RESEARCH PAPER

Predation risk and the evolution of a vertebrate stress response: Parallel evolution of stress reactivity and sexual dimorphism

Jerker Vinterstare[1] | Gustaf M. O. Ekelund Ugge[1,2] | Kaj Hulthén[1] | Alexander Hegg[1] | Christer Brönmark[1] | Per Anders Nilsson[1] | Ursula Ronja Zellmer[3] | Marcus Lee[1] | Varpu Pärssinen[1] | Yongcui Sha[1] | Caroline Björnerås[1] | Huan Zhang[4] | Raphael Gollnisch[1] | Simon D. Herzog[1] | Lars-Anders Hansson[1] | Martin Škerlep[1] | Nan Hu[1] | Emma Johansson[1] | Randall Brian Langerhans[5]

[1]Aquatic Ecology Unit, Ecology Building, Department of Biology, Lund University, Lund, Sweden

[2]School of Bioscience, University of Skövde, Skövde, Sweden

[3]Animal Ecology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

[4]State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

[5]Department of Biological Sciences, W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC, USA

Correspondence
Jerker Vinterstare, Aquatic Ecology Unit, Ecology Building, Department of Biology, Lund University, SE-223 62 Lund, Sweden.
Email: jerker.vinterstare@biol.lu.se

Abstract

Predation risk is often invoked to explain variation in stress responses. Yet, the answers to several key questions remain elusive, including the following: (1) how predation risk influences the evolution of stress phenotypes, (2) the relative importance of environmental versus genetic factors in stress reactivity and (3) sexual dimorphism in stress physiology. To address these questions, we explored variation in stress reactivity (ventilation frequency) in a post-Pleistocene radiation of live-bearing fish, where Bahamas mosquitofish (*Gambusia hubbsi*) inhabit isolated blue holes that differ in predation risk. Individuals of populations coexisting with predators exhibited similar, relatively low stress reactivity as compared to low-predation populations. We suggest that this dampened stress reactivity has evolved to reduce energy expenditure in environments with frequent and intense stressors, such as piscivorous fish. Importantly, the magnitude of stress responses exhibited by fish from high-predation sites in the wild changed very little after two generations of laboratory rearing in the absence of predators. By comparison, low-predation populations exhibited greater among-population variation and larger changes subsequent to laboratory rearing. These low-predation populations appear to have evolved more dampened stress responses in blue holes with lower food availability. Moreover, females showed a lower ventilation frequency, and this sexual dimorphism was stronger in high-predation populations. This may reflect a greater premium placed on energy efficiency in live-bearing females, especially under high-predation risk where females show higher fecundities. Altogether, by demonstrating parallel adaptive divergence in stress reactivity, we

---

Vinterstare and Ekelund Ugge contributed equally to this study.

----

1 | INTRODUCTION

Predation is a major evolutionary force that has selected for a plethora of anti-predator adaptations in prey (Langerhans 2007; Vamosi 2005). Considerable research has explored morphological traits, such as body shape (Brönmark & Miner, 1992; Cott, 1940; Price et al., 2015; Young et al., 2004), behavioural strategies, including activity and exploration (Heinen-Kay et al., 2016; Hossie et al., 2010; Ydenberg & Dill, 1986), and life-history traits, such as offspring number and size (Hagmayer et al., 2020; Reznick et al., 1990; Riesch et al., 2013) linked to enhanced fitness under varying predation risk. By comparison, fewer studies have examined how predators may drive evolutionary shifts in underlying physiological processes (neuroendocrine and cardiovascular processes) that act to increase survival and maintain physiological homeostasis (Clinchy et al., 2013; Hammerschlag et al., 2017).

The vertebrate stress response involves a complex endocrinological pathway that leads to enhanced glucocorticoid secretion, followed by increased blood glucose levels, as well as enhanced cardiovascular activity and ventilation rate, i.e. the necessities of the fight-or-flight response for predator evasion (Clinchy et al., 2013; Hawlena & Schmitz, 2010; Sapolsky, 1990; Sapolsky et al., 2000). For instance, the hypothalamus–pituitary–adrenal/inter-renal (HPA/HPI) axis ranks among the most prevalent and evolutionarily conserved adaptations to stress (Clinchy et al., 2013; Hawlena & Schmitz, 2010; Sapolsky et al., 2000). Stress responses often show a high degree of intraspecific variability and interspecific variability (Höglund et al., 2000; Sapolsky, 1982, 1990), which can result from environmental stimuli via developmental plasticity (Champagne et al., 2008; Chouinard-Thuly et al., 2018; Denver, 2009). Predation as a driver of stress response differentiation is a topic that has garnered considerable attention, but comparatively few studies have focused on the role of local variation in selection regimes for intraspecific divergence by examining both phenotypic (wild-caught) and genetic (laboratory-reared) patterns, or investigated potential sex differences in this context, even though this could prove important.

Overall, the reactivity and magnitude of the stress response should reflect its relative functional importance, manifested as a trade-off between costs and benefits associated with the specific selective environment. For instance, due to high energy requirements, stress reactivity may be dampened in environments with relatively scarce resource availability (Kitaysky et al., 1999). Physiological stress could incur substantial costs in terms of lost opportunities for foraging and reproduction, reduced growth rates, impaired immunity and inflammatory responses, as well as inhibition of sexual behaviour, and overall increased energy consumption (Gregory & Wood, 1999; McPeek et al., 2001; Oppliger et al., 1998; Sapolsky et al., 2000). For example, stress exposure resulted in a 25% reduction of the metabolic scope for activity in green sturgeon (*Acipenser medirostris*) (Lankford et al., 2005) and a decrease in egg size and offspring survival in brown (*Salmo trutta*) and rainbow (*Oncorhynchus mykiss*) trout (Campbell et al., 1994). Hence, whereas it may increase the chance of successful escape from acute threats, high-stress reactivity also comes with associated costs.

Here, we quantified the change in ventilation frequency over time following exposure to a threat, as a proxy of a physiological stress response (e.g. Barreto et al., 2009; Brown et al., 2005; Di Poi et al., 2016; Hawkins et al., 2004). Respiration among most teleost fishes is based on pumping water through the gills by expanding and contracting the buccal cavity in tandem with opening and closing the opercular valves (Helfman et al., 1997). A common reaction to stressors such as predation risk is increased ventilation frequency to increase oxygen uptake in preparation for behavioural responses, such as escape manoeuvres (Bell et al., 2010; Hawkins et al., 2004). Furthermore, ventilation frequency correlates with plasma cortisol levels and is proven to be a very sensitive measurement of predator-induced stress (Barreto et al., 2003; Barreto & Volpato, 2004; Queiroz & Magurran, 2005) and is both easy to quantify and non-invasive. We measured individuals' ventilation frequency in multiple populations of Bahamas mosquitofish (*Gambusia hubbsi*) that have evolved for thousands of years in either the presence or absence of predatory fish (Langerhans et al., 2007). To evaluate the roles of within-lifetime environmental exposure versus intrinsic, genetically based population differences, we conducted these tests using both field-collected fish and those reared for two generations in a common laboratory environment, for which differential environmental effects should be minimized.

Owing to natural variation in fish communities, inland blue holes (water-filled, vertical caves) on Andros Island, The Bahamas, present an ideal study system to test for predator-induced selection on the vertebrate stress response. Bahamas mosquitofish are small, live-bearing fish (family Poeciliidae) inhabiting numerous blue holes that vary substantially in predation risk due to the presence/absence of a principal piscivorous predator (bigmouth sleeper, *Gobiomorus dormitor*) (Björnerås et al., 2020; Heinen et al., 2013; Langerhans et al., 2007; Martin et al., 2015). Hence, blue holes are easily dichotomized into 'high predation', where predators impose strong mortality and reduce Bahamas mosquitofish densities, and 'low predation' with no predatory

fishes and, consequently, low-predation pressure and low mortality rates by comparison. As a consequence, several studies have demonstrated that Bahamas mosquitofish from high- and low-predation blue holes have repeatedly evolved different suites of phenotypic traits, including morphology, behaviour and life history (e.g. , 2017; Langerhans et al., 2007; Riesch et al., 2020), since the colonization of the blue holes ~15 000 years ago (Fairbanks, 1989). Importantly, gene flow among populations is low and predation regime is not associated with genetic relatedness among populations (Heinen-Kay & Langerhans, 2013; Langerhans et al., 2007; Riesch et al., 2013; Schug et al., 1998). Further, predation risk in blue holes does not systematically covary with environmental variables such as chlorophyll *a* density, zooplankton and phytoplankton densities, salinity, turbidity, water transparency, depth, dissolved oxygen, temperature and pH (Björnerås et al., 2020; Heinen et al., 2013). This scenario allows us to focus explicitly on the role of predation regime in driving divergence in the physiological stress response in Bahamas mosquitofish.

In this study, we test a number of specific predictions regarding the role of predation risk in driving differentiation in the physiological stress reactivity (change in ventilation frequency) of both males and females. We expected that in environments with frequent stressful encounters, evolutionary trade-offs would fine-tune the stress reactivity to prevent unwarranted energy expenditure, while still providing adequate means for escape. Specifically, we hypothesized that populations experiencing higher predation risk would evolve reduced physiological responses to unknown threats. Therefore, we predicted (1) high-predation populations to show a smaller change in ventilation frequency, i.e., lower stress reactivity, over the course of an experimentally induced stressful scenario. This prediction was founded on earlier research and the theory on physiological stress in the wild. Prey populations in high-predation sites often adapt to the specific stress imposed by predator exposure (Romero, 2004) and should have evolved traits that prevent excessive energy expenditure linked to glucocorticoid synthesis (e.g. Romero, 2004; Sapolsky et al., 2000) as shown in, e.g., fish prey living in high-predation localities (e.g. Archard et al., 2012; Brown et al., 2005; Fischer et al., 2014; Jenkins et al., 2021). Moreover, as the predation regimes in the blue holes have been relatively constant for thousands of years (Heinen-Kay & Langerhans, 2013; Langerhans et al., 2007; Martin et al., 2015; Riesch et al., 2013), we hypothesized that differences in stress reactivity would reflect evolutionary divergence rather than phenotypic plasticity. We therefore expected that (2) differences in stress reactivity between predation regimes would be consistent among laboratory-reared and wild-caught individuals. Finally, because sex is a recognized source of variation in responsiveness to stress, we also investigated potential sex-specific trade-offs. Here, we hypothesized that efficient energy utilization would be more important to females than to males, as female Bahamas mosquitofish bear live young and allocate more time towards foraging (Heinen et al., 2013), and, hence, efficient energy utilization would be more important to females than to males. We predicted (3) a lower overall ventilation frequency and a lower stress reactivity in females compared with males, and, in addition, that sex

differences should be larger in fish from high-predation populations with females in these environments showing the smallest stress reactivity given the increased fecundity females have evolved in high-predation populations (Hulthén et al., 2021; Riesch et al., 2013, 2020).

