OXFORD

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Behavioral differences within and among populations of an African cichlid found in divergent and extreme environments

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Abstract

Animals are increasingly faced with human-induced stressors that vary in space and time, thus we can expect population-level divergence in behaviors that help animals to cope with environmental change. However, empirical evidence of behavioral trait divergence across environmental extremes is lacking. We tested for variation in behavioral traits among 2 populations of an African cichlid fish (*Pseudocrenilabrus multicolor victoriae* Seegers, 1990) that experience extremes of dissolved oxygen (DO) and turbidity and are known to vary in a number of physiological and life history traits associated with these stressors. Using a common garden rearing experiment, F1 progeny from wild-caught parents originating from a swamp (low DO, clear) and a river (high DO, turbid) were reared in high DO, clear water. Predator simulation assays were conducted to test for (1) variation in boldness, general activity, and foraging activity between populations, (2) differences in correlations between behaviors within and across populations, and (3) repeatability of behaviors. There was strong evidence for divergence between populations, with swamp fish being more bold (i.e., leaving refuge sooner after a simulated predator attack) and active (i.e., spent more time out of refuge) than river fish. Across populations there were positive correlations between foraging activity and both boldness and general activity; however, within populations, there was only a strong positive relationship between foraging activity and boldness in the river population. Here, we have demonstrated that populations that originate from drastically different environments can produce progeny that exhibit measurable differences in behaviors and their correlated relationships even when reared under common conditions.

Key words: behavioral correlation, behavioral syndrome, boldness, hypoxia, pace of life, turbidity.

Environmental heterogeneity can have a strong impact on phenotypic diversity, driving divergence among populations through plasticity and local adaptation (Hendry 2015; Edelaar et al. 2017). As an environment shifts through time and space, behavioral traits are often a species' first line of defense in response to change. This is especially true under increasingly rapid, human-induced environmental change (Sih et al. 2011). Furthermore, we now understand that behavior is often a repeatable and heritable trait and that individuals consistently differ in personality within populations of a single species (Sih et al. 2004; Mittelbach et al. 2014; Mitchell and Biro 2017). And like many traits, aspects of personality (e.g., boldness, aggressiveness) can be under strong selection when faced with environmental extremes (Dubuc-Messier et al. 2017). For example, Brown et al. (2005) found that populations of a poeciliid fish *Brachyrhaphis episcopi* were consistently bolder if they were from high-predation versus low-predation sites across multiple rivers. Thus, we might expect populations to diverge in personality when they experience opposite ends of environmental extremes.

Personality traits have been increasingly found to covary with physiological traits and together may promote species persistence under environmental extremes (Dingemanse and Wolf 2010; Sih et al. 2015). For example, individuals that display high aggressiveness, high activity, and are bold (e.g., proactive types) have higher resting metabolic rates than less aggressive, less active, and shy individuals (e.g., reactive types) (Careau et al. 2008; Huntingford et al. 2010). Boldness, in particular, has also been shown to positively covary with metabolic scope (e.g., the difference between resting and active metabolic rates) (Killen et al. 2014; Binder et al. 2016). For example, bold bluegill sunfish *Lepomis macrochirus* have greater metabolic scope and exhibit greater aerobic capacity for locomotor activity than shy sunfish (Binder et al. 2016). These associations have provided empirical evidence for the pace-of-life syndrome which predicts that behavior, physiological, and life-history traits coevolve in response to correlational selection pressures (Réale et al. 2007, 2010). Spatial and temporal heterogeneity in environmental conditions thus has the potential to create geographic variation in a suite of behavioral, physiological, and life-history traits (Atwell et al. 2014; Montiglio et al. 2014). Understanding the role of environmental extremes in shaping population divergence in a suite of traits may be particularly important for understanding how species adapt to changing environments.

In aquatic ecosystems, hypoxia (i.e., low dissolved oxygen, DO) and turbidity (i.e., suspended particulates) are metabolically and visually challenging conditions, respectively, that have been shown individually to generate divergence in behavioral, life-history, and physiological traits in aquatic organisms (e.g., van der Sluijs et al. 2011; Chapman 2015). For example, in contrast to normoxic environments, hypoxic environments tend to favor fish that are less active (Abrahams et al. 2005; Gotanda et al. 2011; McNeil et al. 2016), have relatively larger gills and smaller brains (Chapman et al. 2008; Crispo and Chapman 2010), lower metabolic rates (Reardon and Chapman 2010; Crocker et al. 2013), and shorter brooding times due to the challenge of extracting oxygen at very low concentrations. Environments with anthropogenically elevated turbidity levels also impose a suite of potential stressors on fish (e.g., Bruton 1985; Newcombe and MacDonald 1991) and notably pose visual challenges for aquatic organisms (e.g., Utne-Palm 2002; van der Sluijs et al. 2011). For example, Dugas and Franssen (2011, 2012) found that red shiner *Cyprinella lutrensis* populations from turbid waters have larger eyes and more intense nuptial color patterns than clear-water populations, suggesting that such adaptions allow for better vision and more visible signals, respectively, when the water is turbid. In contrast to other traits, however, variation in personality across extremes of these 2 environmental stressors has been overlooked in recent literature.

