Predicting multifarious behavioural divergence in the wild

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Many animals show complex behaviours that can have important ecological and evolutionary consequences. Environmental variation can lead to divergent selection that consistently favours particular behaviours in different environments; but how predictably multiple aspects of animal behaviour diverge in response to different environmental conditions remains unclear. We tested whether populations evolving under different levels of predation risk show predictable and repeatable population-level behavioural differences in all five primary components of animal personality: aggression, sociability, boldness, activity and exploration. We formulated and tested a priori predictions of divergence for each behaviour using the adaptive radiation of Bahamas mosquitofish, *Gambusia hubbsi* (family Poeciliidae), inhabiting vertical water-filled caves (blue holes) where they have evolved for thousands of years in either the presence or absence of predatory fish. Mosquitofish behaviours differed consistently, and largely predictably, between predation regimes: low-predation mosquitofish showed reduced sociability and greater exploration of a novel environment compared to high-predation counterparts. However, some differences were sex dependent: only females showed greater boldness and only males displayed reduced aggressiveness in low-predation populations. Activity levels did not differ between predation regimes. All populations showed a behavioural syndrome characteristic of either proactive or reactive stress-coping styles with regard to exploration. Exploration behavioural syndromes were more similar among populations that evolved in similar predation regimes, regardless of genetic relatedness. Using laboratory-born, high-predation mosquitofish, we confirmed that exploratory behaviours have a genetic basis and show significant within-individual repeatability. Our results suggest that environmental variation, such as chronic predation risk, can lead to repeatable, and often predictable, changes in multifarious animal behaviours, and that various aspects of behaviour can diversify more or less independently of one another. Considering the ecological importance of these behaviours, the ability to forecast behavioural shifts in a rapidly changing world could serve as a valuable conservation tool.

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Many animals regularly display distinct behaviours that have important fitness consequences and influence ecological patterns (Sih, Cote, Evans, Fogarty, & Pruitt, 2012; Westneat & Fox, 2010; Wolf & Weissing, 2012). Selection from environmental factors, especially stressors such as predation risk, could consistently and simultaneously act on multiple behaviours, favouring different suites of behavioural types under different conditions (Bell, 2005; Dingemanse et al., 2007). Predation risk is known to affect complex social behaviours in diverse prey taxa (Langerhans, 2006; Lima & Dill, 1990; Réale & Festa-Bianchet, 2003) and may concurrently

drive repeatable and predictable evolution in multifarious animal behaviours. However, we currently have a poor understanding of how predictably multiple aspects of animal behaviour evolve in the wild.

Réale, Reader, Sol, McDougall, and Dingemanse (2007) described five broad behavioural categories of animal 'temperament' or 'personality': (1) aggression, (2) sociability, (3) boldness, (4) activity and (5) exploration (for definitions, see Table 1). Although these behaviours are frequently investigated within the context of animal personality per se (i.e. within-individual repeatability), they appear to capture the major axes of variation in complex animal behaviour more broadly. Recent years have witnessed growing interest in understanding why individual animals, populations or species may differ in these behavioural categories. Yet to our knowledge, no prior study has investigated

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Table 1
Description of the five categories of animal behaviour examined in this study (from Réale et al., 2007), and our a priori predictions of divergence (see text for details and references)

Behavioural category	Definition and prediction rationale
Aggression	An individual's agonistic reaction toward conspecifics
Prediction: lower in LP	Reduced selection for grouping behaviours that can lead to aggression in LP Greater survivorship of aggressive individuals in HP
Sociability	An individual's tendency to avoid or associate with conspecifics
Prediction: lower in LP	Increased intraspecific competition in LP Survival advantages of social grouping in HP
Boldness	An individual's reaction to a risky, but not novel, situation
Prediction: greater in LP	Absence of lethal agents in LP Selection for wariness in HP, especially with abundant resources
Activity	An individual's general level of activity in a non-novel situation
Prediction: no difference	Similar overall needs for foraging, mating, social interactions
Exploration	An individual's reaction to a novel situation
Prediction: greater in LP	Foraging advantages of exploration in LP Mortality risks of exploratory behaviours in HP

LP: low predation; HP: high predation.

population-level responses of all five behavioural categories to environmental variation within a single study system. Such a study could provide critical insights into the sources of among-population variation in diverse aspects of animal behaviour, the extent to which different behaviours show adaptive responses versus physiological or genetic constraints and our ability to predict complex behavioural changes a priori.

As a first step in the evaluation of the predictability of multifarious behavioural changes in response to ecological variation between populations, we should (1) generate clear, a priori predictions of changes in all five behavioural categories based on theory, prior empirical study and natural history of the relevant system, (2) investigate intraspecific divergence to avoid confounding variables inherent in among-species comparisons, (3) examine multiple, independent populations to avoid confounding effects of shared ancestry and gene flow and (4) use a relatively simple natural system where populations vary in a small number of readily identifiable ecological factors to avoid confounding effects of other environmental variables (e.g. see Langerhans, 2010; Martin, McGee, & Langerhans, 2015). We accomplished this by developing and testing predictions of divergence in all five behavioural aspects of personality within a model system for adaptive diversification: Bahamas mosquitofish, *Gambusia hubbsi*, inhabiting vertical water-filled caves (blue holes) across Andros Island. *Gambusia* fishes are well studied in personality research, showing many individually consistent complex behaviours readily amenable to field and laboratory study (e.g. Biro & Adriaenssens, 2013; Blake & Gabor, 2014; Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010, 2011; Ward, 2012; Wilson, Godin, & Ward, 2010). However, we know little about how populations might jointly diverge in mean values of multiple behavioural components of personality when evolving in environments with different selective pressures.

