to exit the refuge during the pretrial period. Once the focal fish exited the refuge, several measures of its boldness and antipredator behaviors were quantified over a 10-min trial. Boldness measures quantified included total time spent active in the arena, total time spent inspecting the predator, and total time spent in the zone closest to the predator model. Predator inspection was defined as an individual approaching a potential predator in a tentative, saltatory series of movements interrupted by short stationary pauses, during which time the inspector is visually fixated on the predator (Dugatkin and Godin 1992). Each inspection approach was terminated by the prey fish moving away from the predator.

Repeatability of behavior: laboratory testing of marked–recaptured fish

To assess the repeatability of boldness behavior in the bluegill sunfish of Patterson Lake, we conducted a mark–recapture experiment in which all fish that completed the laboratory experiments on Days 1, 2, and 3 were tagged and released at their point of capture in the lake. Tagging occurred the day after the conclusion of the 3-day experimental period using visible elastomer implant tags (Northwest Marine Technology Inc.) and following a standardized protocol (Ward A, personal communication). This protocol involved using unique combinations of 4 colored tags and 6 different body locations along the dorsal musculature. Prior to tagging, fish were anesthetized using tricaine methanesulfonate (MS-222, 1:10 000 dilution) buffered with sodium bicarbonate and then injected subcutaneously with a small amount of elastomer using a syringe (29 gauge, 1 cc) in 2 locations along the dorsal fin area (e.g., left/right side of first dorsal spine). All tagged fish were then wet weighed, external parasites (e.g., black spot, *Uvulifer ambloplitis*) counted, and morphometric measurements (e.g., standard length, girth) made. After tagging, all fish were held in the laboratory for 7 days, to allow for recuperation and to ensure tag retention, before being released into their lake of origin at the same location of initial capture.

Previous studies using this type of elastomer tag have revealed 99% tag retention periods for up to 6 months (Dewey and Zigler 1996). We confirmed these findings with additional bluegill sunfish from our study site in the laboratory and found 100% tag retention and 0% mortality among 33 tagged juvenile sunfish over a 6-month period (Wilson ADM, Godin J-GJ, unpublished data).

Marked individuals were recaptured 1–3 months after initial release (depending on release date) using a beach seine in the littoral zone of Patterson Lake. Any individuals that were recaptured prior to the final 2 weeks of recapturing attempts were weighed, measured, and released to allow time for maximum growth over the course of the summer–early fall season. Individuals recaptured during the final 2 weeks were brought back to the laboratory in the same manner as their initial arrival, except that recaptured fish were only exposed to the third experiment (Day 3; described above) and not those conducted on Days 1 and 2 of initial experimentation. Four behavioral traits were quantified for repeatability (r): 1) latency to exit the refuge in the presence of a predator; 2) time spent in the area of the arena closest to the predator; 3) time spent inspecting the predator; and 4) total time spent active.

Statistical analyses

First, comparisons of individual behavioral traits within and across experimental days were made using the nonparametric Spearman's rank correlation test. These correlations were made to assess individual consistency in related measures of boldness behavior. To avoid an inflated chance of Type 1 error, our alpha level for this analysis was adjusted to be more conservative ($P < 0.0014$) using the Bonferroni correction.

Second, to test for the presence of a behavioral syndrome incorporating activity, risk taking, and exploratory behavior contexts, individual traits that best represented each of these behavioral contexts were collapsed into first principal component scores for each axis of interest using Principal Components Analysis (PCA) (Table 1). Across-context correlations between these scores were then calculated using Spearman's rank correlation test as above. To avoid an inflated chance of Type 1 error, our alpha level for this second analysis was also adjusted to be more conservative ($P < 0.0166$) using the Bonferroni correction.

Lastly, to quantify behavioral repeatability (r) in recaptured individuals, we followed the protocol described in Lessells and Boag (1987). Data were normalized by log transformation, where necessary. To establish the significance of our measures of repeatability, we calculated the 95% confidence intervals (CIs) for each measure as recommended by Nakagawa and Cuthill (2007).

RESULTS

All pairwise correlations that were significant, or suggest strong trends ($P < 0.05$), are shown in Table 2. Only those correlations that were significant at the appropriate adjusted alpha level (see Methods) are discussed in further detail below.