2 | MATERIALS AND METHODS

2.1 | Wild-caught specimens

We captured adult male ($n = 91$) and female ($n = 85$) Bahamas mosquitofish from three high-predation and three low-predation focal blue holes (populations C, S, W, E, H, and R in Figure 1; see Table S1) during 27 February–7 March 2018, using hand-held dip nets while snorkelling. Sex was easily determined visually by the detection of fully developed gonopodia in males. Blue holes were selected a priori as representative of the larger set of blue holes on Andros Island and are characterized by independent colonization events and low gene flow between mosquitofish populations (e.g. Heinen-Kay & Langerhans, 2013; Langerhans, 2017; Langerhans et al., 2007; Langerhans & Rosa-Molinar, 2021; Riesch et al., 2013). Despite varying resource availability (e.g. food and nutrients), there is a lack of covariation between known environmental parameters and the presence or absence of the predatory bigmouth sleeper (Björnerås et al., 2020; Heinen et al., 2013; Hulthén et al., 2021). After collection, each experimental subject was immediately transferred to a transparent plastic cube ($5 \times 5 \times 5$ cm; approximately 1.5–2.0 × fish body length) filled to a depth of 2 cm with water from the same blue hole as the focal fish was captured from (cubes were always rinsed and fresh blue hole water added between trials). To decrease environmental disturbance and to facilitate behavioural analysis by standardizing light conditions during trials, the cube was placed inside a white Neewer® photographic tent ($80 \times 80 \times 80$ cm) on an electronic tablet (Apple iPad, model A1822) set to full light intensity on white background. Trials were conducted near the shore of each blue hole, during daylight while shaded by white curtains, and were recorded from above using a tripod mounted DSLR camera (Canon EOS 70D; Canon Inc.) equipped with a macro lens (Canon EF 100 mm f/2.8 USM Macro 1:1). Videos were used to visually determine ventilation frequency (see Video S1 example in Appendix S1), a straightforward and established method for measuring stress in fish (see, e.g., Barreto et al., 2009; Brown et al., 2005; Di Poi et al., 2016; Hawkins et al., 2004; Queiroz & Magurran, 2005). Video recording of each trial began immediately after the cube was placed on the tablet (<40 s after the fish entered the cube), to capture immediate stress responses elicited from capture and placement into a novel, confined environment. To standardize stress exposures, all fish were also subjected to chasing as an additional stressor (e.g. Reid et al., 1994; Marentette et al., 2013, Samaras et al., 2018). After 60 s of recording, fish were chased for 15 s by a hand-held wooden spatula. We then continued to record each fish for an additional 5 min.

FIGURE 1 Map of the location of the eight focal blue holes used in this study. Blue: low predation, red: high predation. Study site abbreviations follow Table S1

2.2 | Laboratory-reared specimens (F2)

For the common-garden experiment, we examined the stress reactivity using the same protocol as for the wild-caught populations. All fish were raised under common laboratory conditions in a large, recirculating aquarium system comprising 72 115-L aquaria with biological, mechanical and ultraviolet filtration. Water was maintained at approximately 25°C, a conductivity of 2850 µS/cm and a pH of 8.3, with a 14-h light/10-h dark photoperiod. Fish were fed daily with a mixture of TetraPro Tropical Crisps, Fluval Bug Bites for Tropical Fish and Hikari freeze-dried *Daphnia*, blood worms and brine shrimp. To generate the experimental animals, we collected the parental generation (F0) as newborns (to minimize maternal and environmental effects) from eight blue hole populations (Figure 1). Wild-caught F0 specimens were transported to the laboratory facilities at North Carolina State University, United States, raised to adulthood, and each female was mated with a single male from the same population, with no fish used more than once (on average, 19 unique male–female pairings were used from each locality, range: 10–26). Offspring (F1) were then raised to adulthood in the same recirculating aquarium system, and females from each population were

mated with multiple males from the same population (to maintain high genotypic diversity in laboratory populations). Offspring from these matings (F2) were raised separately by sex and served as experimental subjects for stress trials (male: $n = 84$, female: $n = 87$; see Table S1). All fish were reared and tested at approximately 25.0°C.

2.3 | Data treatment and statistical analysis

Recordings were viewed blind by a single observer (URZ) who was not involved in the recordings. The observer used the VLC media player software (3.0.8) with optional slow-motion analysis to extract the time taken for 60 opercular beats to occur at six different time-points in each recording: start of the recording (t_0), immediately following the additional acute stressor (t_1), and then in 1-min increments until 4 min after the acute stressor (t_2–t_5). If the experimental subject was swimming/moving in the arena, we paused the counting of opercular beats and continued as soon as the individual stopped moving. Paused time was subtracted from the total time in the analyses. Across all six time-points, the average proportion of trials that required pausing were as follows, field data: 0.266; laboratory data: 0.113.

To measure body size, we captured still frames from the videos and measured standard length (SL in mm) of each fish using the image analysis software ImageJ (version 1.52, https://imagej.nih.gov/ij/). Size of each image was calibrated from a ruler placed in the experimental cube (see Figure S1 in the Appendix S1).

Data were transformed into ventilation frequency (beats per minute, BPM) and used as the dependent variables (BPM from the six repeated time-points) in a mixed-model repeated-measures analysis of covariance (ANCOVA) to test for differences in stress reactivity between sexes, predation regimes and rearing environments. Predation regime, sex, rearing environment and their interactions served as independent variables. To properly treat population as the unit of replication for tests of predation regime, we included population nested within predation regime as a random effect. Also, we included $\log_{10}$-transformed (for normality) SL (mean $\pm$ SE; 24.32 $\pm$ 0.22 mm for males, and 29.94 $\pm$ 0.55 mm for females) as a covariate to statistically adjust for body size effects on ventilation frequency (we expected a negative correlation, see, e.g. Brown et al., 2005) and thus examined size-independent aspects of stress reactivity. We included an interaction term between standard length and time point to test whether size dependence varied over the course of the trial; interactions between standard length and all other effects were initially included but excluded from the final model due to unimportance (all $p > 0.17$).

Whereas this repeated-measures analysis provided an appropriate overall test of (1) within-subjects effects (Time), (2) whether changes in ventilation frequency over time might differ between groups (e.g. Time $\times$ Predation Regime), and (3) whether average ventilation frequencies differ between groups (e.g. Sex), we calculated two additional metrics to more explicitly evaluate variation in the stress response per se and the recovery ventilation rate. First, we measured the scope of ventilation frequency for each fish as an estimate of the relative magnitude of the stress response: average BPM during t_0 and t_1 minus the minimum BPM during the trial. This metric is designed to capture each fish's reactivity to the mild stressors of capture, confinement and chasing. Second, we calculated the recovery ventilation frequency as the average BPM during t_3 to t_5, estimating the ventilation frequency exhibited soon after a startling experience. Using these two metrics as dependent variables, we conducted separate general linear mixed models with predation regime, sex, rearing environment and their interactions as independent variables. We again included $\log_{10}$-transformed SL as a covariate to adjust for effects of body size, as well as population nested within predation regime as a random effect. These two dependent variables (scope and recovery BPM) were not correlated with one another ($r = 0.07$, $p = 0.17$). To ensure robust results, we additionally performed all analyses excluding the two populations only examined in the laboratory (not in the wild); all results were qualitatively similar (see Tables S2 and S3). Statistical analyses were performed using the Proc Mixed procedure in SAS software (version 9.3).

Because our results indicated that an additional environmental factor (not included in our models) might play an important role in explaining among-population variation in stress response (see Results),

we conducted two more analyses. Owing to the energetic costs of physiological stress responses (see Introduction), we suspected that resource availability might serve as an important selective agent, favouring reduced stress responses with scarce resources and elevated stress responses when high-quality food resources are readily available (see Discussion). We estimated resource availability in these eight blue holes using previously published estimates of zooplankton density. Specifically, previous work has documented that the availability of major prey of Bahamas mosquitofish (zooplankton, mainly copepods; Araujo et al. 2014; Gluckman and Hartney 2000; Riesch et al. unpubl. ms) consistently varies among blue holes (e.g. Heinen et al., 2013; Hulthén et al., 2021; Sha et al., 2020) and has led to population variation in male coloration (Martin et al., 2014) and evolutionary divergence in some life-history traits, such as juvenile growth rate (Hulthén et al., 2021). Zooplankton density was estimated using a 60-m tow of a zooplankton net (20-cm diameter, 153-µm mesh) at 0.5-m depth within habitat where Bahamas mosquitofish were abundant within all sites (Heinen et al., 2013). We conducted a general linear mixed model separately for wild-caught and laboratory-raised fish that used the scope of ventilation frequency as the dependent variable; and predation regime, sex, zooplankton density ($\log_{10}$-transformed for normality) and their interactions as independent variables; and $\log_{10}$-transformed SL as a covariate. Population nested within predation regime was included as a random effect. Because the interaction between zooplankton density and sex, and the three-way interaction (zooplankton density $\times$ predation regime $\times$ sex) were uninformative (all $p > 0.22$), we excluded those terms from our models.

3 | RESULTS

Our mixed-model repeated-measures ANCOVA revealed a number of important effects of model terms on ventilation frequency (Table 1). This analysis revealed that fish from high-predation populations tended to show a dampened stress reactivity (Predation Regime $\times$ Time interaction; Table 1; Figure 2), in line with our first prediction. In low-predation populations, ventilation frequency generally dropped more precipitously over time compared with fish from high-predation populations (Figure 2). Whereas this analysis did not identify any strong evidence that the stress response differed between the wild and the laboratory within either predation regime (i.e. PR $\times$ ENV $\times$ Time interaction), consistent with our second prediction, inspection of the time-course stress reactivity curves (Figure 2) suggested that some variation may have indeed occurred: (1) in the laboratory, females appeared to show little difference between predation regimes in their average time-course curves even though they exhibited strong differences in the wild (Figure 2a) and (2) males in low-predation populations seemed to show differences between the wild and laboratory, resulting in a shift of the time-points in which the greatest differences between predation regimes occurred (i.e. during t_2-t_5 in the wild, during t_0-t_2 in the laboratory; Figure 2b). Overall, fish from different predation regimes, as well as wild-caught

TABLE 1 Results from mixed-model repeated-measures ANCOVA examining variation in ventilation frequency of Bahamas mosquitofish across the six time-points of the mild stressor assays

Effect	d.f.	F	p
Time	5, 960	0.60	0.7000
Log$_{10}$ standard length (SL)	1, 2063	265.94	**< 0.0001**
Predation regime (PR)	1, 7.95	0.21	0.6614
Sex	1, 2058	120.84	**< 0.0001**
Rearing environment (ENV)	1, 2070	1.28	0.2582
PR × Sex	1, 2056	14.39	**0.0002**
PR × ENV	1, 2063	2.88	**0.0897**
Sex × ENV	1, 2059	18.02	**< 0.0001**
PR × Sex × ENV	1, 2056	3.64	**0.0565**
SL × Time	5, 960	0.36	0.8774
PR × Time	5, 960	2.46	**0.0318**
Sex × Time	5, 960	2.33	**0.0407**
ENV × Time	5, 960	1.01	0.4096
PR × Sex × Time	5, 960	0.89	0.4847
PR × ENV × Time	5, 960	1.49	0.1899
Sex × ENV × Time	5, 960	0.24	0.9466
PR × Sex × ENV × Time	5, 960	0.66	0.6509

Fixed effects included Time, Predation Regime (PR), Sex and Rearing Environment (ENV), as well as their interactions. Standard Length (SL, log$_{10}$-transformed) was included as a covariate, and the SL × Time interaction was included to control for possible size effects on stress reactivity over time. Population nested within Predation Regime was included as a random effect.
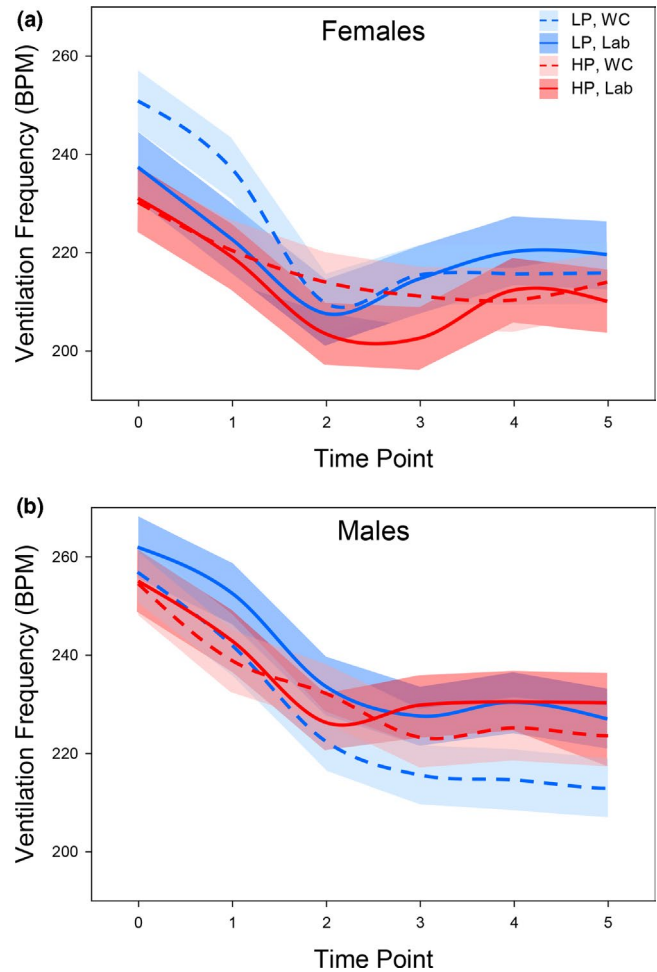
P values < 0.1 are in bold type.