Bahamas mosquitofish are small, livebearing fish that have repeatedly evolved different adaptive traits in either the presence (high predation) or absence (low predation) of predatory fish

during the past ~15 000 years (e.g. Heinen-Kay & Langerhans, 2013; Langerhans, 2010; Langerhans, Gifford, & Joseph, 2007; Martin, Riesch, Heinen-Kay, & Langerhans, 2014; Riesch, Martin, & Langerhans, 2013). Variation in the presence of piscivorous fish represents the primary source of environmental variation in these populations, resulting in two major categories of populations of Bahamas mosquitofish: (1) low-predation populations experience low mortality rates, and consequently face high levels of resource competition due to elevated conspecific densities, and (2) high-predation populations experience high levels of mortality from predatory fish (primarily bigmouth sleeper, *Gobiomorus dormitor*) and have relatively low conspecific densities (Heinen et al., 2013). No other environmental factor measured to date systematically covaries with the presence of predatory fish (e.g. productivity, salinity, turbidity, water transparency, depth, dissolved oxygen, temperature, pH; Heinen et al., 2013; Langerhans & Gifford, 2009; Langerhans et al., 2007). This allows us to focus more or less exclusively on the effects of predation regime in driving phenotypic divergence in this system.

For all five focal behaviours, we explicitly sought a simple, directional a priori prediction of population-level differences between predation regimes based on hypotheses of natural selection, assuming that each behavioural category could independently respond to selection (Table 1). For low-predation populations, we predicted lower aggressiveness to contend with living in high densities (monopolization or defence of resources are not particularly relevant in this system), combined with greater survivorship often experienced by aggressive individuals under threat of predation (Bell, 2005; Bell & Sih, 2007; Lung & Childress, 2007; Quinn & Kokorev, 2002). We predicted lower sociability in low-predation populations owing to increased intraspecific competition for resources in high-density conditions (Heinen et al., 2013), as well as the survival advantages of social grouping (e.g. schooling or shoaling) in the presence of predators (Krause & Ruxton, 2002; Pitcher & Parrish, 1993). We predicted that low-predation populations would show greater boldness in the presence of a potentially threatening stimulus due to the absence of selection from lethal agents, along with selection favouring wariness under chronic predation risk, particularly when resources are abundant (Bell, 2005; Herczeg, Gonda, & Merila, 2009; Riechert & Hall, 2000). For activity, we predicted no change across predation regimes, as selection should favour similar levels of overall activity for foraging, mating and other social interactions since Bahamas mosquitofish are highly social fish and activity is not known to affect predation risk from bigmouth sleepers. We predicted increased exploratory behaviours in low-predation populations due to foraging advantages of exploration under high resource competition, as well as selection against exploratory behaviours in environments with abundant ambush predators (Bell, 2005; Riechert & Hall, 2000). Alternative predictions could be made for some of the traits using different assumptions or different hypotheses of natural selection. For instance, aggression could be higher in low-predation populations if correlation with boldness or sociability prevents an independent response (Archard & Braithwaite, 2011a; Herczeg et al., 2009; Magurran & Seghers, 1991), and selection could favour increased boldness in high-predation populations as has been previously observed in some systems (Brown, Jones, & Braithwaite, 2005; Godin & Davis, 1995; Harris, Ramnarine, Smith, & Pettersson, 2010; Smith & Blumstein, 2010). However, we wished to assess our ability to predict changes in behaviour using simple predictions (i.e. assuming trait independence, ample genetic variance, closed system) most suitable for this particular study system. In this way, we can begin to gauge how accurately researchers can predict how multiple behaviours change in response to ecological variation.

METHODS

We investigated each type of behaviour in six focal blue holes: three high-predation blue holes (Cousteau's, Stalactite and West Twin) and three low-predation blue holes (East Twin, Hubcap and Rainbow). We selected these blue holes as representative of the larger set of blue holes on Andros Island, characterized by independent colonization events, low gene flow with outside populations and similarity in most environmental variables other than the presence of the predatory bigmouth sleeper. For one aspect of behaviour, exploration, we further examined laboratory-born progeny of mosquitofish captured from Cousteau's blue hole, a high-predation population. All animals were treated ethically in accordance with Institutional Animal Care and Use Committee protocol number 13-101-O at North Carolina State University. The Bahamas government granted permission to conduct the work.

Aggression

To assess mosquitofish aggression, we analysed data collected during a previous study. In brief, using underwater visual observations within each of the six focal blue holes, we recorded the frequency that six different behaviours (see Activity below) were displayed during ~90 s observation periods (240 total fish; see Heinen et al., 2013, for details). Behavioural observations were conducted between 1035 and 1540 hours in June 2011. For the current study, we estimated aggression as the frequency of agonistic behaviours shown by Bahamas mosquitofish (20 males, 20 females) towards conspecifics of the same sex. These data were never previously directly analysed on their own, as the prior study only included these data within multivariate analyses including all six behaviours measured. By focusing on intrasexual aggression, we sought to measure agonistic social behaviours exclusive of sexual and interspecific interactions. We employed a linear mixed model (LMM) using restricted maximum likelihood (REML) to test for differences in aggression between predation regimes, sex, and their interaction; population served as a random effect, representing the unit of replication for the predation regime test. All statistical tests were conducted in JMP (SAS Institute, Cary, NC, U.S.A.), and data met assumptions of linear models.