In this study, we compared behavioral traits (e.g., general activity, foraging behavior, and boldness under predation risk) of 2 populations of the haplochromine cichlid *Pseudocrenilabrus multicolor victoriae* Seegers, 1990, from divergent environmental conditions. We also examined whether individuals exhibited consistent individual differences in these behavioral traits (i.e., personality) both within and across populations. This species is broadly distributed across the Nile River basin of East Africa where it is found living under a variety of environmental conditions, the extremes of which include the dense interior of hypoxic (low DO), clear swamps to normoxic (high DO) but turbid lake edges and rivers (e.g., Chapman et al. 2002; Crispo and Chapman 2008; McNeil et al. 2016). Additionally, river sites experience fluctuating environmental conditions due to degradation of the surrounding landscape leading to excessive sediment run-off that is pulsed into the system during intense rain events. Swamps, on the other hand, tend to offer more stable environmental conditions due the buffering effects of dense aquatic vegetation.

Populations of *P. multicolor* show strong phenotypic differences in morphological, physiological, and life-history traits in response to extreme differences in DO and turbidity. For example, in response to low DO, *P. multicolor* from swamps tend to have on average 10% smaller brains and 56% larger gill surface area (Chapman and Chapman 2003; Chapman et al. 2008; Crispo and Chapman 2010; Weins et al. 2014). Swamp fish also have lower resting metabolic rates and higher metabolic scope, the latter likely afforded by larger gill surface areas and thus a higher capacity for metabolic performance (Reardon and Chapman 2010). Fish from the swamp and those reared under hypoxic conditions also have smaller broods and brood for a shorter period of time compared with populations from high DO, turbid rivers (Reardon and Chapman 2009). The pace-of-life hypothesis would therefore predict that *P. multicolor* from low DO/low turbidity swamp populations would fall closer to the slow end of the pace-of-life continuum, and river fish (or fish reared under normoxic conditions) closer to the fast end of the continuum. However, by rearing fish from both populations in a common environment free of low DO and turbidity stressors, we predicted that swamp fish would show behaviors more consistent with a relatively faster pace of life due to their adaptations for life under energetically constraining low DO conditions. From a physiological standpoint, *P. multicolor* from swamp populations show enhanced metabolic abilities when tested under high DO conditions, compared with river fish (Reardon and Chapman 2010). Thus, swamp fish should be, on average, more bold and active than high DO/high turbidity river populations when reared under common garden conditions. This might be especially true if river fish also have higher perceived predation risk in a clear environment compared with the turbid environments they typically encounter and thus may reduce activity, increase vigilance, and be shyer in clear waters than they would normally be. Precedent use of common garden experimental designs to reduce the effects of environmental stressors when testing for personality and pace of life syndromes have been suggested and applied in previous studies. For example, Urszán et al. (2015) used agile frog tadpoles *Rana dalmatina* as a model organism to provide evidence for pace of life and behavioral consistencies at different ontogenetic stages. Additionally, a common garden experimental design has been used to study the pace of life of stonechat *Saxicola torquata* populations (Wikelski et al. 2003). By raising individuals from divergent environments in a common garden experimental design, environmental stressors originally present in the home environment are released from these individuals, thus any observed differences may allow us to draw some conclusions about the adaptive nature of trait divergence.

Finally, boldness and activity have frequently been found to be repeatable (i.e., consistent individual differences in behavior; Bell et al. 2009) and also correlated within populations among a number of species (Wilson and McLaughlin 2007; Mazué et al. 2015); however, we know much less about whether there is geographic variation in personality and correlated behaviors within species (except see Bell 2004, van Dongen et al. 2010). In 1 example, Fraser et al. (2001) found a positive correlation between boldness and movement (with higher growth rate in bold individuals) in Trinidadian killifish *Rivulus hartii* from high predation areas, but a lack of correlation in populations from predator-absent sites. Therefore, we also examined whether boldness under predation risk and activity are

correlated behaviors within *P. multicolor* populations and whether these correlations were different across populations. Our experimental design therefore allowed us to test for population-level variation in behavioral traits and assess whether there is geographic variation in personality and correlated behaviors. Together, this research adds to the body of work to date on *P. multicolor* that has focused on population-level responses to individual stressors (e.g., DO or turbidity), while also examining within-population, individual variation in behavioral traits that are important in responses to rapidly changing environments (Sih et al. 2011; Tuomainen and Candolin 2011).

Materials and Methods

Collection and rearing of study organisms

Adult *P. multicolor* were collected from 2 sites within the Mpanga River basin (Uganda, Africa) that differ dramatically in levels of DO and turbidity. Bwera is a dense *Cyperus papyrus* swamp (hereafter *swamp*) characterized by low DO (monthly mean $\pm SE = 0.28$ mgL^{-1} O$_2$ ± 0.1; Crispo and Chapman 2008) and clear, but tannin-stained water, typically <5.0 NTU (mean $\pm$ SE point-in-time measurements during time of fish collection: 1.09 NTU ± 0.03). Bunoga is a river site characterized as having, on average, higher DO (monthly mean $\pm$ SE: 8.5 mgL^{-1} O$_2$ ± 0.1; McNeil et al. 2016) and higher turbidity (mean $\pm SE$ point-in-time measurements during time of fish collection: 13.25 NTU ± 0.1) compared with the swamp. Fish collected from swamp and river sites were transported from Uganda to The Ohio State University in July 2014 and held by population under ambient light: dark cycles in aquaria located within Kottman Hall greenhouse (2021 Coffey Rd., Columbus Ohio), approved under The Institutional Animal Care and Use Committee (IACUC) Protocol # 2014A00000055.