Behavioral correlations

In general, individuals that were more exploratory and emerged faster from the refuge had shorter latencies to touch a novel object (following exit from refuge) and were more active than less exploratory individuals. Individuals that were more exploratory and more active were also more willing to take risks by spending longer periods of time near the surface and closer to the predator model than less active/exploratory individuals. Similarly, those more active individuals also spent more time in the zone closest to the predator and spent more time inspecting the predator than less active individuals (Table 1).

PCA (across-context syndrome analyses)

Spearman rank correlation tests between the first PCA scores for all 3 axes of behavior suggest strong across context correlations between activity, risk taking, and exploratory behaviors. In particular, risk taking was strongly positively correlated with activity ($r_s = 0.615$, $P < 0.001$, Figure 2). Individuals that were more active were also more willing to take risks when confronted with a potential predation threat. Similarly, exploratory behavior was strongly negatively correlated with both activity ($r_s = -0.614$, $P = 0.003$, Figure 2) and risk taking ($r_s = -0.564$, $P = 0.008$, Figure 2), suggesting that more exploratory individuals (e.g., shorter latency-to-exit refuge) were also more active and spent more time performing risky behaviors than less exploratory individuals.

Behavioral repeatability (mark–recapture experiment)

During the course of our field season, 19 of a total of 43 marked and released individuals were recaptured. Six of these individuals were released prior to our final collection period in an attempt to allow for maximal growth during the field season and were not recaptured. The remaining 13 fish were recaptured in the final collection period of the study and were brought back to the laboratory to test for repeatability of their exploratory–antipredator behavior previously exhibited on Day 3 of the initial laboratory experiments (on average 36 ± 4 days earlier). Of the 4 behavioral traits considered, only latency to exit the refuge ($r = 0.63$, 95% CI $= 0.15–1.33$) and time spent in the area of the arena closest to the predator ($r = 0.83$, 95% CI $= 0.59–1.77$) were repeatable. In contrast, time spent being

Table 1

PCA loadings of within-context behavioral variables used to generate a principal component scores (PC1) to assess across-context correlations in activity, exploration, and risk-taking behavior in the bluegill sunfish (D = Day)

Behavioral context	Behaviors within each context	Loadings for PC1	% Variation explained
Exploration	Latency to emerge from refuge (D1)	0.6608	44.7
	Latency to emerge from refuge (D2)	0.5612	
	Latency to emerge from refuge (D3)	0.4843	
	Latency to touch object (D2)	0.1179	
Activity	Activity (D1)	0.7071	51.2
	Activity (D3)	0.7071	
Risk taking	Time spent in upper water zone (D1)	0.3517	50.4
	Time spent close to predator (D3)	0.6804	
	Time spent inspecting predator (D3)	0.6429	

Table 2

Across- and within-context Spearman (r_s) correlations in measures of activity, exploration, and risk-taking behaviors in the bluegill sunfish

Behavioral trait 1	Behavioral trait 2	r_s	P value	N[a]
Latency to emerge (Day 1)	Time spent in upper water zone (Day 1)	−0.458	0.0012[b]	47
Latency to emerge (Day 1)	Activity (Day 1)	−0.592	<0.0001[b]	47
Activity (Day 1)	Time spent in upper water zone (Day 1)	0.483	0.0006[b]	47
Latency to emerge (Day 1)	Latency to emerge (Day 2)	0.474	0.0009[b]	46
Latency to emerge (Day 2)	Time spent in upper water zone (Day 1)	−0.369	0.0116	46
Latency to emerge (Day 2)	Activity (Day 1)	−0.427	0.0031	46
Latency to touch novel object/food item (Day 2)	Latency to emerge (Day 1)	0.450	0.0356	22
Latency to emerge (Day 3)	Time spent in upper water zone (Day 1)	−0.305	0.0439	44
Latency to emerge (Day 3)	Latency to emerge (Day 1)	0.497	0.0006[b]	44
Latency to emerge (Day 3)	Latency to emerge (Day 2)	0.524	0.0003[b]	44
Activity (Day 3)	Latency to emerge (Day 2)	−0.313	0.0460	41
Activity (Day 3)	Latency to emerge (Day 3)	−0.408	0.0081	41
Time spent close to predator (Day 3)	Latency to emerge (Day 2)	−0.415	0.0079	41
Time spent close to predator (Day 3)	Activity (Day 3)	0.795	<0.0001[b]	41
Time spent inspecting predator (Day 3)	Activity (Day 3)	0.569	0.0001[b]	41
Time spent inspecting predator (Day 3)	Time spent close to predator (Day 3)	0.610	<0.0001[b]	41

All Spearman correlations at $P < 0.05$ are shown.