Sociability

We assessed shoaling intensity as a proxy for sociability by conducting underwater visual observations within 20 quadrats (each measuring 1 m³) equally spaced around the perimeter of six blue holes (120 total quadrats). To measure shoaling intensity, we used a 0—3 scale based on the following criteria where at least four fish were present within the quadrat: 0 = no shoaling, with no evidence of any social grouping behaviours; 1 = slight shoaling, with some noticeable degree of group swimming for two or more fish; 2 = moderate shoaling, with several fish to a small group showing obvious coordinated group swimming; 3 = strong shoaling, with several fish to a large group showing tight, cohesive and coordinated group swimming. Because conspecific density could affect shoaling behaviour (Hensor, Cousin, James, & Krause, 2005), we also measured mosquitofish density within each quadrat. To confirm interobserver reliability of shoaling intensity estimates, two snorkellers scored the same 15 quadrats in a pilot test; all shoaling scores were identical, indicating high reliability. A single observer (M.T.C.) then conducted shoaling assays in all six focal blue holes. Both sociability and boldness assays (see below) were conducted on a single day in each blue hole between 1230 and 1300 hours in June 2014, taking care to measure different fish for the two behaviours. We tested for differences in shoaling between predation regimes using a mixed-model ordinal logistic regression. Fixed factors included predation regime, log-transformed mosquitofish density, and their interaction; population served as a random effect.

Boldness

Within each of the six blue holes, we assessed boldness by measuring how close an individual mosquitofish would allow a human to approach prior to fleeing, termed 'flight initiation distance' ($N = 148$). While snorkelling in each blue hole, one person slowly approached a single fish with an outstretched arm and pointed their index finger until the fish rapidly swam away. A second snorkeller observed this interaction from the side and nearby (<2 m) and measured flight initiation distance as the distance between the tip of the index finger and the fish at the time of retreat (immediately swam to the location and used a centimetre-marked string for measurement). Two observers (D.A.S. and A.T.S.) measured flight initiation distance of 20—34 mosquitofish within each of the six focal blue holes (10—17 per sex per site; 148 total trials). We used an LMM with REML to test for differences between predation regimes, sexes, and their interaction; population served as a random effect.

Activity

We calculated mosquitofish activity levels within each of the six blue holes as the sum of six behaviours recorded in a previous study (see Aggression above; Heinen et al., 2013). These activity-level data have never before been analysed. While snorkelling, we recorded the frequency of six major behaviours displayed by 20 male and 20 female Bahamas mosquitofish within each blue hole: (1) feeding, (2) prey inspection, (3) copulation attempts, (4) male—female chase, and (5) intra- and (6) intersexual aggressive behaviours ($N = 240$ fish; see Heinen et al., 2013, for details). These six behaviours capture most unambiguously observable behaviours in situ for these fish, with most individuals showing 2—10 behaviours per minute and no fish showing zero behaviours (mean = 5.77). We again employed an LMM with REML to examine variation in activity levels between predation regimes, sexes, and their interaction, while population provided a random effect.

Exploration

Field trials

We conducted novel tank diving tests with ~20 male and ~20 female Bahamas mosquitofish on the shore of each of the six focal blue holes (249 total mosquitofish) following standard methods for small fish such as poeciliids and zebrafish (Cachat et al., 2010; Wong et al., 2012). Briefly, we captured individual fish from each blue hole using hand-held dip-nets, and immediately transferred them to the novel tank (22 × 9.5 × 12 cm, L × W × H) to minimize handling time (<30 s). For each trial, we introduced a single fish into the novel tank filled with 2.5 litres of water from the blue hole (rinsed and replaced between trials; otherwise empty) and video-recorded the tank for 5 min. A white drop cloth over the experimental arena prevented the fish from witnessing any potentially distracting stimulus during a trial. From the videos, we recorded seven behaviours previously identified as associated with stress-coping style and anxiety in fish (Cachat et al., 2010; Egan et al., 2009): (1) latency to upper half of the tank, (2) duration of time spent in upper half, (3) number of transitions to upper half, (4) number of quadrat transitions (i.e. movements between nine quadrats formed by a 3×3 grid on the side of the tank), (5) number of freezing bouts (lack of movement, except pectoral fins, gills, or

eyes, for longer than 1 s), (6) duration of time spent frozen and (7) number of erratic behaviours (rapid, darting bursts).