FIGURE 2 Visualization of variation in the physiological stress response (ventilation frequency) of Bahamas mosquitofish from low- and high-predation populations for (a) females and (b) males (sample sizes in Table S1). Illustration depicts a spline fit to the least-squares means for each of the six time-points, and the shaded regions depict ±1 SE. Red: high predation, blue: low predation; light shade and dashed lines: wild-caught (WC), dark shade and solid line: laboratory-raised (Lab)

and laboratory-raised fish, showed little consistent differences in ventilation frequency averaged across time-points (Predation Regime term, Rearing Environment term, respectively, Table 1; Figure 3a). Related to our third prediction, we uncovered strong evidence of sexual dimorphism in ventilation frequency, which varied among predation regimes, rearing environments and time-points (Table 1). Specifically, females typically exhibited a lower average ventilation frequency than males (Sex term; Table 1), a pattern that was more evident in fish from high- than from low-predation populations (Sex × Predation Regime interaction; Table 1, Figure 3a), as well as in laboratory vs. field conditions (Sex × Rearing Environment interaction; Table 1, Figure 3a). In the wild, males exhibited a ventilation frequency 7% higher, on average, than females within high-predation environments, but only showed an average difference of 1% in low-predation environments (Figure 3a). However, in the laboratory, males clearly showed higher ventilation frequencies than females within all but one population (on average, 11% higher in high-predation, 8% higher in low-predation; Figure 3a). Also, females tended to show smaller changes in ventilation frequency over the time-course of the assay, that is a lower stress reactivity as compared to males (Sex × Time interaction, Table 1, Figure 2). Average sex differences in ventilation frequency were greatest early in the assay (females ~ 19 BPM slower than males during t_0–t_2) and quickly

declined towards the end of the assay (females ~ 13, 11 and 8 BPM slower than males in t_3, t_4 and t_5, respectively). Finally, as expected there was a strong, negative relationship between ventilation frequency and body size (Table 1, Figure S2), which did not vary over the time-course of the assay (SL × Time interaction, Table 1).

Examination of the scope of ventilation frequency uncovered that several factors influenced this estimate of stress reactivity to the mild stressors imposed in our assay (Table 2). Smaller fish typically exhibited a larger scope, whereas the strongest influence on size-independent scope involved a dependence of the predation regime effects on the rearing environment (Predation Regime × Rearing Environment interaction): on average, low-predation populations exhibited a larger scope than high-predation populations in the wild (75% higher in females, 42% higher in males), but these differences declined in laboratory-raised F2 fish (1% higher in females,
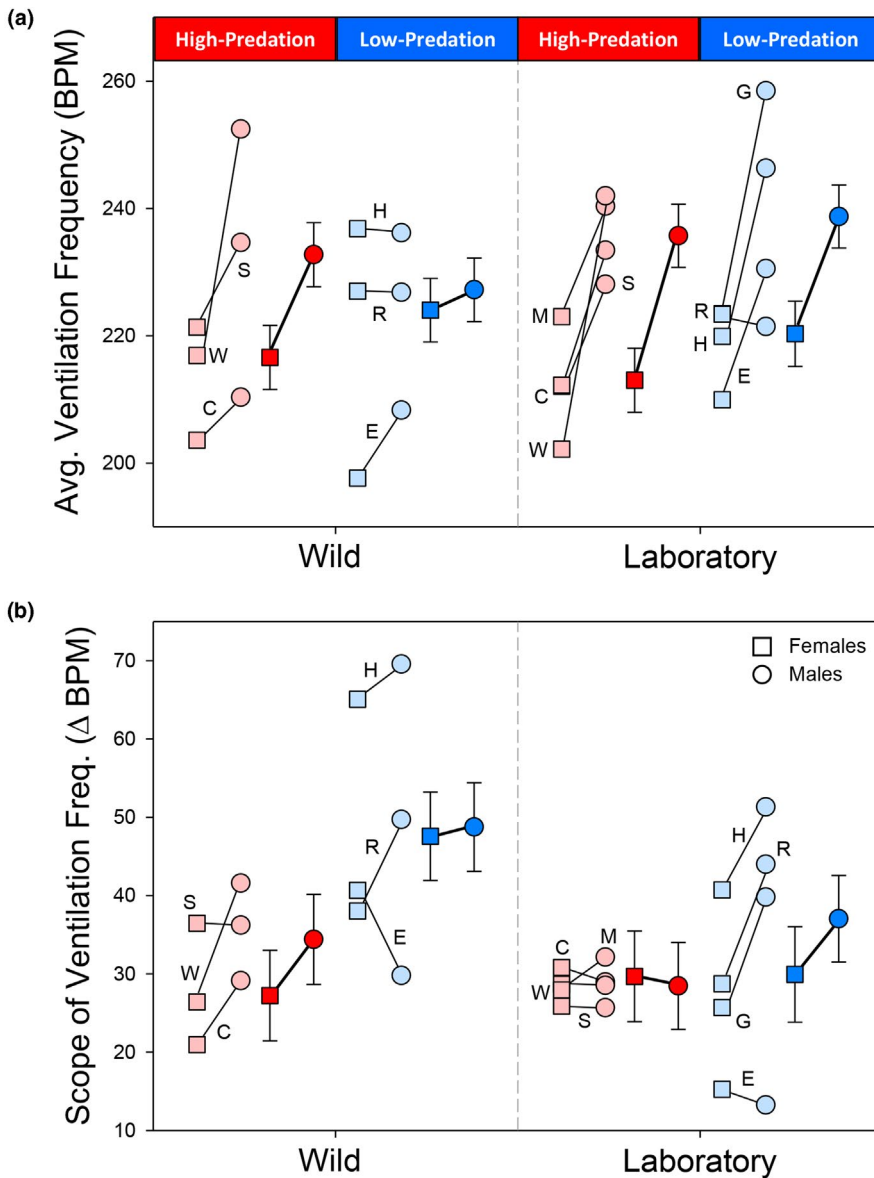
FIGURE 3 Variation in (a) average ventilation frequency and (b) the scope of ventilation frequency (change during the mild stressor assay) for females (square) and males (circle) in all populations of Bahamas mosquitofish examined in the wild and after two generations of laboratory rearing (least-squares means ±1 SE depicted; population abbreviations follow Figure 1; samples sizes in Table S1). Light shaded symbols: population means, dark shaded symbols: predation-regime means

30% higher in males) (Figure 3b). This general pattern was consistent with the suggestive time-course patterns observed in the repeated-measures analysis above. Inspection of the variation among populations within each predation regime revealed a clear pattern not apparent when only inspecting average differences: low-predation populations exhibited more variation in scope among one another than high-predation populations (Figure 3b). High-predation populations all showed relatively similar scope, especially in F2 laboratory-raised fish where very little variation among populations was observed (Figure 3b). For instance, the average coefficient of variation among population means in the wild was 22.9% vs. 35.6% in high- compared with low-predation populations, and in the laboratory, it was 8.2% vs. 41.4% in high- compared with low-predation populations.

These results suggested that an unmeasured factor might help explain among-population patterns, and thus, we performed the additional analyses that tested for the influence of resource availability

on the scope of ventilation frequency. Despite low statistical power to detect among-population trends, especially in the wild (three populations within each predation regime), the results depicted relatively clear patterns. In the wild, populations with greater zooplankton density tended to exhibit higher ventilatory scope (Figure 4a), although the trend was not strong and had little statistical support (Table S4). Laboratory-reared fish, however, showed an especially clear pattern where low-predation populations showed a strong positive relationship between scope of ventilation frequency and zooplankton density, but high-predation populations showed no association (Figure 4b; Table S4).

Results for recovery ventilation rate largely paralleled findings for average ventilation rate from the mixed-model repeated-measures analysis. Statistically adjusting for effects of body size, we found that sex differences in recovery ventilation rate depended on the predation regime (larger in high predation) and rearing environment (larger in the laboratory) (terms involving Sex, other than the

TABLE 2 Results from general linear mixed models examining variation in the scope of ventilation frequency (i.e. magnitude of the stress response) and the recovery ventilation frequency (i.e. relative baseline ventilation frequency soon after a startling experience)

Effect	Scope of ventilation frequency			Recovery ventilation frequency		
	d.f.	F	p	d.f.	F	p
Log$_{10}$ standard length (SL)	1, 335.08	5.63	**0.0182**	1, 336.90	52.84	**<0.0001**
Predation regime (PR)	1, 6.30	2.45	0.1659	1, 5.30	0.01	0.9191
Sex	1, 333.55	1.96	0.1628	1, 334.11	14.48	**0.0002**
Rearing environment (ENV)	1, 337.79	7.89	**0.0053**	1, 336.20	2.25	0.1343
PR × Sex	1, 332.84	0.08	0.7825	1, 332.57	6.75	**0.0098**
PR × ENV	1, 337.18	8.04	**0.0049**	1, 323.57	1.21	0.2714
Sex × ENV	1, 333.75	0.07	0.7944	1, 334.55	4.66	**0.0316**
PR × Sex × ENV	1, 332.81	2.90	**0.0894**	1, 332.51	0.13	0.7197

Population nested within predation regime was included as a random effect.

P values < 0.1 are in bold type.

FIGURE 4 Association between resource availability (zooplankton density in the field) and the scope of ventilation frequency in Bahamas mosquitofish from low-predation and high-predation populations examined (a) the wild and (b) after two generations of laboratory rearing (least-squares means ±1 SE depicted). Regression lines drawn for each predation regime (sexes pooled) to illustrate the trends
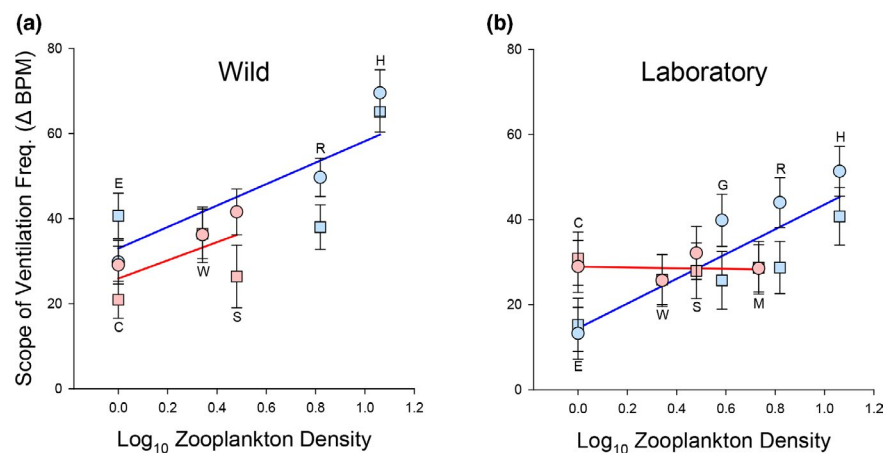
three-way interaction; Table 2). Consistent with our third prediction, females usually showed lower recovery ventilation frequencies than males, especially in high-predation populations (Figure S3). Similar to the average ventilation frequencies (Figure 3a), wild-caught males exhibited a recovery ventilation frequency 6% higher, on average, than females within high-predation environments, but only showed an average difference of 1% in low-predation environments (Figure S3). In the laboratory, males showed higher ventilation frequencies than females within all but one population (on average, 11% higher in high-predation, 6% higher in low-predation; Figure S3). Again, we found the expected strong, negative relationship between ventilation frequency and body size (Table 2).