[a] N denotes number of fish tested.

[b] Correlations that are significant at Bonferroni's adjusted alpha = 0.0014.

active ($r = -0.15$, 95% CI $= -0.74$ to 0.44) and time spent inspecting the predator ($r = -0.20$, 95% CI $= -0.7894$ to 0.3924) were not significantly repeatable.

DISCUSSION

Our results showed that juvenile bluegill sunfish exhibited consistent and repeatable individual differences in behavior and that these differences were reflected in a behavioral syndrome incorporating aspects of activity, risk taking (e.g., antipredator), and exploratory behavior. We characterize the observed behavioral syndrome as being a boldness syndrome because individual willingness to take risks (e.g., predator inspection) and explore novelty is significantly linked across contexts. Generally, bolder individuals were more active, more willing to explore novel objects/environments and more willing to inspect a potential predator and spend time in risky areas than shy individuals.

Our study addresses 3 important areas of current research need, namely, animal personality, behavioral consistency/repeatability, and the nature of behavioral syndromes in wild-caught animals. As predicted, using pairwise correlation analysis, we found strong behavioral correlations across contexts, across individual traits and, more importantly, across each of the principal component scores representing the 3 behavioral axes of interest (e.g., exploration, activity, and risk taking). These results provide compelling evidence for behavioral corre-

Figure 2
Behavioral syndrome showing correlations between 3 component behaviors (activity, exploration, and risk taking) in bluegill sunfish. Numbers shown indicate results from Spearman rank (r_s) correlations tests. Levels of significance are indicated by (*) for each pair of behaviors (*$P = 0.008$, **$P = 0.003$, *** $P < 0.001$).

lations across multiple observations and thus for behavioral syndromes (sensu Sih et al. 2004; Sih and Bell 2008) in bluegill sunfish. In addition, the individual behavioral traits that we used as a basis for our PCA analyses also demonstrated strong correlations, and therein consistency, in multiple measures of similar behaviors (Table 2). One such behavior that appears to play a prominent role in this boldness syndrome is that of emergence behavior. Emergence from a refuge into an open habitat carries a certain level of risk of predation and uncertainty about ambient ecological conditions in that habitat (Godin 1997; Sih 1997). Similarly, although latency to emerge from a shelter is commonly used as a laboratory measure of animal personality (Hedrick 2000; Brown and Braithwaite 2004; Lopez et al. 2005; Wilson and McLaughlin 2007), the importance of this behavior and its relationship to fitness remains unclear, though some studies do exist (e.g., Brown et al. 2005; Brown, Jones, and Braithwaite 2007). For example, Brown, Jones, and Braithwaite (2007) found that boldness behaviors, including refuge emergence, were strongly influenced by both body size and predation pressure in the poeciliid fish *Brachyraphis episcopi*. In our study, latency to emerge from a refuge was highly correlated across all 3 experimental days as well as with activity, novel object exploration, water column position, and time spent in close proximity to the predator model. We also found that emergence behavior was highly repeatable over the long term based on our mark–recapture study. The fact that emergence behavior was consistent across all 3 experimental days, as well as repeatable among those fish recaptured, suggests that this behavior has some ecological and/or evolutionary importance.

From an adaptive standpoint, poor or uncertain information about the environment outside a refuge pose a serious constraint on the ability of potential prey to respond adaptively to ambient conditions, as represented, for example, by food availability, predation risk, and novel situations (e.g., new environments), outside of its refuge (Sih 1992, 1997). Alternatively, emergence behavior may represent some underlying genetic predisposition arising through natural selection pressures favoring it (e.g., independent selection) or other traits that it may be correlated with (e.g., correlational selection). Bell (2005) demonstrated experimentally that selection regimes, such as predation, can favor the development of behavioral syndromes. As such, activity, risk taking, and exploratory
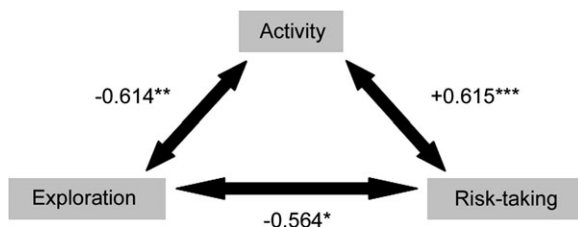
behavior in bluegill sunfish may similarly be shaped by high-predation intensity at our study site (Patterson Lake). Correlations between activity and exploratory behavior have been shown to be relatively common across species, lending support to the notion that this correlation is shaped by natural selection (Sih et al. 2004; Réale et al. 2007; Biro and Post 2008). This proposition is further supported by recent studies that have shown that boldness behavior can have a genetic component (Wright et al. 2006) and may therefore be heritable in some species (Drent et al. 2003; Sinn et al. 2006; Brown, Burgess, and Braithwaite, 2007).