We used principal components analysis (PCA) to reduce dimensionality and discover major axes of behavioural variation. We retained the first four PCs, explaining ~88% of the behavioural variance (see Results, Table 3). We first inspected the PCs for evidence of behavioural syndromes characterizing proactive (many quadrat transitions, many transitions to the upper half, low frozen duration, few freezing bouts and short latency to the upper half) versus reactive (few quadrat transitions, few transitions to the upper half, high frozen duration, many freezing bouts and high latency to the upper half) stress-coping styles, as commonly observed in other taxa (Koolhaas, de Boer, Coppens, & Buwalda, 2010; Sih, Bell, & Johnson, 2004). Second, we conducted separate LMMs using REML for each PC to test for differences in exploration behaviours between predation regimes, sexes, and their interaction; population served as a random effect (P values adjusted to control for a false discovery rate of 5%, following Benjamini & Hochberg, 1995). Third, we examined overall similarity in exploration behavioural syndromes (i.e. behavioural covariance matrices) among populations using random skewers analysis. This procedure compares each population's predicted evolutionary response to random vectors of selection (Cheverud, 1996; Cheverud & Marroig, 2007). For each population pair, we generated 10 000 random selection vectors ('skewers'), applied them to both matrices and calculated the average vector correlation between their responses to selection using PhyTools in R version 3.1.2 (R Development Core Team, 2014). If fish in different predation regimes have experienced different patterns of correlational selection on exploration behaviours, then populations within similar predation regimes should show more similar behavioural syndromes (higher vector correlations) than populations inhabiting different predation regimes, irrespective of genetic relatedness. To test this hypothesis, we conducted a partial Mantel test using 9999 randomizations that examined the association between the matrix of pairwise vector correlations and a habitat matrix (0 = same predation regime, 1 = different predation regime), while holding a matrix of genetic relatedness constant (genetic distance data from Heinen-Kay & Langerhans, 2013).

Genetic basis

To test for genetic variation (i.e. heritability) in exploration behaviours, we executed the same novel tank diving test procedure described above with adult laboratory-raised mosquitofish (N = 70) representing the offspring of eight different females captured as adults from a single high-predation blue hole (Cousteau's). Because we used a relatively small number of families, this represents a conservative first test for heritability of exploration behaviours in Bahamas mosquitofish. Wild-caught pregnant females were housed in the laboratory for at least 2 months prior to acquiring offspring for laboratory rearing to minimize maternal effects. Laboratory-born fish were raised in 10-litre aquaria within a recirculating system at approximately 25 °C in a temperature-controlled room and fed a varied diet of live brine shrimp, freeze-dried daphnia and bloodworms, and TetraMin Pro flakes. To avoid confounding any tank effects with family effects, we split all families among multiple tanks (two to six tanks, mean = 4, SD = 1.5). To test for a genetic basis of behaviours observed in the field, we projected laboratory-raised individuals onto the PC axes constructed for wild-caught fish. This allowed us to directly test for genetic variation in the multivariate behavioural axes of interest, and to directly compare behavioural scores between laboratory-raised and wild-caught fish on the same multivariate axes. We tested for differences between full-sib families by conducting separate analysis of variance models (ANOVAs) for each PC with

full-sib family and sex as factors (P values adjusted to control for a false discovery rate of 5%, following Benjamini & Hochberg, 1995). We used Model II ANOVA with restricted maximum likelihood to calculate an upper-bound estimate of narrow-sense heritability ($h^2 = V_A/V_P$) based on full-sib families and assuming no dominance or shared environmental effects on phenotypic variance (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Owing to our sample size, we only provide this as a rough upper-bound estimate of heritability, with greater emphasis on the significance test. Following experimental trials, all fish were returned to their home tanks.

Within-individual repeatability

While previous work has shown within-individual repeatability for several behaviours in *Gambusia* fishes (e.g. Biro & Adriaenssens, 2013; Blake & Gabor, 2014; Cote et al., 2010, 2011), we tested for behavioural consistency in exploration behaviour in Bahamas mosquitofish. To do so, we subjected 26 adult, second-generation, laboratory-born, high-predation Bahamas mosquitofish (15 female, 11 male) to two novel tank diving test trials, ~5 weeks apart (mean ± SD = 37.9 ± 3.42 days). Rearing conditions, novel tank diving test methods and final disposition of mosquitofish were the same as above, but different individuals were used. We again projected laboratory-raised fish onto the PC axes derived from wild-caught fish and tested for within-individual repeatability of exploration behaviours by conducting an ANOVA for each PC (P values adjusted to control for a false discovery rate of 5%, following Benjamini & Hochberg, 1995) and by calculating the intraclass correlation coefficient (Lessells & Boag, 1987). This procedure estimates the proportion of behavioural variation explained by differences among individuals in their mean behaviours (i.e. individual consistency).

RESULTS

Aggression

Low-predation males, but not females, showed much lower frequencies of intrasexual aggression than high-predation counterparts (Fig. 1, Table 2). The sexes did not differ in their frequency of intrasexual aggression in low-predation blue holes, but males had much higher aggression than females in high-predation blue holes.

Sociability

Low-predation mosquitofish displayed much lower shoaling intensity (i.e. reduced sociability) than high-predation counterparts, and only high-predation fish showed density-dependent shoaling behaviours (Fig. 1, Table 2).

Boldness

Male mosquitofish showed greater boldness (shorter flight initiation distance) than females, but only females showed increased boldness in low-predation blue holes (Fig. 1, Table 2).

Activity

Mosquitofish showed no differences in activity levels between predation regimes or sexes (Table 2).