To create F1 progeny from wild-caught *P. multicolor*, 1 male and 3 female fish from the same population were placed in an isolated aquarium to allow for natural reproduction. The maternal mouth brooding behavior of *P. multicolor* allowed us to visually determine when a female started holding a brood (e.g., widely distended jaw, refusal of food, and anti-social behavior). Once a female was noted to be mouth brooding she was removed and allowed to further develop her brood in an isolated aquarium. The male was also replaced to maintain independent broods (i.e., because only one male was available per reproduction aquarium we knew the identity of each parent for each brood). This process allowed us to create eight independent F1 broods (four broods with different parents per population) of *P. multicolor* using $N = 16$ fish (4 males: 4 females per population). Offspring were housed in a common garden environment for 3–6 months under normoxic (mean DO $\pm SE$: 8.70 mgL^{-1} O$_2$ ± 0.76) and low turbidity (mean turbidity $\pm SE$: 0.49 NTU ± 0.03 SE) conditions. Juvenile fish were fed Hikari® First Bites daily *ad libitum* for 1 month, then weaned onto adult food (crushed Tetra® TetraMin Tropical Crisps) for the remainder of the study.

Individuals from single broods were housed together in a single aquarium ($N = 8$ brood aquaria) until fish reached 3 months of age, after which 5 fish per brood per population (5 fish × 4 broods × 2 populations; $N = 40$ fish total) were randomly selected to be housed individually in 19-L glass aquaria (Figure 1). Each aquarium contained a sponge filter and refuge (small plastic plant) placed at one end of the aquarium such that one-third of the aquarium contained refuge habitat and the remaining two-thirds was open water. Once placed in an experimental aquarium, fish were allowed to acclimate to the new aquarium for 2 weeks before trials began. All fish housed in experimental aquaria were held in the same controlled greenhouse unit, under equivalent ambient light conditions, and were fed the same food once a day *ad libitum*. Prior to the start of the behavioral assays, food was withheld for 24-h to standardize hunger. To reduce stress from individually housing a social cichlid species, experimental aquaria were positioned to allow for visual interactions between individuals in neighboring aquaria. To eliminate the ability of fish to observe behavioral trials of other fish, an opaque barrier was used to visually isolate the selected experimental aquarium from surrounding aquaria housed on the same shelf. This barrier was placed surrounding the aquarium before the acclimation period began and was not removed until the conclusion of the behavioral trial.

Trial procedure

Before the start of a trial, a movable transparent, acrylic barrier was placed into the aquarium, dividing it into 2 zones: an accessible area where the fish can swim freely to and from refuge ("refuge zone"; 1/3 refuge + 1/3 open space), and an inaccessible area which the fish could not enter ("food zone", 1/3 open space) (Figure 1, Appendix Figure A1). Once the clear barrier was in place, fish were given 15 min to acclimate. The trial began by adding food (0.275 g ± 0.02 SE food added per trial) to the isolated "food zone" in order to promote fish to leave refuge. Once the fish was observed to leave refuge and swim towards the partition and food, we simulated a predator attack using a model invertebrate predator [i.e., a large plastic insect (~9.5 cm long) resembling a natural predator, *Belostomatidae* spp.] to illicit a fear response and scare the fish back into refuge. To initiate the predator attack, the model predator was introduced into the "open water" zone of the aquarium where it was moved back and forth to mimic searching behavior of the predator and thus scare the fish. Once the fish retreated into refuge, the predator remained in the aquarium for an additional 5–10 s before being removed. Upon predator removal the clear barrier was also removed to allow fish access to introduced food. All trials were video-recorded with a Canon® Vixia HF R600 HD camcorder for post-trial analysis. Each trial lasted 300 s from the time the simulated predator attack ended and barrier restricting food access was removed.

Behavior assays

To quantify and compare the behavior of *P. multicolor* individuals from each population, boldness, general activity, and foraging activity were quantified following a simulated predator attack as in previous studies (Fraser and Gilliam 1987; Fraser et al. 2001; Wright et al. 2006; Harcourt et al. 2009; Toms et al. 2010). We measured boldness as the fish's latency to leave refuge following the simulated predator attack. We calculated boldness as 1 minus the proportion of time before the fish emerged from the refuge following a simulated predator attack.

The fish was considered outside of refuge when it moved 1.5 body lengths (measured in cm) away from the refuge. To measure general activity, we measured the proportion of time that an individual spent outside of refuge once it emerged from the refuge following the simulated predator attack.

Finally, we quantified foraging activity as the number of food pecks counted following the simulated predator attack and initial emergence from refuge. A single food peck was defined as a fast, forward strike at a food particle with an open mouth (masticating food which was already in the mouth was not considered a food peck).
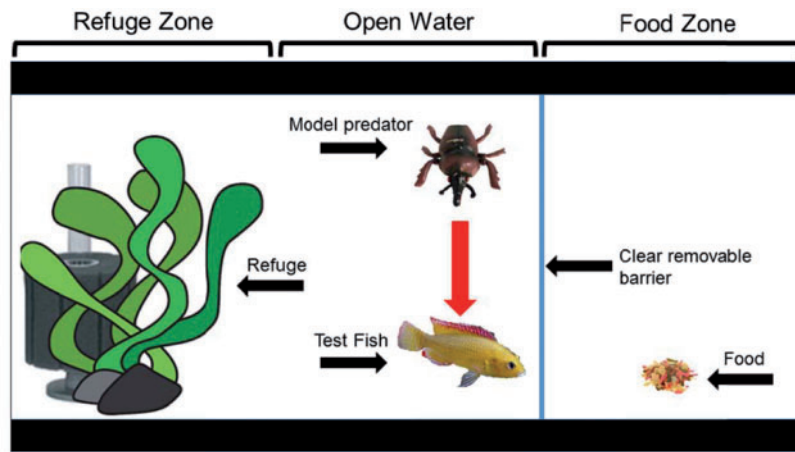
Figure 1. Visual representation of the experimental aquaria. Aquaria are set up so 1/3 of the aquarium is an area of refuge (refuge zone), 1/3 of the aquarium is open water where the model predator is introduced to scare fish back into refuge, and the remaining 1/3 of the aquarium is the area where food is introduced (food zone). The food zone is initially blocked by a transparent removable barrier, then after the predator has been removed, the barrier is removed to allow fish to have access to food.