The importance of predation in shaping risk-taking behavior associated with boldness was a primary consideration in our choosing to repeat the simulated predator experiment as a test of behavioral repeatability. As such, we expected antipredator behavior to remain temporally consistent over the course of our 3-month experimental period due to apparently high predation pressures present at our field site for the duration of the study. Interestingly, we found that only emergence behavior and time spent near the predator model were repeatable. Neither activity level nor predator inspection was repeatable. There are several plausible explanations for these differences. Firstly, as advocated by Bell and Stamps (2004), these differences may reflect adaptive temporal variation in trait combinations that are influenced by variation in developmental and/or ecological circumstances over time. Although difficult to assess in one population over a short time period, Dingemanse et al. (2007) have provided evidence for the adaptive evolution of favorable trait combinations with an interpopulation comparison of the 3-spined stickleback (*Gasterosteus aculeatus*).

Alternatively, it is also possible that our nonsignificant repeatability findings above may be a result of a sample size effect or, more likely, habituation. Juvenile sunfish demonstrate high levels of learning and rapidly recognize previously encountered stimuli (Gotceitas and Colgan 1988; Colgan et al. 1991; Kieffer and Colgan 1992). It is therefore possible that our recaptured fish remembered their initial encounter with the predator model (on Day 3 of the initial experiments) and no longer considered it a significant threat when retested several weeks later. As such, their behavior (activity, predator inspection) toward a novel threat stimulus and one to which they have been acclimated would likely be different, as we observed and has been observed in other fish species (e.g., Brown 2001). In contrast, the observed repeatability of refuge emergence behavior might be attributable to its importance in the first critical moments of information gathering when exiting a refuge, prior to predator stimulus recognition.

In addition to consistency, repeatability of behavior, the nature of behavioral correlations across contexts, and the link between personality and fitness remain topics in need of further research. A recent meta-analysis of personality traits suggests that boldness (in males) appears to favor increased reproductive success at the expense of long-term survival (Smith and Blumstein 2008). Although the underlying mechanistic basis for this trade-off remains unclear, Smith and Blumstein (2008) suggest that both shy and bold behavioral types are maintained in natural populations because of equal respective lifetime reproductive success. For example, shy individuals might experience a short-term reproductive disadvantage compared with bold individuals but, because of their greater longevity, their overall lifetime reproductive success might be similar to that of bold individuals. This pattern, though not explicitly tested, is consistent with other current models explaining the evolution and maintenance of personality differences among individuals in populations (Bell and Stamps 2004; Stamps 2007; Wolf et al. 2007). Additionally, any fitness differences between bold and shy individuals may shed some light on the origin and maintenance of the behavioral differences evident between different reproductive and life-history strategies, such as that exhibited between parental and cuckolder males in fishes (Gross 1982), and perhaps even the ecological diversification seen in resource polymorphisms (Skulason and Smith 1995; Wilson and McLaughlin 2007).

Our current study illuminates for the first time the nature of a boldness syndrome in bluegill sunfish and the temporal repeatability and consistency of the behaviors involved. More research is needed to better understand the ecology and evolution of such a behavioral syndrome in sunfish in particular and in other animals in general. Though not presented formally here, we did quantify parasite load, hepatic–somatic index, and instantaneous growth rates in our recaptured sunfish and found that some fitness traits were correlated, even with our small sample size (Wilson ADM, Godin J-GJ, unpublished data). However, the connection between these fitness correlates and boldness behavior remains unclear and is currently the subject of a larger ongoing study.

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