4 | DISCUSSION

In this study, we utilized the post-Pleistocene radiation of Bahamas mosquitofish to test several key hypotheses regarding the role of predation risk in moulding the vertebrate stress response. By investigating male and female stress reactivity in field-collected and laboratory-reared individuals from multiple populations, we gained several important insights into the evolution and expression of

stress reactivity in a vertebrate. Interestingly, our observations did not completely align with our a priori predictions but point towards an unexpected future research direction.

Our first major result was partially in line with our prediction that populations inhabiting high-predation environments, where encounters with stressors are frequent and prolonged, should evolve a relatively lower stress response to sudden, mild stressors. We hypothesized that the frequent predator encounters experienced by *Gambusia* in high-predation environments would select for an adaptive fine-tuning of stress reactivity that reduces accumulating costs, consistent with the adaptive hypothesis for animals living in high-risk environments (Cooke et al., 2003). Previous studies have demonstrated that this pattern can result from acclimation, where individuals that frequently experience stressful encounters are more familiar with stressors and, accordingly, show reduced stress responses in order to minimize associated costs, such as damage from excessive levels of circulating glucocorticoids (Barcellos et al., 2010; Dobrakovova et al., 1993; Romero, 2004). Our results are partially consistent with these findings, as only wild-caught, but not second-generation laboratory-reared, Bahamas mosquitofish showed strong differences between predation regimes in physiological responses (i.e. scope of ventilation frequency) to mild

stressors. Yet, this resulted from an intriguing pattern of among-population variation within predation regimes that we argue (1) highlights the strong role of predation risk in driving the evolution of stress reactivity and (2) points to another important selective agent (resource availability) in the evolution of the stress response.

Considering that these blue hole populations are genetically isolated, largely evolving independently from one another (Heinen-Kay & Langerhans, 2013; Langerhans et al., 2007; Riesch et al., 2013; Schug et al., 1998), our findings in second-generation laboratory-raised fish suggest that evolutionary trajectories have differed between predation regimes. First, high-predation populations appear to have converged on highly similar magnitudes of stress responses (see Figures 3 and 4b). This is consistent with the notion that in the presence of predators, strong and consistent selection has caused parallel evolution. Meanwhile, low-predation populations exhibited considerable variation in their ventilatory scope and appear to have strongly responded to resource-mediated selection, with larger stress responses evident in populations that have evolved in environments with higher levels of resource availability (zooplankton density). Previous work has independently suggested important roles for predation risk and food availability/quality in stress reactivity, but these factors have not been simultaneously considered, and most prior research involved plastic (or potentially plastic) responses rather than evolutionary divergence in the stress response (e.g. Clinchy et al., 2013; Hammerschlag et al., 2017; Hawlena & Schmitz, 2010; Herring et al., 2011; Kitaysky et al., 1999, 2007; Romero & Wikelski, 2001). Together, our results suggest a scenario where three key attributes might together largely explain evolutionary patterns of the vertebrate stress response based on the relative costs and benefits of reacting to stressful events: (1) intensity of stressful encounters, (2) frequency of stressful encounters and (3) food availability. We briefly describe this hypothesis, present a simple conceptual model to illustrate its general evolutionary predictions (see Appendix S1 for details) and suggest that future studies should explore this hypothesis both theoretically and empirically.

If we combine the predicted influence of these key agents, we find that the predicted patterns of stress-response evolution closely resemble our findings in this study. First, selection should more strongly favour a large-magnitude stress response when encounters are more intense, where 'intensity' can range from mild to severe consequences of encounters, such as energy expenditure, lost feeding or mating opportunities, injury and death. Second, owing to the costs of mounting a stress response (e.g. Gregory & Wood, 1999; McPeek et al., 2001; Oppliger et al., 1998; Sapolsky et al., 2000), the relative fitness benefits of strong reactivity should decrease with increasing frequencies of stressful encounters. Third, as high-quality food becomes less readily available, selection should favour reduced magnitudes of stress responses owing to the lower availability of energy to fuel the responses and other needs (maintenance, growth, reproduction). If these three factors fully explain the evolution of the magnitude of the vertebrate stress response in a simple, non-interactive manner (selection from one agent is independent of other agents), then we expect to find that (1) populations under high-predation risk, where both the intensity and frequency of stressful encounters are high, evolve low-magnitude stress responses with little divergence between environments that vary in food availability (Figure 5), whereas (2) populations under low-predation risk, where the intensity and frequency of stressful encounters are both low, evolve low-magnitude stress responses when food is scarce, but high-magnitude responses under high food availability (Figure 5; see Figures S4 and S5 and Appendix S1). These expectations correspond well to the patterns observed here in Bahamas mosquitofish; that is, all high-predation populations showed similar, low-to-moderate ventilatory scope in response to
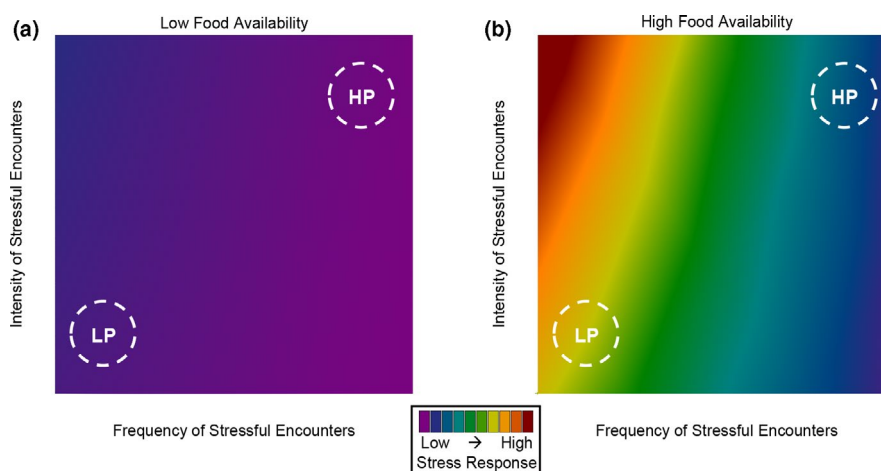

FIGURE 5 Predictions for the evolution of the stress response under varying intensity and frequency of stressful encounters in environments with (a) low food availability and (b) high food availability based on our simple conceptual model that assumes its evolution depends solely on selection from these three factors (see text, Appendix S1; 0.1 and 1.0 food levels depicted; full range of frequency of stressful encounters depicted; intensity of stressful encounters range from 0.25 to 1.0). Approximate regions for each predation regime for Bahamas mosquitofish denoted with LP (low predation) and HP (high predation)

a mild stressor, whereas low-predation populations showed low-magnitude stress responses when accustomed to low resource levels but high-magnitude ventilatory scope when accustomed to high resource levels. The generality of this conceptual model requires further investigation, and we suggest future studies address this topic.

The weak evidence for differences in scope of ventilation frequency between rearing environments for high-predation populations matched our prediction that evolutionary divergence, not phenotypic plasticity, explains variation in stress responses—but this only applied to high-predation populations, as low-predation populations generally showed smaller scope in the laboratory compared with the field. Blue hole populations have experienced relatively constant predation threat for thousands of generations with little gene flow across blue holes (e.g. Heinen-Kay & Langerhans, 2013; Riesch et al., 2013). This scenario should elicit little selection for plasticity in stress reactivity (Tollrian & Harvell, 1999; West-Eberhard, 2003). In line with these findings, genetic adaptation, and not developmental plasticity, has been found to underlie divergence in stress reactivity (ventilation frequency) between marine and freshwater three-spined sticklebacks (*Gasterosteus aculeatus*) (Di Poi et al., 2016). Yet, low-predation populations in this study showed considerable variation between the wild and the laboratory in their stress response, suggesting these fish are more sensitive to certain aspects of environmental variation in this regard. Whereas prior work has shown temporal repeatability in primary productivity and resource availability in the Bahamian blue holes (e.g. Heinen et al., 2013; Hulthén et al., 2021), these factors do show some variation over time, and food quality and quantity surely differed between the wild and the laboratory, suggesting that resource-related factors may partially explain these findings. One potential phenomenon that could be involved is counter-gradient variation, and future studies could directly examine this hypothesis. That is, reduced food levels might induce higher stress responses (Kitaysky et al., 2007; Romero & Wikelski, 2001), but select for lower stress responses. Considering that laboratory-raised fish from low-predation populations tended to show lower stress responses than in the wild, and food quality is probably greater in the laboratory, this pattern seems at least plausible at this point. Thus, it seems that whereas predation by piscivorous fish drives parallel evolution of stress responses in Bahamas mosquitofish, other factors such as food availability become the driving factors affecting stress responses in the absence of predators.

In contrast to patterns of ventilatory scope, fish from different predation regimes did not show differences in average or recovery ventilation frequency. In a previous study, wild-caught female fish (*Brachyrhaphis episcopi*) from high-predation sites in Panama similarly showed a lower ventilation frequency and a smaller scope than their low-predation counterparts when exposed to an experimental stressor, but, in contrast, a higher ventilation frequency under normal activity levels (Brown et al., 2005). Consequently, the evolution of stress responses is complex and has proven not always consistent across species. For example, wild three-spined sticklebacks facing a high risk of predation had higher opercular beat rates in response to confinement stress as compared to conspecifics from low-predation sites (Bell et al., 2010). However, these fish were juveniles and there was no effect of sex in the final model (Bell et al., 2010). Hence, we argue that it is of importance to address both biological and ecological contexts to gain a more thorough understanding of the proximate and ultimate factors affecting development and evolution of stress responses (see, e.g., Archard et al., 2012; Reeder & Kramer, 2005). Moreover, size-specific differences in ventilation frequency between predation regimes on the scale observed here (~14–20 BPM in the wild) could certainly translate to fitness-relevant differences in energy usage and is similar to or exceeds findings in previous work of about 10-BPM differences between predatory environments in similarly sized fish (Bell et al., 2010; Brown et al., 2005).

The sex-specific effects on ventilation frequency indicate that stress reactivity can evolve differentially among males and females, and independently of sex differences in overall body size. Sex-specific stress responses have been demonstrated in earlier studies (e.g. Donelan & Trussell, 2020), where female responses to stress have been suggested to build on processes related to attachment and caregiving that ultimately would downregulate the HPA-axis (Sapolsky et al., 2000; Taylor et al., 2000). The fact that females showed lower overall ventilation frequencies than males, especially in laboratory-raised fish and in high-predation populations, suggests that the sexual dimorphism has a strong genetic basis and that predation threat has influenced its evolution. We suggest a high premium is placed on energy efficiency in females compared with males. Bahamas mosquitofish females are viviparous, breed year-round, invest heavily into reproduction (embryos make up ~12%–13% of the body weight), are almost constantly pregnant and even provide nutritional provisioning to embryos during pregnancy in some populations (Riesch et al., 2013). These great energetic demands should result in stronger selection for energy efficiency in the stress response of females compared with males. A possible explanation for why sex differences were greater in the laboratory derives from sex-specific housing in the rearing environment; laboratory-reared females did not contend with male harassment, whereas laboratory-reared males may have experienced elevated male–male interactions. Avoiding wasteful energy expenditure may be even more important in high-predation localities, where fecundity is higher (Hulthén et al., 2021; Riesch et al., 2013) and energy-demanding rapid locomotor performance is critical for surviving predatory threats (Langerhans, 2009). Similar patterns were recently described in the foraging behaviour of mosquitofish, with stronger divergence between predation regimes in females than males (Pärssinen et al., 2021). Overall, female energy efficiency is therefore a potential explanation to why the sexes differ more strongly in high-predation compared with low-predation populations.