Exploration

PC1 from the novel tank diving test trials (43% of behavioural variance) captured the major features of an exploration behavioural
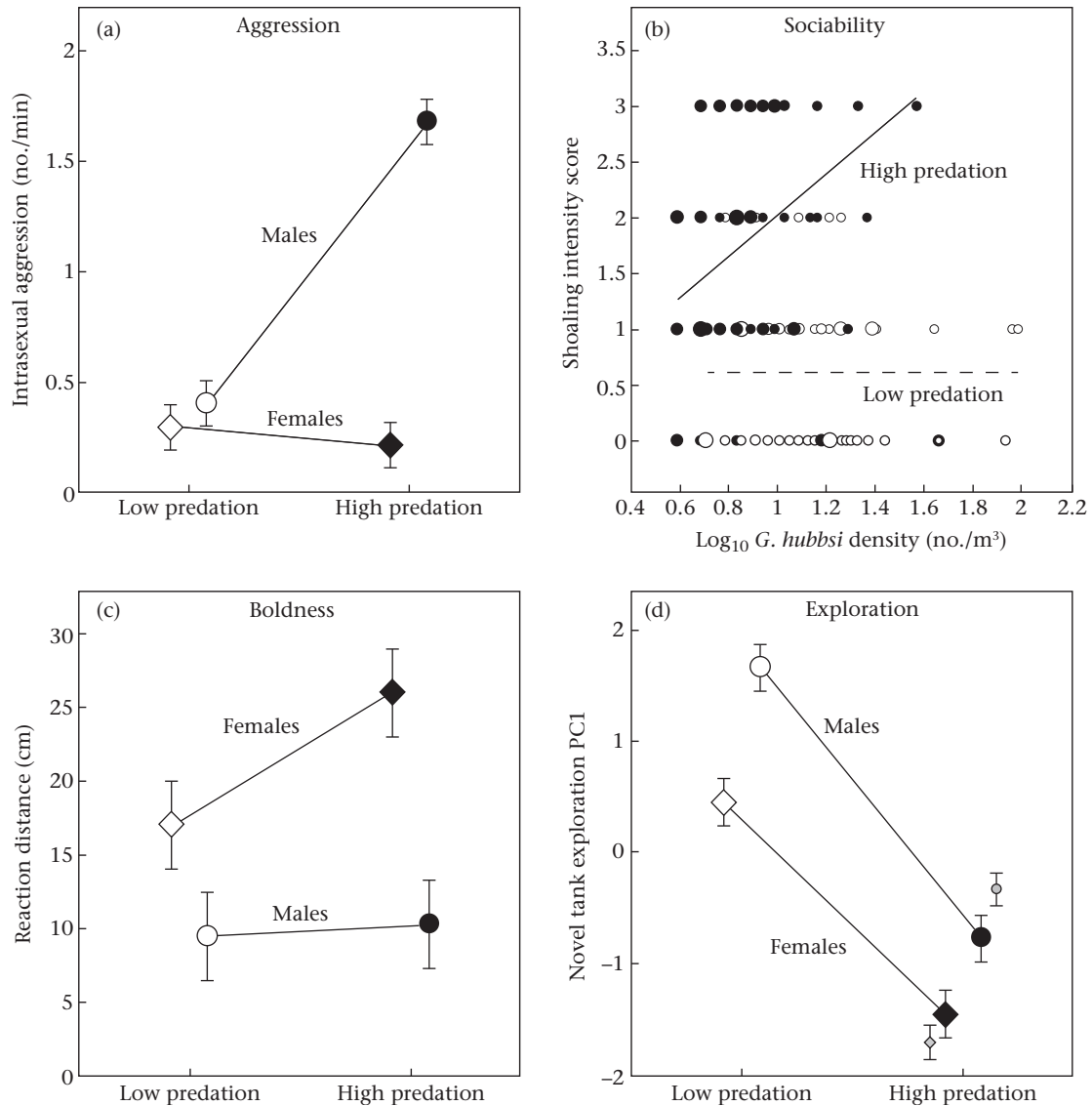
Figure 1. Variation in four aspects of animal behaviour in Bahamas mosquitofish: (a) aggression; (b) sociability; (c) boldness; (d) exploration. Means ± SE are depicted. In (b), the size of the symbols reflects sample size. Grey symbols in (d) depict laboratory-raised individuals.

Table 2
Results of separate mixed models examining variation in intrasexual aggression, sociability (shoaling intensity), boldness (flight initiation distance), activity and exploration (PC1 scores of seven behaviours measured in the novel tank diving tests) in wild Bahamas mosquitofish across six blue holes

Behavioural category	Source	F	df	P
Aggression	Predation regime (PR)	41.11	1, 4	0.0030
	Sex	50.61	1, 232	<0.0001
	Sex × PR	37.61	1, 232	<0.0001
Sociability	PR	53.85*	1	<0.0001
	Log density	7.43*	1	0.0064
	Log density × PR	6.54	1	0.0106
Boldness	PR	1.49	1, 4.064	0.2885
	Sex	80.19	1, 140.1	<0.0001
	Sex × PR	9.80	1, 140.1	0.0021
Activity	PR	0.78	1, 4	0.4277
	Sex	0.09	1, 232	0.7592
	Sex × PR	0.01	1, 232	0.9155
Exploration	PR	75.51	1, 4.03	0.0014
	Sex	35.26	1, 241.2	<0.0001
	Sex × PR	2.82	1, 241.2	0.0946