To quantify the repeatability (i.e., consistent individual differences in behavior) of these 3 behaviors (boldness, general activity, and foraging activity), we tested each individual fish 3 times, with a minimum of 1 day between trials. At the conclusion of the final trial, we measured the mass (g) and standard length (SL; cm) of each fish so that we could account for differences in age and size between populations. We also calculated Fulton's condition factor, $K = 100 \frac{W}{L^3}$ [where W = mass (g) and L = standard length (cm); Fulton 1904; Froese 2006], to test if broods reared in the greenhouse at different times of the year displayed similar weight–length ratios.

Statistical analyses

Fish used for experimental trials varied significantly in age due to logistical constraints; on average swamp fish were younger than river fish (*swamp:* 116 days ± 3.22 SE; *river:* 161 days ± 4.28 SE; analysis of variance (ANOVA): $F_{1,38} = 69.274$, $P < 0.001$). This meant that, on average, fish from each population were also of unequal size when tested: swamp fish were significantly smaller (mean mass: 0.91 g ± 0.06 SE, mean standard length (SL): 2.95 cm ± 0.06 SE) than river fish (mean mass: 1.27 g ± 0.04 SE, mean SL: 3.38 cm ± 0.04 SE; ANOVA: mass: $F_{1,38} = 22.522$, $P < 0.001$; SL: $F_{1,38} = 34.502$, $P < 0.001$). Although the age and size of the 2 populations were different, there was no significant difference in condition: *Fulton's condition*, $K = 3.45 ± 0.07$ SE for swamp fish and for river fish $K = 3.29 ± 0.08$ SE (ANOVA: $F_{1,38} = 2.127$, $P = 0.153$). Although this demonstrates that condition factor was similar across populations, we decided to include standard length as a covariate in the following analyses to account for the size differences apparent between populations and any ontogenetic behavioral shifts (see Bell and Stamps 2004).

To test for population-level differences in behavioral responses to the simulated predator attack, individual fish behaviors (i.e., boldness, general activity, and foraging activity) were averaged over 3 trials conducted per fish. For each behavioral response, we used an analysis of covariance (ANCOVA) with log-transformed SL as a covariate to test for differences in mean behaviors between swamp and river fish. Nonsignificant interaction terms (population × log SL) were removed from the models. Because some individuals were genetically related (i.e., siblings randomly selected from each brood), we also included brood identification ($N = 4$ broods per population) as a random effect in our ANCOVA analysis. If the random effects (i.e., brood

identification) were determined to be nonsignificant, they were subsequently removed from the model as well.

To test if there were correlations between the 3 measured behaviors across populations (while accounting for differences in body size), we used separate multiple linear regressions (including log SL as an additional predictor variable) to test each pair of behaviors. To evaluate if the correlations between these behaviors differed between the swamp and river populations, we calculated Pearson correlation coefficients separately for each population and then used Fisher's z-transformation to compare the 2 correlation coefficients from each population (Zar 1999).

To test whether there were consistent individual differences in behavior, we calculated the repeatability, r, for each of the 3 behaviors via univariate ANOVA with individual as the fixed factor and behaviors as the dependent variables. We used the resulting mean square values (MS_{within} and MS_{among}) in the equation: $r = s_A^2/(s^2 + s_A^2)$, where s_A^2 is the variance among individuals, $(MS_A - MS_W)/n_0$, and s^2 is the variance within individuals, MS_W (Bell et al. 2009). It is important to include the term n_0 for the number of observations per individuals because repeatability changes systematically as the number of measurements per group increases, thus not incorporating the number of observations per individual calculates repeatability incorrectly (Lessells and Boag 1987). The standard error of repeatability was calculated using the following equation:

$$ SE\ r = \sqrt{\frac{2(1-r)^2 * (1 + (k-1)r)^2}{[k(k-1)(N-1)]}} $$

where r is repeatability, k is number of measurements per individual (or n_0), and N is the number of individuals tested (Becker 1984). Analyses were performed in SPSS (version 24) and R statistical software (using Rstudio; version 1.1.419).

Results

Behavioral differences between populations

Variances for boldness and general activity were not homogeneous between populations, with behavioral variances being larger in the river relative to the swamp population (Levene's test for homogeneity of variances: *boldness*: $F_{1,38} = 6.113$, $P = 0.018$; *activity*: $F_{1,38} = 5.451$,

$P = 0.025$), thus values for both were arcsine transformed, improving variance homogeneity for ANCOVA analysis. Foraging activity was not transformed since variances were homogeneous between the swamp and river population (Levene's test for homogeneity of variances: $F_{1,38} = 0.035$, $P = 0.852$). We included log SL as a covariate in the ANCOVA models to account for any differences in size between populations (see first paragraph of *Statistical analysis* for description of size differences). The interaction between population and log SL was not significant for boldness and foraging activity and was therefore removed from those models; however, population × SL was a significant interaction for general activity and was retained in the model (see Table 1). The random effect of brood number was not significant for boldness, general activity, or foraging activity and was therefore removed from all models (Table 1).