We however only found inconclusive support for reduced stress reactivity (i.e. scope of ventilation frequency) in females compared with males, indicating that whereas females have clearly evolved lower ventilation rates, the relative sex differences in the physiological stress response are more variable and minor. In

contrast, a recent study demonstrated that in another live-bearing fish species (Trinidadian guppies, *Poecilia reticulata*), females have a lower cortisol release than males when exposed to a stressor (Chouinard-Thuly et al., 2018). Overall, it seems likely that life-history differences between the sexes may largely explain differential stress responses, as these can be tightly linked to individual variation in physiological stress responsiveness in vertebrates (Furtbauer et al., 2015; Reeder & Kramer, 2005; Sapolsky, 1990). In Bahamas mosquitofish, the sexes differ in a large range of morphological and behavioural traits, and genetic correlations among these traits may prove important in sex-specific patterns of stress physiology and reactivity.

5 | CONCLUSIONS

Natural populations of Bahamas mosquitofish inhabiting blue holes provide an excellent model system for evolutionary studies of local selection and adaptation. In the current study, we utilized both wild-caught and laboratory-reared mosquitofish originating from multiple, independent high-predation and low-predation sites to provide evidence that predation risk is indeed a major factor moulding stress responses in vertebrates. High-predation pressure appears to have driven very similar stress responses in high-predation populations, whereas low-predation populations show more variation among populations, apparently responding to site-specific resource availabilities. We argue that these patterns are likely explained by evolutionary trade-offs to reduce stress-related energy expenditure among environments that differ in frequency and intensity of stressful encounters, as well as in food availability. Furthermore, we demonstrate significant sexual dimorphism in ventilation frequency and propose that this is strongly influenced by evolution under varying predation risk. In a broader context, our results suggest that animals exposed to frequent, high-intensity stressful encounters benefit from a dampened acute stress response, especially for individuals that require high energy intake (e.g. reproductively active, live-bearing females). Meanwhile, a dampened stress response should also evolve in environments with low resource availability, but this effect may be strongest when encounters are less frequent and potentially less intense. The link between the vertebrate stress response and overall energy allocation, as well as potential effects thereon from predation risk and sex, remains important issues for future research.

ORCID

Jerker Vinterstare https://orcid.org/0000-0001-6998-4632
Gustaf M. O. Ekelund Ugge https://orcid.org/0000-0002-2418-1163
Per. Anders Nilsson https://orcid.org/0000-0002-3541-9835
Marcus Lee https://orcid.org/0000-0002-3320-3010
Varpu Pärssinen https://orcid.org/0000-0002-9189-9765
Caroline Björnerås https://orcid.org/0000-0002-3506-0367
Raphael Gollnisch https://orcid.org/0000-0001-6177-8877
Lars-Anders Hansson https://orcid.org/0000-0002-3035-1317
Martin Škerlep https://orcid.org/0000-0001-5812-2383
Emma Johansson https://orcid.org/0000-0001-5300-9672
Randall. Brian Langerhans https://orcid.org/0000-0001-6864-2163

REFERENCES

Archard, G. A., Earley, R. L., Hanninen, A. F., & Braithwaite, V. A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. *Functional Ecology, 26*, 637–645. https://doi.org/10.1111/j.1365-2435.2012.01968.x

Araujo, M. S., Langerhans, R. B., Giery, S. T., & Layman, C. A. (2014). Ecosystem fragmentation drives increased diet variation in an endemic livebearing fish of the Bahamas. *Ecology and Evolution, 4*, 3298–3308.

Barcellos, L. J. G., Ritter, F., Kreutz, L. C., & Cericato, L. (2010). Can zebrafish Danio rerio learn about predation risk? The effect of a

previous experience on the cortisol response in subsequent encounters with a predator. *Journal of Fish Biology*, 76, 1032–1038. http://dx.doi.org/10.1111/j.1095-8649.2010.02542.x

Barreto, R. E., Luchiari, A. C., & Marcondes, A. L. (2003). Ventilatory frequency indicates visual recognition of an allopatric predator in naïve Nile tilapia. *Behavioural Processes*, 60, 235–239. https://doi.org/10.1016/S0376-6357(02)00127-4

Barreto, R. E., & Volpato, G. L. (2004). Caution for using ventilatory frequency as an indicator of stress in fish. *Behavioural Processes*, 66, 43–51. https://doi.org/10.1016/j.beproc.2004.01.001

Barreto, R. E., Volpato, G. L., de Brito Faturi, C., Giaquinto, P. C., Gonçalves de Freitas, E., & Fernandes de Castilho, M. (2009). Aggressive behaviour traits predict physiological stress responses in Nile tilapia (Oreochromis niloticus). *Marine and Freshwater Behaviour and Physiology*, 42, 109–118. https://doi.org/10.1080/10236240902850392

Bell, A. M., Henderson, L., & Huntingford, F. A. (2010). Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. *Journal of Comparative Physiology B*, 180, 211–220. https://doi.org/10.1007/s00360-009-0395-8

Björnerås, C., Škerlep, M., Gollnisch, R., Herzog, S. D., Ekelund Ugge, G., Hegg, A., Hu, N., Johansson, E., Lee, M., Pärssinen, V., Sha, Y., Vinterstare, J., Zhang, H., Hulthén, K., Brönmark, C., Hansson, L.-A., Nilsson, P. A., Rengefors, K., & Langerhans, R. B. (2020). Inland blue holes of The Bahamas – chemistry and biology in a unique aquatic environment. *Fundamental and Applied Limnology*, 194, 95–106. https://doi.org/10.1127/fal/2020/1330

Brönmark, C., & Miner, J. G. (1992). Predator-induced phenotypical change in body morphology in crucian carp. *Science*, 258, 1348–1350. https://doi.org/10.1126/science.258.5086.1348

Brown, C., Gardner, C., & Braithwaite, V. A. (2005). Differential stress responses in fish from areas of high- and low-predation pressure. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, 175, 305–312. https://doi.org/10.1007/s00360-005-0486-0

Campbell, P. M., Pottinger, T. G., & Sumpter, J. P. (1994). Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. *Aquaculture*, 120, 151–169. https://doi.org/10.1016/0044-8486(94)90230-5

Champagne, D. L., Bagot, R. C., van Hasselt, F., Ramakers, G., Meaney, M. J., de Kloet, E. R., Joëls, M., & Krugers, H. (2008). Maternal care and Hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *The Journal of Neuroscience*, 28, 6037–6045.

Chouinard-Thuly, L., Reddon, A. R., Leris, I., Earley, R. L., & Reader, S. M. (2018). Developmental plasticity of the stress response in female but not in male guppies. *Royal Society Open Science*, 5, 172268. https://doi.org/10.1098/rsos.172268

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, 27, 56–65. https://doi.org/10.1111/1365-2435.12007

Cooke, S. J., Steinmetz, J., Degner, J. F., Grant, E. C., & Philipp, D. P. (2003). Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. *Canadian Journal of Zoology*, 81, 699–709.

Cott, H. B. (1940). *Adaptive coloration in animals*. Methuen.

Denver, R. J. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. *General and Comparative Endocrinology*, 164, 20–31. https://doi.org/10.1016/j.ygcen.2009.04.016

Di Poi, C., Lacasse, J., Rogers, S. M., & Aubin-Horth, N. (2016). Evolution of stress reactivity in stickleback. *Evolutionary Ecology Research*, 17, 395–405.

Dobrakovova, M., Kvetnansky, R., Oprsalova, Z., & Jezova, D. (1993). Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology*, 18, 163–174. https://doi.org/10.1016/0306-4530(93)90001-2

Donelan, S. C., & Trussell, G. C. (2020). Sex-specific differences in the response of prey to predation risk. *Functional Ecology*, 34, 1235–1243. https://doi.org/10.1111/1365-2435.13569

Fairbanks, R. G. (1989). A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. *Nature*, 342, 637–642.

Fischer, E. K., Harris, R. M., Hofmann, H. A., & Hoke, K. L. (2014). Predator exposure alters stress physiology in guppies across timescales. *Hormones and Behavior*, 65, 165–172. https://doi.org/10.1016/j.yhbeh.2013.12.010

Furtbauer, I., Pond, A., Heistermann, M., & King, A. J. (2015). Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. *Functional Ecology*, 29, 931–940.

Gluckman, T. L., & Hartney, B. K. (2000). A trophic analysis of mosquitofish, Gambusia hubbsi Breder, inhabiting blue holes on Andros Island, Bahamas. *Caribbean Journal of Science*, 36, 104–111.

Gregory, T. R., & Wood, C. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology*, 72, 286–295.

Hagmayer, A., Furness, A. I., Reznick, D. N., Dekker, M. L., & Pollux, B. J. A. (2020). Predation risk shapes the degree of placentation in natural populations of live-bearing fish. *Ecology Letters*, 23, 831–840. https://doi.org/10.1111/ele.13487

Hammerschlag, N., Meyer, M., Seakamela, S. M., Kirkman, S., Fallows, C., & Creel, S. (2017). Physiological stress responses to natural variation in predation risk: evidence from white sharks and seals. *Ecology*, 98, 3199–3210. https://doi.org/10.1002/ecy.2049

Hawkins, L. A., Armstrong, J. D., & Magurran, A. E. (2004). Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. *Journal of Fish Biology*, 65, 88–100. https://doi.org/10.1111/j.0022-1112.2004.00543.x

Hawlena, D., & Schmitz, O. (2010). Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist*, 176, 537–556. https://doi.org/10.1086/656495

Heinen, J. L., Coco, M. W., Marcuard, M. S., White, D. N., Peterson, M. N., Martin, R. A., & Langerhans, R. B. (2013). Environmental drivers of demographics, habitat use, and behavior during a post-Pleistocene radiation of Bahamas mosquitofish (Gambusia hubbsi). *Evolutionary Ecology*, 27, 971–991.

Heinen-Kay, J. L., & Langerhans, R. B. (2013). Predation-associated divergence of male genital morphology in a livebearing fish. *Journal of Evolutionary Biology*, 26, 2135–2145. https://doi.org/10.1111/jeb.12229

Heinen-Kay, J. L., Schmidt, D. A., Stafford, A. T., Costa, M. T., Peterson, M. N., Kern, E. M. A., & Langerhans, R. B. (2016). Predicting multifarious behavioural divergence in the wild. *Animal Behaviour*, 121, 3–10. https://doi.org/10.1016/j.anbehav.2016.08.016

Helfman, G. S., Collette, B. B., & Facey, D. E. (1997). *The diversity of fishes*. Blackwell Science.

Herring, G., Cook, M. I., Gawlik, D. E., & Call, E. M. (2011). Food availability is expressed through physiological stress indicators in nestling white ibis: a food supplementation experiment. *Functional Ecology*, 25, 682–690. https://doi.org/10.1111/j.1365-2435.2010.01792.x

Höglund, E., Balm, P. H. M., & Winberg, S. (2000). Skin darkening, a potential social signal in subordinate Arctic charr (*Salvelinus alpinus*): The regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. *Journal of Experimental Biology*, 203, 1711–1721.

Hossie, T. J., Ferland-Raymond, B., Burness, G., & Murray, D. L. (2010). Morphological and behavioural responses of frog tadpoles to

perceived predation risk: A possible role for corticosterone mediation? *Ecoscience*, 17, 100–108. https://doi.org/10.2980/17-1-3312

Hulthén, K., Hill, J., Jenkins, M., & Langerhans, R. (2021). Predation and resource availability interact to drive life-history evolution in an adaptive radiation of livebearing fish. *Frontiers in Ecology and Evolution*, 9, 619277. https://doi.org/10.3389/fevo.2021.619277

Jenkins, M. R., Cummings, J. M., Cabe, A. R., Hulthén, K., Peterson, M. N., & Langerhans, R. B. (2021). Natural and anthropogenic sources of habitat variation influence exploration behaviour, stress response, and brain morphology in a coastal fish. *Journal of Animal Ecology*. https://doi.org/10.1111/1365-2656.13557

Kitaysky, A. S., Piatt, J. F., & Wingfield, J. C. (2007). Stress hormones link food availability and population processes in seabirds. *Marine Ecology Progress Series*, 352, 245–258. https://doi.org/10.3354/meps07074

Kitaysky, A. S., Wingfield, J. C., & Piatt, J. F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. *Functional Ecology*, 13, 577–584.

Langerhans, R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in Gambusia affinis. *Journal of Evolutionary Biology*, 22, 1057–1075.