* χ^2 values from ordinal logistic regression.

syndrome in Bahamas mosquitofish, describing stress-coping styles that range from reactive to proactive (Wong et al., 2012) (Table 3). Low-predation fish showed a proactive stress-coping style, with high levels of exploration (many quadrat transitions, transitions to the upper half, low frozen duration, few freezing bouts and short latency to the upper half), while high-predation fish showed a more reactive stress-coping style, with low levels of exploration in the novel environment (Fig. 1, Table 2). Compared to females, males displayed a more proactive stress-coping style (Fig. 1). Low-predation females showed more freezing bouts (i.e. stopping–-starting behaviours) than high-predation counterparts (PC4; Supplementary Table S1). Summary statistics for all behaviours are provided in Supplementary Table S2.

All high-predation populations showed generally similar covariance structure, while low-predation populations were slightly less similar to one another, and populations having different predatory environments had even less similar covariance matrices (Table 4). Partial Mantel test confirmed that populations in similar predatory environments showed more similar behavioural syndromes than populations living in dissimilar predatory environments, controlling for genetic relatedness ($P = 0.0280$).

Table 3
Principal component loadings summarizing variation in behaviours during novel tank diving test trials in wild-caught Bahamas mosquitofish

Behavioural variable	PC1	PC2	PC3	PC4
Latency to upper half	**−0.51**	**−0.64**	0.18	0.07
Upper half duration	0.24	**0.73**	−0.41	0.27
Transitions to upper half	**0.93**	−0.10	0.13	−0.04
Quadrat transitions	**0.96**	−0.13	0.12	−0.05
Freezing bouts	**−0.55**	0.37	0.01	**−0.71**
Frozen duration	**−0.78**	0.17	0.17	0.43
Erratic behaviours	0.11	0.49	**0.85**	0.05
% Variance explained	43.10	19.79	14.06	10.92

Loadings greater than |0.5| are shown in bold. PC1 explained more than twice as much variance as subsequent PCs, capturing the prominent features of a behavioural syndrome ranging from a reactive stress-coping style (negative scores: few quadrat transitions, few transitions to the upper half, high frozen duration, many freezing bouts and high latency to the upper half) to a proactive stress-coping style (positive scores: many quadrat transitions, many transitions to the upper half, low frozen duration, few freezing bouts and short latency to the upper half).

Table 4
Similarity in exploration behavioural syndromes among populations

	East Twin	Hubcap	Rainbow	Cousteau	Stalactite	West Twin
East Twin		**0.0125***	**0.0036***	0.0249*	0.0187*	0.0249*
Hubcap	**0.78***		**0.0098***	0.1599	0.1381	0.1546
Rainbow	**0.87***	**0.81***		0.0451*	0.0401*	0.0519
Cousteau	0.73*	0.40	0.64*		*0.0005**	*0.0004**
Stalactite	0.75*	0.43	0.66*	*0.93**		*0.0004**
West Twin	0.71*	0.41	0.62	*0.96**	*0.97**	

Average vector correlations from random skewers are shown below the diagonal; *P* values are shown above diagonal. Population pairs from similar predation regimes are denoted by bold (low-predation regimes) and italics (high-predation regime). *$P < 0.05$.

We found a significant genetic basis for variation in stress-coping style among laboratory-raised mosquitofish (Supplementary Table S3). Specifically, we revealed significant heritability of PC1 scores ($F_{7,61} = 2.46$, $P = 0.0272$). Upper-bound narrow-sense heritability was estimated as 0.30 ± 0.24. Furthermore, PC1 scores of laboratory-raised fish were very similar to those observed in the wild for high-predation mosquitofish (Fig. 1). We also found significant within-individual repeatability of stress-coping style (PC1: $F_{25,26} = 3.82$, $P = 0.0023$, r (intraclass correlation coefficient) $= 0.58$), and expression of erratic behaviours (PC3: $F_{25,26} = 2.37$, $P = 0.0328$, $r = 0.41$). Results for repeatability of behaviours associated with use of the upper half of the tank (PC2) and the number of freezing bouts (PC4) were nonsignificant (PC2: $F_{25,26} = 1.74$, $P = 0.1124$, $r = 0.27$; PC4: $F_{25,26} = 1.41$, $P = 0.1947$, $r = 0.17$).

DISCUSSION

Populations of Bahamas mosquitofish that evolved under different levels of predation risk showed consistent and largely predictable, although sometimes sex-dependent, differences in aggression, sociability, boldness and exploration. Only one behavioural category, activity, did not differ between predation regimes, and this lack of differentiation matched our prediction. Moreover, we demonstrated that exploration behaviours are repeatable within an individual (i.e. reflects personality) and show significant genetic variation within a population (i.e. heritability). In light of prior work demonstrating personality in mosquitofish (e.g. Biro & Adriaenssens, 2013; Blake & Gabor, 2014; Cote et al., 2010, 2011; Ward, 2012; Wilson et al., 2010) and a genetic basis to divergence in many traits within this study system (e.g. Anderson & Langerhans, 2015; Heinen-Kay & Langerhans, 2013; Langerhans &

Makowicz, 2013; Martin et al., 2014; Riesch et al., 2013), it seems likely that observed behavioural differences between predation regimes at least partially reflect divergent evolution. Regardless of the extent to which our results reflect genetic divergence or phenotypic plasticity, our findings suggest that complex behaviours are subject to natural selection and can differ predictably in response to an ecological stressor.