We found behavioral differences between the 2 populations despite being reared under common garden conditions and largely consistent with pace of life predictions based on known divergent characters. First, we found that swamp fish left refuge significantly earlier than did river fish (ANCOVA: $F_{1,37} = 12.36$, $P = 0.001$) (Table 1, Figure 2A) suggesting that on average swamp fish are more bold than river fish. Second, there was a significant difference between populations for general activity, with swamp fish being more active than river fish (ANCOVA: $F_{1,36} = 5.390$, $P = 0.026$) (Figure 2B). Finally, foraging activity did not differ among populations (ANCOVA: $F_{1,37} = 1.888$, $P = 0.178$) (Figure 2C). However, the result becomes significant (ANOVA: $F_{1,38} = 4.50$, $P = 0.04$) if we remove the nonsignificant log SL covariate ($F_{1,37} = 0.045$, $P = 0.833$), suggesting that swamp fish had the tendency to peck at food particles more often when out of refuge than did river fish (Table 1).

Correlated behaviors within and across populations

Results of the multiple linear regression indicated that there was a significant positive relationship between boldness and foraging activity across populations (Pearson's $r = 0.399$, $t_{37} = 2.172$, $P = 0.036$), but no effect between log SL and foraging activity (Pearson's $r = -0.248$, $t_{37} = -0.597$, $P = 0.554$). Within each population, we only found a positive correlation between foraging activity and boldness for river fish (Pearson's $r = 0.633$, $t_{18} = 3.467$, $P = 0.003$) (Figure 3A), but not for the swamp fish (Pearson's $r = -0.028$, $t_{18} = -0.118$, $P = 0.907$) (Figure 3A). The relationship between boldness and foraging activity was significantly different between the 2 populations ($Z = -2.26$, $P = 0.024$).

Across populations, there was a significant positive relationship between general activity and foraging activity (Pearson's $r = 0.376$, $t_{37} = 2.40$, $P = 0.021$), but no relationship between log SL and foraging activity (Pearson's $r = -0.248$, $t_{37} = -1.47$, $P = 0.151$). Within each population, there was no significant correlation between foraging activity and general activity for either population (*swamp*: Pearson's $r = 0.408$, $t_{18} = 1.894$, $P = 0.074$; *river*: Pearson's $r = 0.293$, $t_{18} = 1.301$, $P = 0.210$) (Figure 3B). The relationship between general activity and foraging activity was not significantly different between the swamp and river populations ($Z = 0.38$, $P = 0.701$)

Finally, we found no relationship between boldness and general activity across populations (Pearson's $r = 0.222$, $t_{37} = 1.28$, $P = 0.207$), but there was a significant negative relationship between log SL and general activity (Pearson's $r = -0.417$, $t_{37} = -2.73$, $P = 0.01$). Within each population there was not a significant relationship between boldness and general activity for either population (*swamp*: Pearson's $r = 0.304$, $t_{17} = 1.24$, $P = 0.232$; *river*: Pearson's $r = -0.033$, $t_{17} = -0.789$, $P = 0.44$) (Figure 3C) and the correlation coefficients did not differ ($Z = 1.01$, $P = 0.311$).

Table 1. Results of ANCOVAs testing population-level differences in boldness, general activity, and foraging activity across swamp and river populations.

Behavior	Model	df	F	P*
Boldness	Population	1, 37	12.357	**0.001**
	log SL	1, 37	0.007	0.934
	Brood number	6, 31	1.139	0.363
	Population × log SL	1, 36	2.751	0.106
General Activity	Population	1, 36	5.390	**0.026**
	log SL	1, 36	3.060	0.089
	Brood number	6, 30	1.576	0.219
	Population × log SL	1, 36	4.782	**0.035**
Foraging Activity	Population	1, 37	1.888	0.178
	log SL	1, 37	0.045	0.833
	Brood number	6, 31	1.149	0.358
	Population × log SL	1, 36	0.080	0.779