Langerhans, R. B. (2017). Predictability and parallelism of multitrait adaptation. *Journal of Heredity*, 109, 59–70. https://doi.org/10.1093/jhered/esx043

Langerhans, R. B., Gifford, M. E., & Joseph, E. O. (2007). Ecological speciation in Gambusia fishes. *Evolution*, 61, 2056–2074. https://doi.org/10.1111/j.1558-5646.2007.00171.x

Langerhans, R. B. (2007). Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. In: Elewa, A. M. T. (Ed.), *Predation in Organisms: A Distinct Phenomenon* (pp. 177–220). Heidelberg, Germany: Springer-Verlag.

Langerhans, R., & Rosa-Molinar, E. (2021). A novel body plan alters diversification of body shape and genitalia in live-bearing fish. *Frontiers in Ecology and Evolution*, 9, 619232. https://doi.org/10.3389/fevo.2021.619232

Lankford, S. E., Adams, T. E., Miller, R. A., & Cech, J. J. Jr (2005). The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiological and Biochemical Zoology*, 78, 599–609. https://doi.org/10.1086/430687

Marentette, J. R., Tong, S., & Balshine, S. (2013). The cortisol stress response in male round goby (*Neogobius melanostomus*): Effects of living in polluted environments? *Environmental Biology of Fishes*, 96, 723–733. https://doi.org/10.1007/s10641-012-0064-8

Martin, R. A., McGee, M. D., & Langerhans, R. B. (2015). Predicting ecological and phenotypic differentiation in the wild: A case of piscivorous fish in a fishless environment. *Biological Journal of the Linnean Society*, 114, 588–607. https://doi.org/10.1111/bij.12449

Martin, R. A., Riesch, R., Heinen-Kay, J.L., & Langerhans, R. B. (2014). Evolution of male coloration during a post-Pleistocene radiation of Bahamas mosquitofish (Gambusia hubbsi). *Evolution*, 68, 397–411.

McPeek, M. A., Grace, M., & Richardson, J. M. L. (2001). Physiological and behavioral responses tp predators shape the growth/?predation risk trade-off in damselflie. *Ecology*, 82, 1535–1545.

Oppliger, A., Clobert, J., Lecomte, J., Lorenzon, P., Boudjemadi, K., & John-Alder, H. B. (1998). Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard Lacerta vivipara. *Ecology Letters*, 1, 129–138. https://doi.org/10.1046/j.1461-0248.1998.00028.x

Pärssinen, V., Hulthén, K., Brönmark, C., Björnerås, C., Ekelund Ugge, G., Gollnisch, R., Hansson, L.-A., Herzog, S. D., Hu, N., Johansson, E., Lee, M., Rengefors, K., Sha, Y., Škerlep, M., Vinterstare, J., Zhang, H., Langerhans, R. B., & Nilsson, P. A. (2021). Variation in predation regime drives sex-specific differences in mosquitofish foraging behaviour. *Oikos, 130*, 790–797. https://doi.org/10.1111/oik.08335

Price, S. A., Friedman, S. T., & Wainwright, P. C. (2015). How predation shaped fish: the impact of fin spines on body form evolution across teleosts. *Proceedings of the Royal Society B: Biological Sciences*, 282(1819), 20151428. https://doi.org/10.1098/rspb.2015.1428

Queiroz, H., & Magurran, A. E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biology Letters*, 1, 155–157. https://doi.org/10.1098/rsbl.2004.0267

Reeder, D. M., & Kramer, K. M. (2005). Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. *Journal of Mammalogy*, 86, 225–235.

Reid, S. G., Furimsky, M., & Perry, S. F. (1994). The effects of repeated physical stress or fasting on catccholamine storage and release in the rainbow trout, *Oncorhynchus mykiss*. *Journal of Fish Biology*, 45, 365–378. https://doi.org/10.1111/j.1095-8649.1994.tb01319.x

Reznick, D. A., Bryga, H., & Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature, 346*, 357–359. https://doi.org/10.1038/346357a0

Riesch, R., Martin, R. A., & Langerhans, R. B. (2013). Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *The American Naturalist*, 181, 78–93. https://doi.org/10.1086/668597

Riesch, R., Martin, R. A., & Langerhans, R. B. (2020). Multiple traits and multifarious environments: integrated divergence of morphology and life history. *Oikos*, 129, 480–492. https://doi.org/10.1111/oik.06344

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution, 19*, 249–255. https://doi.org/10.1016/j.tree.2004.03.008

Romero, L. M., & Wikelski, M. (2001). Corticosterone levels predict survival probabilities of Galápagos marine iguanas during El Niño events. *Proceedings of the National Academy of Sciences*, 98, 7366–7370.

Samaras, A., Espírito Santo, C., Papandroulakis, N., Mitrizakis, N., Pavlidis, M., Höglund, E., Pelgrim, T. N. M., Zethof, J., Spanings, F. A. T., Vindas, M. A., Ebbesson, L. O. E., Flik, G., & Gorissen, M. (2018). Allostatic load and stress physiology in European Seabass (*Dicentrarchus labrax* L.) and Gilthead Seabream (*Sparus aurata* L.). *Frontiers in Endocrinology*, 9, 451.

Sapolsky, R. M. (1982). The endocrine stress-response and social status in the wild baboon. *Hormones and Behavior*, 16, 279–292. https://doi.org/10.1016/0018-506X(82)90027-7

Sapolsky, R. M. (1990). Adrenocortical function, social rank, and personality among wild baboons. *Biological Psychiatry*, 28, 862–878.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21, 55–89.

Schug, M. D., Downhower, J. F., Brown, L. P., Sears, D. B., & Fuerst, P. A. (1998). Isolation and genetic diversity of Gambusia hubbsi (mosquitofish) populations in blueholes on Andros Island, Bahamas. *Heredity*, 80, 336–346. https://doi.org/10.1046/j.1365-2540.1998.00302.x

Sha, Y., Zhang, H., Lee, M., Björnerås, C., Škerlep, M., Gollnisch, R., Herzog, S. D., Ekelund Ugge, G., Vinterstare, J., Hu, N., Pärssinen, V., Hulthén, K., Nilsson, P. A., Rengefors, K., Brönmark, C., Langerhans, R. B., & Hansson, L.-A. (2020). Diel vertical migration of copepods and its environmental drivers in subtropical Bahamian blue holes. *Aquatic Ecology*. https://doi.org/10.1007/s10452-020-09807-4

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychological Review*, 107, 411–429.

Tollrian, R., & Harvell, C. D. (1999). *The ecology and evolution of inducible defenses*. Princeton University Press.

Vamosi, S. M. (2005). On the role of enemies in divergence and diversification of prey: a review and synthesis. *Canadian Journal of Zoology*, 83, 894–910. https://doi.org/10.1139/z05-063

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press, Oxford.

Ydenberg, R. C., & Dill, L. M. (1986). The economics of fleeing from predators. In: J. S. Rosenblatt, C. Beer, M.-C. Busnel, & P. J. B. Slater (Eds.), *Advances in the study of behavior*, Vol. *16* (pp. 229–249). Academic Press.

Young, K. V., Brodie, E. D. Jr, & Brodie, E. D. III (2004). How the horned lizard got its horns. *Science*, *304*, 65. https://doi.org/10.1126/science.1094790

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary Material for

"Predation risk and the evolution of a vertebrate stress response: parallel evolution of stress reactivity and sexual dimorphism"

<u>**Supporting Text**</u>

Simple Conceptual Model for the Evolution of Stress Responses
We created a simple model for the expected evolution of the magnitude of the physiological stress response based solely on selection from three agents: 1) intensity of stressful encounters, 2) frequency of stressful encounters, and 3) food availability (see main text). We did this as an early guide to the possible combinatorial roles of these factors in the evolution of stress responses. Further work is needed to more fully assess their influence.

We assumed that these three factors, and only these three factors, fully explain the evolution of the stress response. First, the intensity of stressful encounters describes the potential severity of the consequence for the average encounter within a given environment. For instance, a stressful encounter's potential consequence could range from energy expenditure to lost feeding or mating opportunities to injury to death. Thus, failure to appropriately respond to a stressful encounter could typically result in minor fitness consequences in some environments, but death in others. We assumed that selection would more strongly favor a large-magnitude stress response when encounters were more intense. Second, owing to the various costs of mounting a stress response (e.g. Gregory & Wood, 1999, McPeek et al., 2001, Sapolsky et al., 2000, Cooke et al., 2003, Romero, 2004, Hawlena & Schmitz, 2010, Oppliger et al., 1998, Busch et al., 2008), the relative fitness benefits of strongly reacting to stressful events should generally decrease with increasing frequencies of stressful encounters. That is, animals cannot afford to mount large stress responses with each encounter if encounters occur with great regularity, assuming energy is finite and limited. Third, as high-quality food becomes less readily available, selection should favor reduced magnitudes of stress responses owing to the lower availability of energy to fuel the responses and other needs (maintenance, growth, reproduction). While low levels of food could induce higher stress levels (e.g. Kitaysky et al., 2007, Herring et al., 2011), here we are concerned with how selection might shape the evolution of the stress response and not patterns of phenotypic plasticity—indeed, future work might investigate possible counter-gradient variation for stress responses across food-availability gradients.

To explore the parameter space of the predicted evolution of stress responses for these three factors, we examined a range of values designed to capture considerable variation from low to high levels of each factor. For each factor, we spanned the arbitrary range of 0 to 1, representing low to high levels, and assigned a particular selection value to these levels assuming linear relationships between the level of each factor and the corresponding selection for stress response experienced by a population. We calculated the expected magnitude of the stress response as the product of the three selection values (Intensity, Frequency, and Food Availability), and visualized this surface using contour plots (i.e. expected stress response magnitude represented by the Z axis).

For intensity, we assumed that the benefits of the stress response increased linearly with increasing intensity, with selection values ranging from 1 to 2 along the intensity axis. Thus, selection favoured a larger magnitude of stress response under higher intensity. For frequency, we assumed that the accumulative costs of mounting stress responses increased with the frequency of encounters, resulting in a negative linear relationship between

frequency and selection favouring stress responses—we used selection values ranging from 1 to 0.1 along the frequency axis. For food availability, we assumed that the energetic constraints on stress responses scaled proportionally with the availability of food, with selection values ranging from 0 to 1 along the food-availability axis.

This simple model revealed several important patterns for the expected evolution of the stress response (Fig. S4). First, it unsurprisingly indicated that the largest magnitude of stress responses should evolve in environments with low frequencies and high intensities of stressful encounters that have high food availability, while the lowest magnitude of stress responses should evolve in the opposite combination of factors. However, it also showed that the greatest amounts of divergent evolution in the magnitude of the stress response across environments with different frequencies and intensities of stressful encounters should occur in environments with high food availability, while very little variation in stress response should evolve among environments with low food availability (Fig. S4). It also indicated that food availability has little influence on the evolution of the magnitude of stress response in environments with high frequencies of stressful encounters, especially when the average encounter has a low intensity (Fig. S4: little change in right-side, and especially the bottom-right corner, across figure panels).

To place these findings into the context of the present study system, we assumed that Bahamas mosquitofish inhabiting blue holes with predatory fish reside in environments with a high frequency of relatively intense stressful encounters, while low-predation populations reside in environments with a low frequency of stressful encounters that generally have a low-moderate intensity, and both of the predation regimes span a range of food availability (see HP and LP depictions in Fig. S4; Fig. S5). For this illustration, we used frequency values of 0.1 (LP) and 0.85 (HP), and intensity values of 0.35 (LP) and 0.85 (HP). This means that 1) high-predation populations are expected to evolve relatively low magnitudes of stress responses with relatively little divergence between environments that vary in food availability, while 2) low-predation populations are expected to evolve a low magnitude of stress response when food is scarce, but relatively large stress responses under high food availability (Fig. S4, S5). These qualitative expectations correspond well to the patterns observed in Bahamas mosquitofish in this study (see text).