As predicted, high-predation fish showed greater sociability, as measured by shoaling intensity, suggesting that this confers antipredator advantages in this system. This is consistent with previous work demonstrating that fish shoals are more cohesive in riskier environments (Kelley & Brown, 2011; Magurran, 2005). Social grouping can provide survival benefits in the face of predation threat, but it comes with various costs (Krause & Ruxton, 2002; Pitcher & Parrish, 1993). These costs, such as enhanced resource competition, may help explain reduced sociability in low-predation blue holes, but prior work also suggests that costs can be lessened in populations adapting to high risk of predation (Herczeg et al., 2009). The strength of conspecific attraction increased with conspecific density in the presence of predators in the present study, suggesting that net advantages of social grouping in high-predation environments might increase with group size (Pitcher & Parrish, 1993). Some animals can improve foraging success or lessen energy expenditure of movement through social grouping (Herskin & Steffensen, 1998; Pitcher, Magurran, & Winfield, 1982), but these potential gains appear relatively unimportant in this system, as shoaling intensity was much lower in the high resource competition environment in low-predation blue holes.

We predicted that Bahamas mosquitofish would diverge in aggression and boldness between predation regimes, but only low-predation males reduced aggression and only low-predation females increased boldness (females displayed low levels of aggression and males showed high levels of boldness across both predation regimes). Perhaps selection in high-predation environments only favours aggression when individuals are also bold. For instance, aggression may confer no advantage to females in high-predation blue holes, where aggressive behaviours might draw unwanted attention from predators (and pregnant females likely have reduced fast-start swimming abilities, which are used to evade predatory attacks; Ghalambor, Reznick, & Walker, 2004), and retreating from a distant threat likely enhances survivorship. Meanwhile, displaying aggressive behaviours may enhance survivorship or mating success for males, as males probably need to maintain high boldness across all sites to maximize mating opportunities regardless of nearby threats (Smith & Blumstein, 2008), especially given strong competition for females in this polygamous mating system.

Alternatively, genetic correlations between aggression and boldness, as suggested by work in other taxa (Réale et al., 2007; Sih & Bell, 2008; Sih et al., 2004), could prevent the independent evolution or expression of these two categories of behaviour. If true, this could partially explain our findings, where neither sex can show opposite directions of change in aggression and boldness due to genetic or physiological associations between the behaviours. Nevertheless, significant shifts in one of these behaviours occurred within each sex, implying at least some level of independence among the traits. Our finding of a less-than-perfect match to a priori predictions generally fits with previous findings on the effects of predation on aggression and boldness, where researchers have observed mixed results regarding the existence and directionality of changes (Archard & Braithwaite, 2011a; Harris et al., 2010; Herczeg et al., 2009).

Low-predation mosquitofish showed a proactive stress-coping style, more readily exploring the novel tank than high-predation counterparts. Our results indicate that these patterns did not

result from higher overall activity levels within low-predation blue holes. Increased exploration in low-predation fish matched predictions based on selection favouring exploration due to foraging advantages, as well as relaxed selection from potential risks, such as predators. Although our results did match our predictions, prior work in other livebearing fishes has often found that high-predation fish show more exploratory (proactive) behaviours and higher activity levels (e.g. Archard & Braithwaite, 2011b; Archard, Earley, Hanninen, & Braithwaite, 2012; Ingley, Rehm, & Johnson, 2014). In these other systems, however, predator presence/density often correlates positively with habitat size and complexity, productivity and biodiversity, all of which can affect an individual's activity level and propensity to explore a novel environment. In the present system of inland blue holes on Andros Island, these factors do not covary with predation regime (e.g. Heinen et al., 2013), suggesting our findings may more accurately reflect the direct effects of predation risk and population density. A corollary of our results that deserves future attention is that high-predation fish might experience higher anxiety in unfamiliar or stressful situations, as well as increased susceptibility to disease owing to their relatively reactive stress-coping style (including laboratory-raised fish) (Cachat et al., 2010; Zozulya, Gabaeva, Sokolov, Surkina, & Kost, 2008). Whether we can predict such important fitness consequences of behavioural shifts remains to be seen.

Exploration behavioural syndromes of Bahamas mosquitofish in a novel environment were more similar among populations of the same predation regime. This suggests that selection may favour different coordinated responses to unfamiliar or stressful situations in different predation regimes. Furthermore, the high consistency of behavioural syndromes in high-predation sites may reflect high consistency of selection regime, whereas varied strategies may prove similarly successful in more benign environments. For instance, previous work has found that particular combinations of behavioural traits enhance survival in the face of predatory fish (Smith & Blumstein, 2010). Moreover, populations living under chronic threat of predation can show much stronger behavioural syndromes than populations in the absence of serious predation threat (Dingemanse et al., 2007).