*Values in bold are significant at $\alpha = 0.05$.
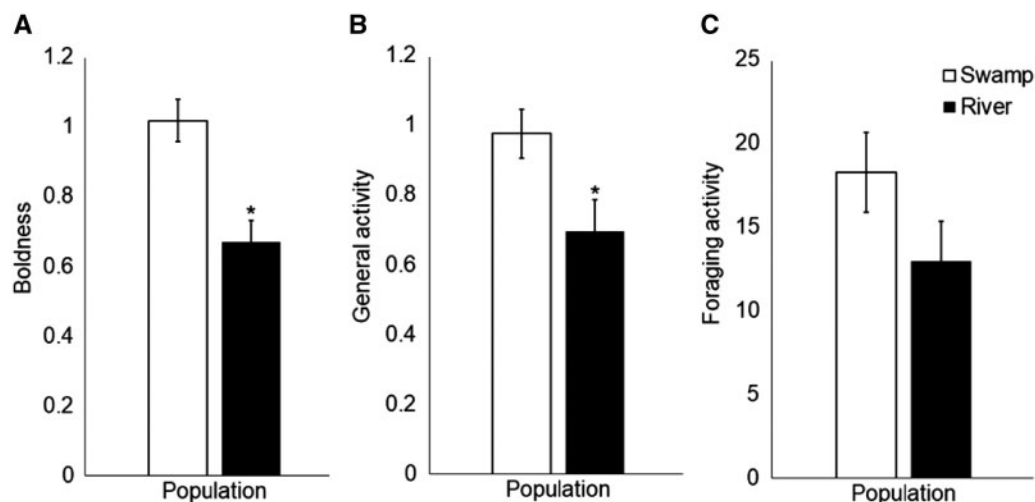
Figure 2. Behavioral responses in 2 different F1 *P. multicolor* populations, swamp (open bars) and river (solid bars). Estimated marginal means (± SE) of (**A**) boldness (arcsine transformed); (**B**) General activity (arcsine transformed); and, (**C**) foraging activity. *Values are significantly different at $\alpha = 0.05$.
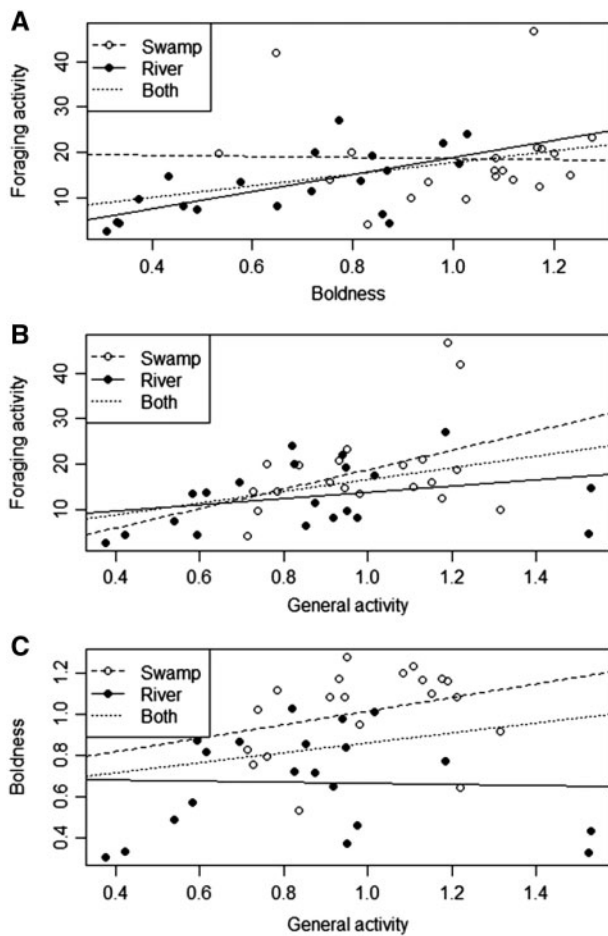
Figure 3. The relationships between (**A**) foraging activity and boldness, (**B**) foraging activity and general activity, and (**C**) boldness and general activity for the swamp (open circles, long dashed lines) and river (closed circles, solid line) populations, and with regression lines for both populations pooled (small dashed line).

Repeatability of boldness and activity

The swamp and river populations both exhibited significant repeatability in at least 1 behavior; however, there were differences between the populations in repeatability values. The swamp population showed significant repeatability in foraging behavior ($r = 0.347 \pm 0.147$ *SE*; ANOVA: $F_{19,60} = 2.596$, $P = 0.006$), but did not show significant repeatability in bold behavior ($r = 0.138 \pm 0.146$ SE; ANOVA: $F_{19,60} = 1.495$, $P = 0.140$) or general activity ($r = 0.128 \pm 0.145$ *SE*; ANOVA: $F_{19,60} = 1.479$, $P = 0.147$). The river population also showed significant repeatability in foraging behavior ($r = 0.374 \pm 0.145$ *SE*; ANOVA: $F_{19,60} = 2.794$, $P = 0.003$) as well as boldness ($r = 0.349 \pm 0.146$ *SE*; ANOVA: $F_{19,60} = 2.632$, $P = 0.005$), but did not show significantly repeatability for general activity ($r = 0.049 \pm 0.138$ *SE*; ANOVA: $F_{19,60} = 1.149$, $P = 0.345$).

Discussion

Animals are increasingly faced with human-induced stressors that vary in space and time, thus we can expect population-level divergence in behaviors that help animals to cope with environmental change that varies across the landscape. We also expect that population differences in behavior might be associated with differences in physiological traits and life-history strategies that may also diverge in response to human-induced rapid environmental change, HIREC (Sih et al. 2011). Here, we found divergent variation in behavioral traits between 2 populations that experience extremes of hypoxia and turbidity and also found evidence that some, but not all, behaviors were repeatable and correlated within populations. Under common garden conditions (i.e., released from major stressors) fish originating from the hypoxic, clear swamp habitat that have been shown to have higher metabolic scope were, on average, more bold and active than fish from the normoxic, turbid river that have lower metabolic scope. Across populations, we found positive correlations between boldness and foraging activity, as well as between foraging and general activity. Thus, individuals that were more active foragers were also bolder and generally more active. However, within populations, boldness and foraging activity were only positively correlated within the river population, but not correlated within the swamp population. Boldness and foraging activity were also repeatable behaviors within the river population, but only foraging activity was a repeatable behavior in the swamp population. Our results suggest that *P. multicolor* populations that experience different extremes of environmental stressors behave differently. We discuss these results in the context of population- and individual-level behavior trait differences in fish known to be divergent in a number of other traits associated with 2 globally important environmental stressors, hypoxia and turbidity.