Supporting References

Busch, D. S., Sperry, T. S., Peterson, E., Do, C. T., Wingfield, J. C. & Boyd, E. H. 2008. Impacts of frequent, acute pulses of corticosterone on condition and behavior of Gambel's white-crowned sparrow (*Zonotrichia leucophtys gambelii*). *General and Comparative Endocrinology* **158**: 224-233.

Cooke, S. J., Steinmetz, J., Degner, J. F., Grant, E. C. & Philipp, D. P. 2003. Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. *Canadian Journal of Zoology* **81**: 699-709.

Gregory, T. R. & Wood, C. M. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72**: 286-295.

Hawlena, D. & Schmitz, O. J. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *American Naturalist* **176**: 537-556.

Herring, G., Cook, M. I., Gawlik, D. E. & Call, E. M. 2011. Food availability is expressed through physiological stress indicators in nestling white ibis: a food supplementation experiment. *Functional Ecology* **25**: 682-690.

Kitaysky, A. S., Piatt, J. F. & Wingfield, J. C. 2007. Stress hormones link food availability and population processes in seabirds. *Marine Ecology Progress Series* **352**: 245-258.

McPeek, M. A., Grace, M. & Richardson, J. M. L. 2001. Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. *Ecology* **82**: 1535-1545.

Oppliger, A., Clobert, J., Lecomte, J., Lorenzon, P., Boudjemadi, K. & John-Alder, H. B. 1998. Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. *Ecology Letters* **1**: 129-138.

Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution* **19**: 249-255.

Sapolsky, R. M., Romero, L. M. & Munck, A. U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* **21**: 55-89.

Figure S1. Video snapshot from a trial, depicting the measurement of body size (standard length) of each fish as the distance between the two landmarks illustrated.
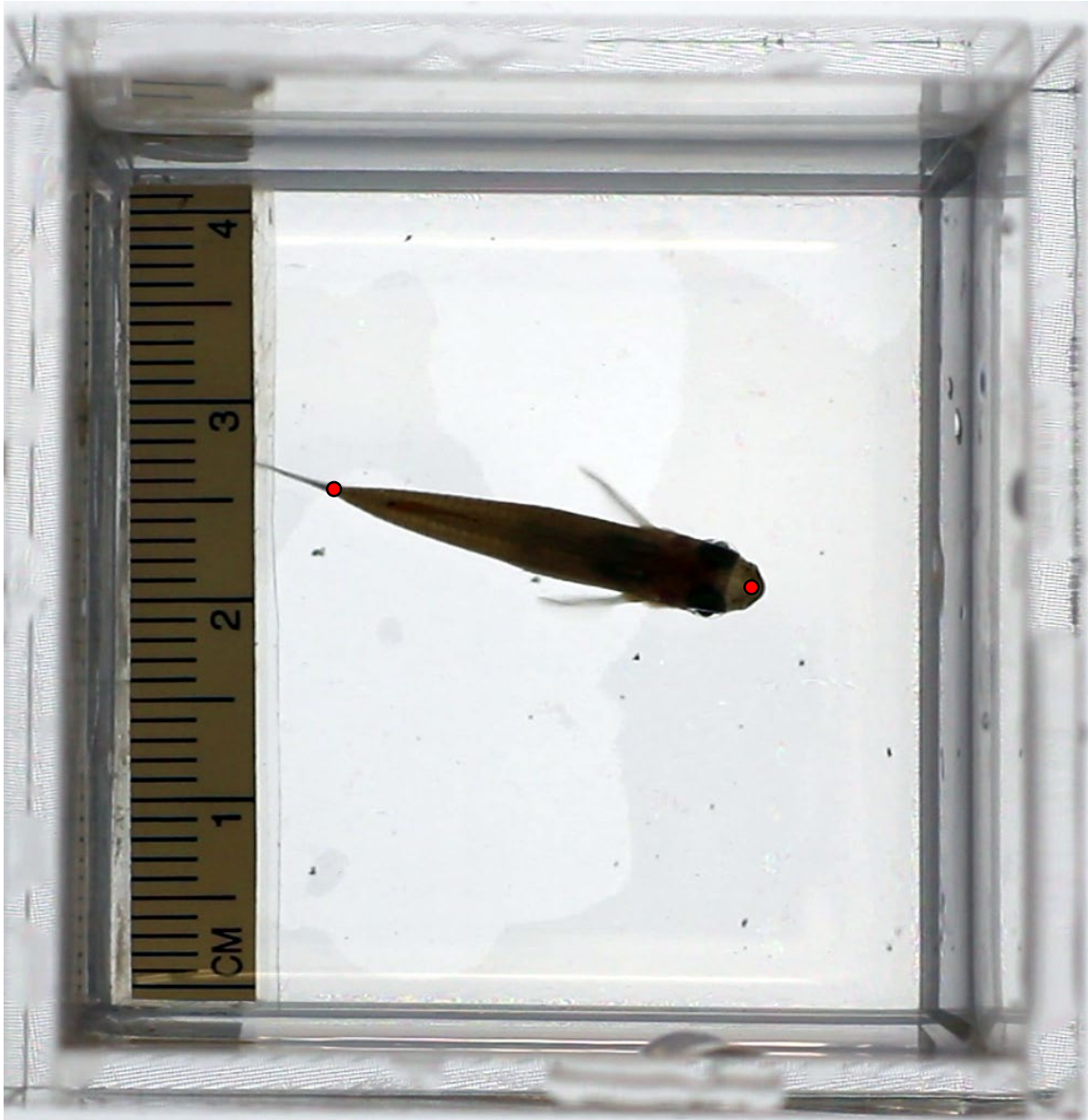
Figure S2. Linear regression of average ventilation frequency on $\log_{10}$-transformed standard length (mm) across all fish in the study, illustrating the body size dependency of ventilation frequency. Linear regression: Mean Ventilation Frequency = -183.76 × $\log_{10}$ SL + 485.47.
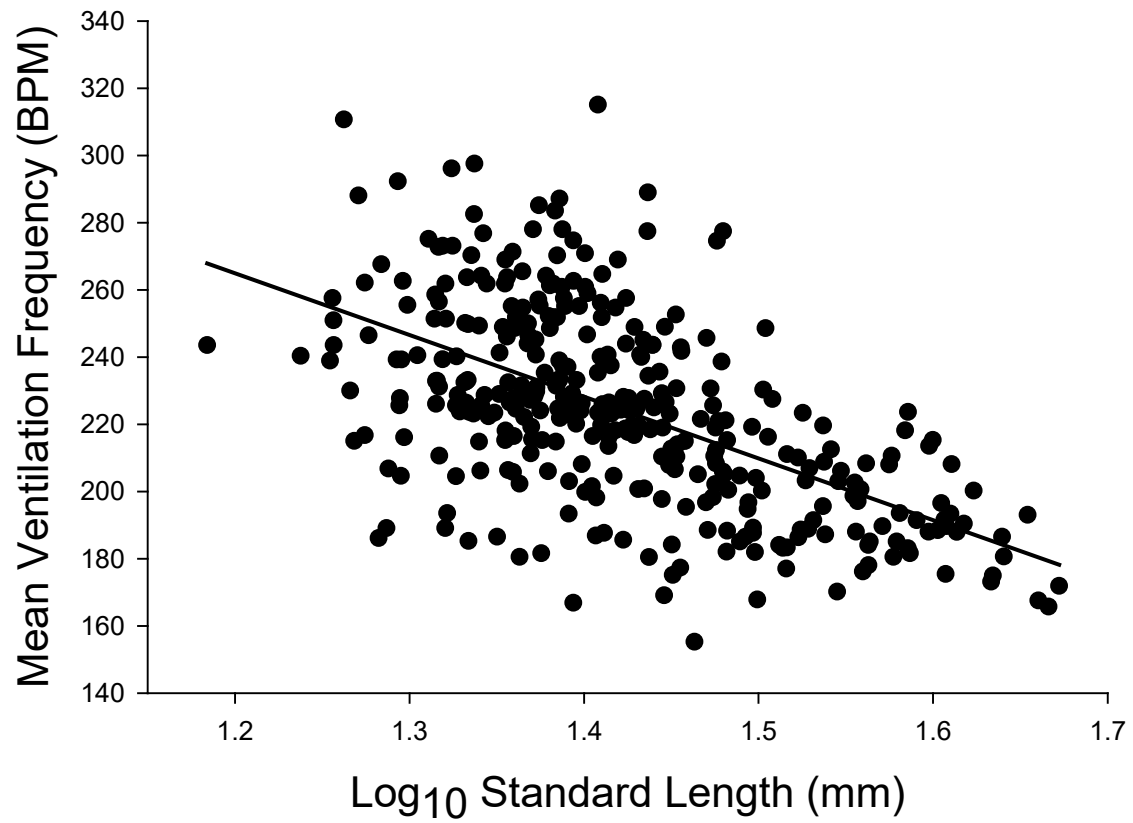
Figure S3. Variation in recovery ventilation frequency for females (square) and males (circle) in all populations of Bahamas mosquitofish examined in the wild and after two generations of laboratory rearing (least-squares means ± 1 SE depicted; population abbreviations follow Fig. 1 and Table S1). Light shaded symbols: population means, dark shaded symbols: predation-regime means.
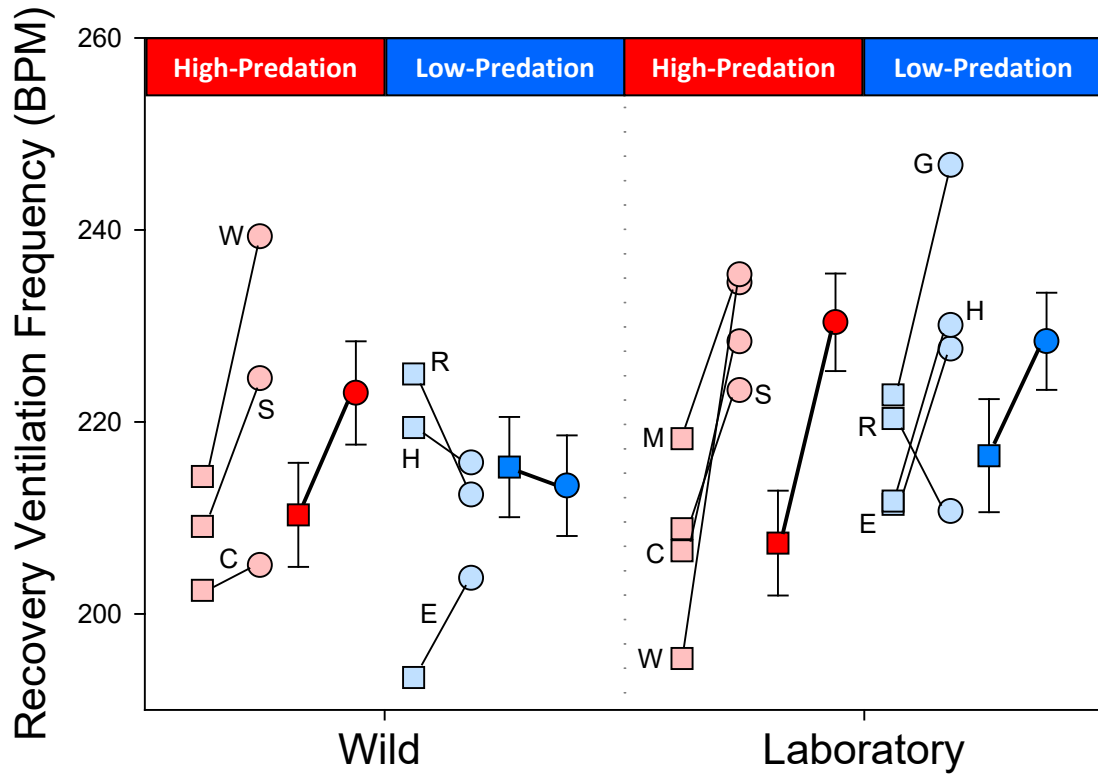
Figure S4. Predictions for the evolution of the magnitude of the stress response (Z axis, elevation) under varying intensity and frequency of stressful encounters in environments of varying food availability based on our simple model. Approximate locations of low-predation (LP: 0.1 Frequency, 0.35 Intensity) and high-predation (HP: 0.85 Frequency, 0.85 Intensity) populations of Bahamas mosquitofish depicted.
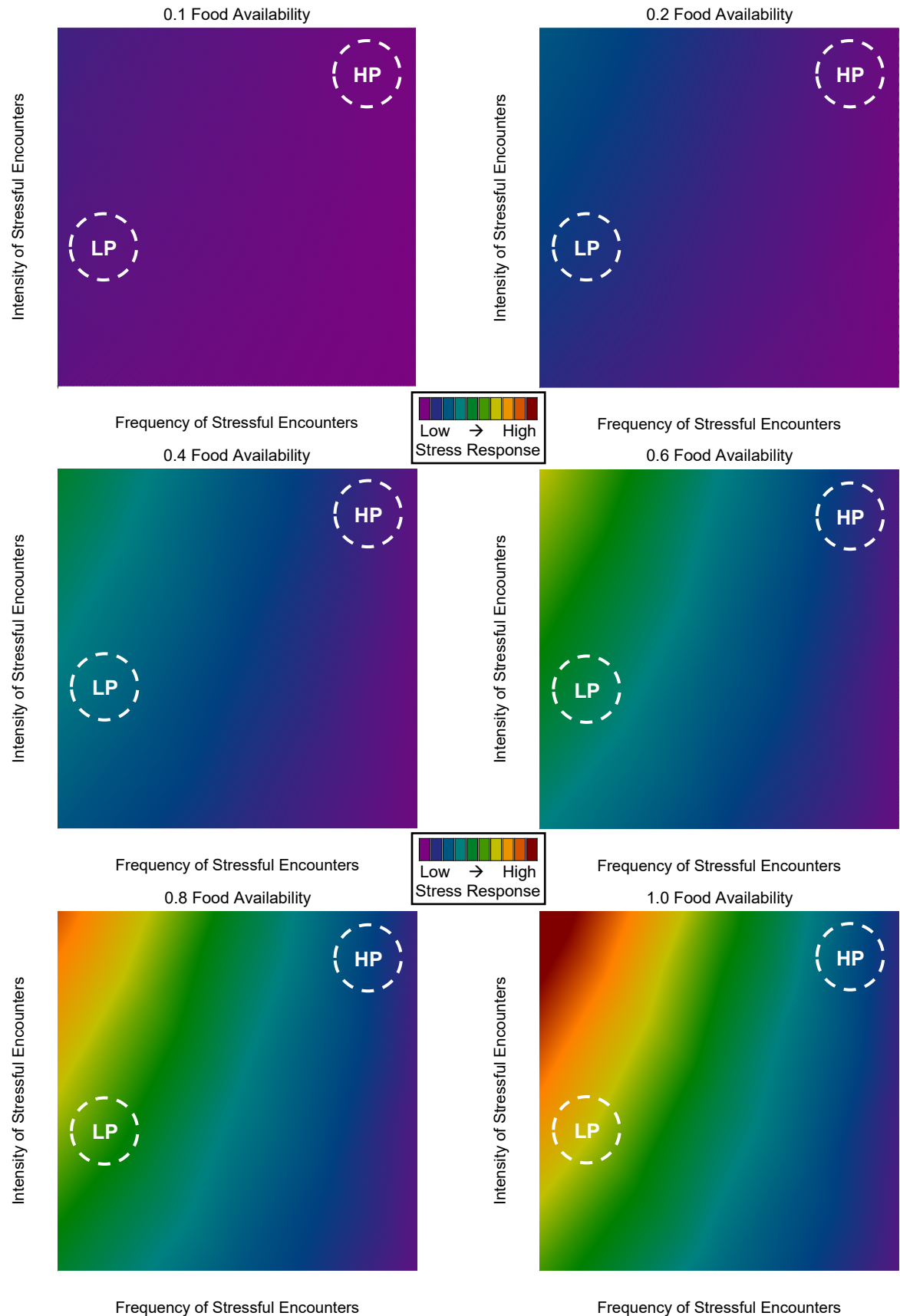
Figure S5. Evolutionary predictions for the magnitude of the stress response in low-predation (blue-shaded region: 0.0-0.2 Frequency, 0.25-0.45 Intensity) and high-predation (red-shaded region: 0.75-0.95 Frequency, 0.75-0.95 Intensity) populations based on our simple model.
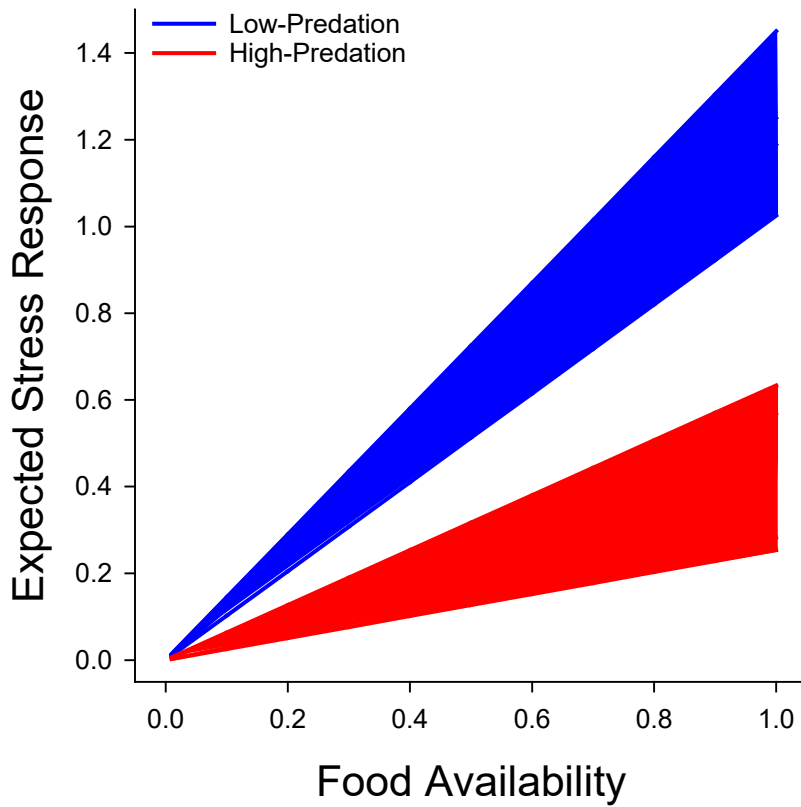
Supplementary Videos