Although we focused on adaptive explanations for observed behavioural divergence because differences between multiple, independent mosquitofish populations largely matched our a priori predictions, multitrait divergence can also reflect genetic associations among traits rather than purely adaptive change (e.g. Losos, 2011; Riesch et al., 2013). Indeed, prior work has suggested that all five categories of animal personality can show positive correlations with one another, partially owing to common genetic and physiological sources (Réale et al., 2007; Sih & Bell, 2008; Sih et al., 2004; Smith & Blumstein, 2010). However, Bahamas mosquitofish did not simply show greater values of all behaviours in one predation regime, but rather showed a complex set of changes: low-predation mosquitofish showed reduced sociability, greater exploration, similar activity, sex-dependent reduction in aggression and sex-dependent increase in boldness compared to high-predation counterparts. Furthermore, the covariance among exploratory behaviours did not reflect intrinsic constraints common to all populations, but rather populations in similar predatory environments showed more similar covariance structure. Altogether, our results add to the growing evidence that the diversity of multifarious animal behaviours may often reflect adaptive differentiation and not inherent constraints (e.g. Dingemanse et al., 2007; Herczeg et al., 2009).

Such consistent and predictable differences in multiple components of behaviour between ecologically distinct populations poses important ecological and evolutionary consequences (Ingley & Johnson, 2014; Smith & Blumstein, 2008; Wolf & Weissing, 2012).

In light of pervasive anthropogenic environmental impacts occurring today, we must better understand how complex behaviours might respond to new conditions to help improve conservation and management efforts (McDougall, Réale, Sol, & Reader, 2006; Sih, 2013). For instance, human-induced environmental change can lead to changes in animal personalities (e.g. Ciuti et al., 2012; Madden & Whiteside, 2014), and our ability to predict such changes can prove critical for a range of conservation applications (Carroll et al., 2014; Smith, Kinnison, Strauss, Fuller, & Carroll, 2014). We suggest that the system studied here could serve as a model for understanding the evolution of personality traits in nature, their ecological consequences and the genetic and neurophysiological mechanisms by which they evolve.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav. 2016.08.016.

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Table S1

Results of separate linear mixed models examining variation in exploratory behaviours of Bahamas mosquitofish across six blue holes

Behavioural variable	Predation regime			Sex			Predation regime × sex		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
PC 2	5.19	1, 3.99	0.1276	5.32	1, 241.1	0.06595	0.11	1, 241.1	0.7421
PC 3	0.00	1, 4.00	0.9727	0.01	1, 241.0	0.9727	2.04	1, 241	0.4637
PC 4	1.29	1, 4.00	0.4788	0.21	1, 241.0	0.6449	14.63	1, 241	0.0005

PC1 results are provided in the main text.

Table S2

Summary statistics from novel tank diving tests of wild-caught Bahamas mosquitofish

Sex	Predation regime	Population	N	Latency to upper half (s)	Upper half duration (s)	Transitions to upper half	Quadrat transitions	Freezing bouts (s)	Frozen duration (s)	Erratic behaviours
Female	LP	East Twin	21	62.00±17.25	100.52±16.34	13.76±3.20	76.05±15.68	6.05±0.73	83.43±13.58	1.14±0.31
		Hubcap	20	12.20±2.56	80.40±10.13	21.05±3.56	112.50±15.70	6.35±0.88	54.80±9.41	5.05±0.82
		Rainbow	21	56.91±12.74	60.19±10.32	16.76±2.84	84.33±10.92	5.81±0.72	46.05±5.82	2.57±0.58
	HP	Cousteau	20	68.80±25.64	95.20±23.92	2.05±0.44	12.05±2.46	6.45±0.55	178.60±15.12	2.00±0.38
		Stalactite	21	83.24±25.16	74.00±16.46	3.62±0.76	20.00±3.71	8.24±0.55	144.57±14.53	3.19±0.67
		West Twin	20	80.70±22.93	117.35±24.31	1.95±0.34	12.80±1.65	5.70±0.57	224.00±13.05	5.40±0.84
Male	LP	East Twin	21	9.57±4.13	141.95±11.60	25.24±4.18	128.76±16.70	3.48±0.82	33.86±9.55	1.57±0.41
		Hubcap	22	12.14±5.12	112.77±8.72	31.27±3.65	158.91±15.64	4.23±0.90	33.32±10.26	6.68±1.01
		Rainbow	21	18.38±6.70	84.67±9.87	23.90±3.50	118.29±15.59	4.81±0.76	30.86±5.73	4.33±0.88
	HP	Cousteau	22	34.50±19.27	125.05±21.53	3.91±1.64	18.64±7.05	6.68±0.49	167.95±13.86	3.55±0.65
		Stalactite	21	48.05±12.34	119.33±12.30	8.95±1.34	47.48±7.10	8.10±0.58	66.81±10.76	2.95±0.57
		West Twin	19	75.95±22.51	132.00±21.68	4.74±1.09	33.79±5.34	7.58±0.62	129.84±17.88	5.47±0.77

Values are means ± SE.

Table S3

Results of linear mixed models testing for genetic variation in exploration of a novel environment in laboratory-raised Bahamas mosquitofish

Behavioural variable	Full-sib family			Sex		
	F	*df*	*P*	*F*	*df*	*P*
PC1	2.46	7, 61	0.0272	42.00	1, 61	<0.0001
PC2	1.35	7, 61	0.4850	0.66	1, 61	0.7983
PC3	0.66	7, 61	0.7082	2.77	1, 61	0.2028
PC4	0.48	7, 61	0.8466	0.12	1, 61	0.8466