The observed differences in boldness and general activity between populations that are physiologically adapted to deal with extremes in oxygen availability and water clarity is consistent with the pace-of-life concept, which predicts that individuals with a higher metabolic scope will be more bold and active than individuals with a lower metabolic scope. However, this finding requires consideration of the testing conditions in our experiment relative to the environmental conditions in the home habitat of each population. In their home environment, swamp fish face energetic challenges associated with low DO (Chapman 2015) and have adapted (through both plastic and genetic mechanisms) to these environments by having lower routine metabolic rates, larger gills, and smaller brains than river fish from high DO habitats (Reardon and Chapman 2009; Crispo and Chapman 2010). Furthermore, swamp fish have also been shown to be less active and display fewer reproductive behaviors than river fish when in their low DO home environment (e.g., Gotanda et al. 2011; McNeil et al. 2016). Therefore, it might make sense to expect swamp fish to demonstrate a slower pace of life relative to river fish, but only under home conditions. In contrast, under the novel rearing conditions (i.e., high DO/clear) of this study, we predicted that when released from the constraint of a low DO environment, swamp fish would be bolder and more active given their adaptations to an energetically challenging environment (i.e., due to adaptations to low DO that would increase performance in high DO conditions, e.g., Reardon and Chapman 2010). Urszán et al. (2015) also suggest that a fixed behavioral strategy, such as we see in the swamp population, would be less energetically expensive compared with greater intra-individual variation. Based on this reasoning, river fish adapted to a more turbid environment might therefore be relatively less active compared with swamp fish in high DO and additionally, may be less bold if clear water induces a fear response (e.g., see Lima and Dill 1990). Indeed, this is what we found: fish from the swamp left refuge sooner after a simulated predator attack, were more active throughout the trial, and displayed marginally higher foraging activity levels than fish originating from the river

(lower metabolic scope, slower life history) (Figure 2). These population differences suggest a relationship between behavior and life history, and that low DO and high turbidity are strong selective agents in this system. Evidence of other environmental extremes driving intraspecific, population-level variation in behavioral traits, and relationships between behaviors and/or physiological and life-history traits is accumulating (e.g., Bell 2004; Moran et al. 2016; Dubuc-Messier et al. 2017).

While our results suggest a relationship between behavior trait differences and population of origin, and a possible link to known divergent physiological traits, they do not allow us to differentiate between the mechanisms driving this association. For example, observed differences in behavior could be due to physiological adaptations to these stressors. In common carp *Cyprinus carpio*, for instance, fish with higher metabolic rates and lower cortisol receptor expression in the brain were found to be bolder and more aggressive, suggesting that variation in physiology is tightly linked to behavioral expression (Huntingford et al. 2010). In a direct test of the link between physiological and behavioral traits, Binder et al. (2016) found that individual bluegill sunfish *L. macrochirus* with higher aerobic capacity were bolder than individuals with lower aerobic capacity. Additionally, there is evidence that oxidative stress resulting from prolonged exposure to elevated stress hormones (i.e., glucocorticoids) is linked to behavioral coping strategies (Costantini et al. 2011). However, in each of these examples, we still do not know if physiological differences drive behavioral expression or vice versa. We know that oxygen concentration and turbidity level are likely strong selective agents driving divergence between swamp and river populations given previous work and the results presented here, and we also know that environmental conditions in the swamp tend to be relatively stable whereas they fluctuate more in rivers. In swamp populations, the relative stability of the environment in concert with consistently low DO would favor traits that help minimize energy expenditure, for example, lower metabolic rates, lower activity, and bold behavior since attaining resources needs to be efficient. However, this combination of an energetically challenging but stable environment is unlikely to favor tight linkages between traits if those linkages might constrain energetic coping mechanisms. On the other hand, in river populations, we might expect that the visual impairment resulting from high turbidity favors decreased predator vigilance or assessment of predation risk (Lima and Dill 1990), but during periods of high water clarity (as imposed in our rearing environment) this might create a higher perceived risk and thus favor shy behavior.

Alternatively, differences in both behavior and physiology could be due to plasticity. Binder et al. (2016) suggest that plasticity in aerobic metabolism could account for the positive correlation between metabolic rate and boldness. While the expression of behavior trait differences here under common garden conditions suggests some heritability, we also know that *P. multicolor* can display behavioral flexibility. For example, in a reciprocal rearing study, *P. multicolor* males displayed higher rates of aggression under turbid conditions regardless of whether they originated from swamp or river populations or if they were reared under clear or turbid conditions (Gray et al. 2012). Similarly, Reardon and Chapman (2010) demonstrate developmental plasticity in routine metabolic rate when swamp fish were reared in high or low DO. Further explorations of how multiple stressors that vary among populations might shape behavior and that directly test for associations between behavioral and physiological traits could be informative under the pace-of-life concept.

It is also possible that other ecological factors, both abiotic (e.g., temperature, flow rate) and biotic (e.g., density, competition effects, predator abundance), may contribute to behavioral trait divergence across *P. multicolor* populations. While our knowledge of the abiotic factors that vary between swamp and river habitats (such as DO, turbidity, temperature) is extensive and comes from monthly sampling over ∼15 years (L.J. Chapman, personal communication), our knowledge of the biological characteristics of each habitat is less complete. For example, while we know that fish from both types of habitat experience similar suites of predators (e.g., wading birds, snakes, and *Clarias* catfish), we do not know whether there are differences in predation pressure exerted by these predators across the 2 habitats (e.g., due to differences in density, habitat complexity, etc.). Variation in predation pressure is well known to drive population-level differences in behavior (e.g., Endler 1980) and in correlations between suites of behavior (e.g., Fraser et al. 2001). Future work distinguishing among these and other mechanisms will greatly enhance our understanding of the variables that shape divergent behavior (Bell 2007).