Video S1. Representative video of a stress-response assay performed in the field. Specifically, this video shows a male mosquitofish from the high-predation Cousteau blue hole.

Table S1. Summary of sample sizes of wild-caught fish (WC, $n = 176$) examined in the field from 6 populations, and second-generation lab-raised fish (F2, $n = 171$) from 8 populations examined in the lab at North Carolina State University. For trials conducted outside in the field for wild-caught fish, we provide the date(s), time range of trials (start of first trial to end of latest trial), and average estimated air temperature (based on hourly air temperature in Nassau, The Bahamas).

Predation Regime	Population	Date(s)	Avg. Air Temp. (°C)	Time of Day	WC Fish F	WC Fish M	F2 Fish F	F2 Fish M
High predation	Cousteau (C)	27 Feb, 3 Mar 2018	25.8	10:41 – 15:01	20	20	10	10
No predation	Murky Brown (M)				0	0	11	12
	Stalactite (S)	2 Mar 2018	29.4	12:26 – 16:59	12	9	12	10
	West Twin (W)	6 Mar 2018	26.2	10:44 – 14:49	7	14	10	10
	East Twin (E)	5 Mar 2018	25.0	10:29 – 15:34	15	15	12	10
	Gollum (G)				0	0	10	10
	Hubcap (H)	1 Mar 2018	27.6	9:43 – 15:14	17	13	11	11
	Rainbow (R)	28 Feb, 7 Mar 2018	27.3	9:42 – 15:42	14	20	11	11

Table S2. Results from mixed-model repeated-measures ANCOVA examining variation in ventilation frequency of Bahamas mosquitofish across the six time points of the mild stressor assays, excluding two populations (Gollum and Murky Brown) that were only examined in the laboratory (not the field). Results are similar to the main text (see Table 1), but with reduced statistical power (e.g. slightly higher P values; only qualitative difference being the lack of any evidence for the PR $\times$ Sex $\times$ ENV effect). Population nested within predation regime was included as a random effect.

Effect	df	F	P
Time	5, 960	0.66	0.6506
Log_{10} Standard Length (SL)	1, 2063	217.49	**< 0.0001**
Predation Regime (PR)	1, 7.95	0.05	0.8354
Sex	1, 2058	96.17	**< 0.0001**
Rearing Environment (ENV)	1, 2070	0.46	0.4977
PR $\times$ Sex	1, 2056	22.73	**< 0.0001**
PR $\times$ ENV	1, 2063	2.28	0.1314
Sex $\times$ ENV	1, 2059	12.88	**0.0003**
PR $\times$ Sex x ENV	1, 2056	0.15	0.6983
SL $\times$ Time	5, 960	0.49	0.7841
PR $\times$ Time	5, 960	1.99	**0.0772**
Sex $\times$ Time	5, 960	1.87	**0.0972**
ENV $\times$ Time	5, 960	0.98	0.4260
PR $\times$ Sex $\times$ Time	5, 960	0.66	0.6533
PR $\times$ ENV $\times$ Time	5, 960	1.21	0.3005
Sex $\times$ ENV $\times$ Time	5, 960	0.2	0.9615
PR $\times$ Sex $\times$ ENV $\times$ Time	5, 960	0.45	0.8118

Table S3. Results from general linear mixed models examining variation in the scope of ventilation frequency (i.e. magnitude of the stress response) and the recovery ventilation frequency (i.e. relative baseline ventilation frequency soon after a startling experience), excluding two populations (Gollum and Murky Brown) that were only examined in the laboratory (not the field). Results are similar to the main text (see Table 2), but with reduced statistical power (e.g. slightly higher P values). Population nested within predation regime was included as a random effect.

Effect	Scope of Ventilation Frequency			Recovery Ventilation Frequency		
	df	*F*	*P*	*df*	*F*	*P*
Log$_{10}$ Standard Length (SL)	1, 292.80	4.18	**0.0419**	1, 294.99	45.17	**< 0.0001**
Predation Regime (PR)	1, 4.03	1.29	0.3187	1, 4.08	0.01	0.9142
Sex	1, 291.71	1.47	0.2270	1, 293.36	11.73	**0.0007**
Rearing Environment (ENV)	1, 292.52	7.25	**0.0075**	1, 294.92	1.23	0.2682
PR × Sex	1, 291.30	0.01	0.9349	1, 292.08	8.49	**0.0038**
PR × ENV	1, 291.41	7.23	**0.0076**	1, 292.50	0.88	0.3498
Sex × ENV	1, 291.82	0.08	0.7780	1, 293.71	3.53	**0.0613**
PR × Sex x ENV	1, 291.25	1.78	0.1835	1, 291.92	0.04	0.8331

Table S4. Results of general linear mixed models examining variation in the scope of the ventilation frequency in our stress-response assays in Bahamas mosquitofish. Population nested within predation regime was included as a random effect.

Effect	Wild-caught Fish			F2 Lab-raised Fish		
	df	*F*	*P*	*df*	*F*	*P*
Log$_{10}$ Standard Length (SL)	1, 168.46	0.72	0.3963	1, 135.30	5.12	**0.0252**
Predation Regime (PR)	1, 2.25	0.77	0.4645	1, 4.09	0.60	0.4821
Sex	1, 168.61	1.57	0.2121	1, 156.00	0.63	0.4271
PR × Sex	1, 168.54	0.64	0.4256	1, 161.28	3.77	**0.0539**
Log$_{10}$ Zooplankton Density (Z)	1, 2.03	3.09	0.2189	1, 3.80	29.45	**0.0065**
Z × PR	1, 2.10	0.00	0.9792	1, 3.81	30.53	**0.0060**