Our results also suggest that correlations between boldness and foraging activity is not a general pattern across *P. multicolor* populations and may be associated with the differences in the selective pressures experienced in swamp and river habitats. Unfortunately, comparing only 2 populations from different environments cannot definitively tell us what factors drive these differences. However, our results in combination with past work demonstrating other trait differences across multiple populations of *P. multicolor* (e.g., Chapman et al. 2000; Reardon and Chapman 2010; Crispo and Chapman 2010) suggest that differences in DO and water clarity might influence whether behaviors are correlated or not. More broadly, this may suggest that correlations between behaviors may depend on context. For example, a positive correlation between boldness and foraging activity might be particularly important in river environments that are much more turbid, on average, than swamps, but experience fluctuating turbidity. For instance, during periods of high turbidity it is possible that the perceived risk of predation is low and so there is an advantage for bold individuals that are more active foragers. Alternatively, periods of lower turbidity, when predation risk might be higher because fish are more visible to predators, individuals with a strong anti-predator response that spend more time hiding than feeding might be favored. It is then possible that the relative stability of the clear, low DO swamp sites do not favor such a trade-off which is why we do not see a correlation between boldness and foraging activity in that population. In a similar examination of boldness and activity coupled with aggression in threespine stickleback *Gasterosteus aculeatus*, Bell (2004) found differences in mean behaviors across very different environments (e.g., high flow and temperature variation in an unregulated stream vs. low variation in a damned and regulated stream). She also discovered that in sticklebacks from the more variable environment, behaviors were often correlated across contexts, whereas there were no significant correlations between behaviors in fish originating from the more stable population. Our results suggest that there are population-level differences in the way that behavioral traits have (co)evolved, similar to the differences found across stickleback populations by Bell (2004).

Consistent behavioral variation, or repeatability, is known to occur within and between individuals across time or ecological contexts (Dall et al., 2004; Sih et al., 2004; Sih and Bell, 2008). In our study, river-origin fish displayed repeatability across 2 contexts (boldness and foraging activity) whereas the swamp-origin fish only

displayed repeatability in foraging activity. Divergent habitats, as well as habitats with large environmental variability, may favor differences in behavioral traits and the repeatability of behavior, assuming limited plasticity. In this system, swamp sites exhibit lower variation in environmental factors (i.e., are more stable), whereas environmental factors tend to fluctuate in the river habitats (e.g., Crispo and Chapman 2010). Behaviors which show relatively low within-individual variance compared with between-individual variance are more repeatable (Bell et al. 2009). If, however, an environment has relatively low environmental variability, it is possible that this would select for a specific behavioral type that is better suited for the environment, thus reducing between-individual variance. Based on our tests for homogeneity of variance between populations, we found that in the swamp population 2 of the 3 behaviors (boldness and general activity) had lower between-individual variance compared with the river population. In the swamp population, low environmental variation in addition to low DO, may especially favor a more fixed behavioral type, given energetic constraints (Urszán et al. 2015). Alternatively, environments with relatively large environmental variation may select for a suite of different behaviors and possibly increase between-individual variance.

Intraspecific divergence in behavior across populations experiencing environmental extremes has been found in a number of taxa (e.g., Brown et al. 2005; Moran et al. 2016). As an example, van Oers et al. (2004) found that great tits *Parus major* show heritable variation in risk-taking behavior when high- and low risk-taking lines were selected in the laboratory over 2 generations. Similarly, Brown et al. (2005) found heritable variation in boldness among populations experiencing different levels of predation in a tropical poecilliid fish, *Brachyraphis episcopi*, when F1s from 2 populations were reared in the laboratory and exposed to different predation regimes. By investigating the underlying behavioral responses from an individual perspective within divergent populations we gain a mechanistic understanding of how behavioral responses, linked to physiological adaptation to environmental change, might contribute to population persistence under human-induced rapid environmental change, HIREC (Koolhaas et al. 1999; Dingemanse and Wolf 2010; Frost et al. 2013; Sih et al. 2015, Wong and Candolin 2015). Here, we have demonstrated behavioral trait divergence between populations and that trade-offs between behavioral traits vary among these populations. Remaining to be investigated is how these behavioral trait differences translate into advantages in the respective home environments of swamp and river fish.

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APPENDIX
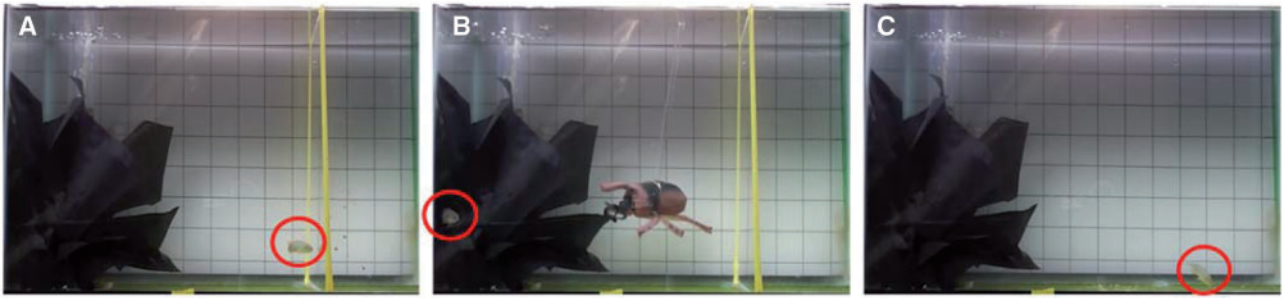
Figure A1. Experimental progression of an actual trial: (**A**) "food addition" phase, which lured the tested fish (circled in red) out of refuge in attempt to consume the introduced food on the adjacent side of the clear barrier, (**B**) "predator introduction" phase introduces the model predator which scares test fish back into refuge, (**C**) "behavioral observation" phase removes the predator and clear barrier to allows fish to have access to food.