

Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure

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Abstract Individual animals of the same species inhabiting environments which differ in the frequency and magnitude of stressors often exhibit different physiological and behavioral responses to stressors. Here, we compare the respiratory response to confinement stress, and behavioral responses to ecologically relevant challenges among sticklebacks from 11 different populations varying in predation pressure. We found that sticklebacks from high predation populations breathed faster in response to confinement stress and were, on an average, more behaviorally responsive to a pike behind glass compared with sticklebacks from low predation populations. These patterns differ from the results of studies on other species, highlighting the need for a conceptual framework to understand the proximate and ultimate factors shaping variable responses to stressors over developmental and evolutionary time. Moreover, physiological and behavioral responses were integrated with each other, both at the individual and population levels. In general, fish that were more aggressive and bold in the presence of a predator breathed faster, independent of body size. These results are consistent with the growing body of evidence that individuals differ in a suite of physiological and behavioral mechanisms for coping with challenges in the environment.

Keywords Aggression · Antipredator behavior · Behavioral syndrome · Geographic variation · Personality · Ventilation rate

Introduction

Not every individual within a species responds to challenges in the same way. When confronted by a predator, for example, some individuals rapidly mobilize a physiological stress response—levels of catecholamines increase in the brain, the animal breathes faster and glucocorticoids are released—while other individuals show a weaker physiological response, or recover faster once the stressor is removed (Cockrem and Silverin 2002b; Ebner et al. 2005; Koolhaas et al. 1999; Koolhaas et al. 1997; Wada et al. 2008). In some cases, intraspecific variation in stress responses can be attributed to differences in body size (e.g., Bell et al. 2007), sex (e.g., Pottinger et al. 1995), age (Heidinger et al. 2006; Otte et al. 2005) and experience (e.g., Bartolomucci et al. 2005; Sloman et al. 2001), or genetic background (e.g., Evans et al. 2006; Pottinger and Carrick 1999). There is also growing evidence that geographic variation in the magnitude and predictability of stressors in the environment is associated with population differences in stress responses. For example, chickadees from disturbed versus undisturbed areas (Lucas et al. 2006), or lizards at the periphery versus the center of the range (Dunlap and Wingfield 1995) differ in physiological responses to stressors (see also Muller et al. 2007).

Three-spined stickleback fish (*Gasterosteus aculeatus*) are especially well-suited for testing the hypothesis that animals inhabiting environments that vary in risk show contrasting physiological responses to stressors. Marine three-spined sticklebacks colonized freshwater environments

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in the northern hemisphere at the retreat of the last glaciers ~12,000 years before present. The radiation created a naturally replicated experiment of independently derived freshwater populations that evolved similar phenotypes in parallel in response to similar selective pressures (Bell and Foster 1994). The replication, therefore, provides an opportunity to compare not just two phenotypically divergent populations, but multiple, independent populations.

Predators are one of the most important selective pressures for sticklebacks, especially when they are small or young. Evolutionary responses to selection by predators have shaped morphological (Reimchen 1994, 2000) and behavioral (Huntingford et al. 1994) traits. Sticklebacks inhabiting water bodies where there are abundant predators ('high predation populations') have more pronounced defensive morphology such as lateral plate armoring and long, serrated spines (reviewed in Reimchen 1994) as well as heightened antipredator behavioral responses compared with sticklebacks from populations with fewer predators in the environment ('low predation populations') (reviewed in Huntingford et al. 1994). Given the important selective pressure exerted by predators, and that predators are stressors (Bell et al. 2007; Blanchard et al. 1998; Carere et al. 1999; Cockrem and Silverin 2002a; Eilam et al. 1999; Scheuerlein et al. 2001), we hypothesize that sticklebacks from high predation populations show contrasting physiological and behavioral responses to stress compared with sticklebacks from low predation populations.

But how should we expect physiological indicators of stress to differ between high and low predation populations? On the one hand, we might hypothesize that sticklebacks from high predation populations will be chronically stressed, and therefore, show continuously increased indicators of stress compared with sticklebacks from low predation populations, even in a 'nonstressed', baseline condition (as in Bonier et al. 2007; Boonstra et al. 1998; Hik et al. 2001). However, chronic exposure to elevated stress hormones such as cortisol has deleterious consequences for health (Sapolsky et al. 2000), so chronic activation of the hypothalamic–pituitary–interrenal (HPI) axis would be selected against in high predation populations (McEwen and Wingfield 2003).

It is more likely that sticklebacks from high predation populations experience a higher frequency of acutely stressful encounters than fish from low predation populations. That is, considering that predators come and go (Lima and Bednekoff 1999), the frequency of encounters with predators will be greater in high predation environments and thus the frequency with which the HPI axis (and sympathetic system) is activated will also be greater than in a low predation environment. Therefore, natural selection by predators might have favored the ability to mount an appropriate acute response to risk. Indeed, several studies

have shown that animals from more dangerous environments show higher acute stress-induced levels of glucocorticoids (Dunlap and Wingfield 1995; Lucas et al. 2006).

Finally, it is possible that animals that have repeatedly been exposed to stressors in the environment (such as in high predation populations) show an attenuated physiological response to stress due to habituation (Brown et al. 2005a; Caldji et al. 2000; Espmark and Langvatn 1985; Romero 2004). Indeed, we know that in trout, repeat exposure to mild stressors can result in acclimation, whereby the animal no longer responds to the stressor because it no longer perceives the stressor as threatening (Pickering and Pottinger 1985). However, if the stressor remains a perceived threat, repeated exposure may result in facilitation, where the response to the original and novel stressors is enhanced (Romero 2004). Our aim in this paper is to generate information that bears on these alternatives by comparing multiple high and low predation populations of sticklebacks. We wish to know whether sticklebacks from high predation populations, which presumably have been repeatedly exposed to predator-induced stress, show a greater or lesser response to challenges compared with sticklebacks from low predation populations.

A sensitive measure of the physiological response to stress is ventilation rate. Ventilation rate is a particularly useful index of stress because it can be measured noninvasively, and, therefore, repeatedly on the same individuals, although its sensitivity as a measure of disturbance means that caution must be exercised in its use (Barreto and Volpato 2004). Ventilation rate is a sympathetic response, which quickly increases in response to stressors (Priede 1985), including predators (Barreto et al. 2003; Cooke et al. 2003; Hawkins et al. 2004; Hojesjo et al. 1999; Johnsson et al. 2001; Metcalfe et al. 1995; Sundstrom et al. 2005), possibly in preparation for a flight response. Baseline ventilation rate, stress-induced ventilation rate and metabolic rate have been linked to different behavioral tendencies (Johnsson et al. 2001; Sloman et al. 2000; Verbeek et al. 2008). For example, individual Atlantic salmon with higher standard metabolic rates tend to be more dominant (Cutts et al. 1998), while 'reactive' personality types have higher parasympathetic responses in great tits (Carere et al. 2003; Carere and Van Oers 2004) and mice (Koolhaas et al. 1999; Veenema et al. 2003). Indeed, individual differences in metabolic rate have recently been proposed as an anchor underlying personality variation (Careau et al. 2008).

Here, we assess whether sticklebacks from a series of populations exposed to high and low predation pressure by fishes exhibit systematic differences in key behavioral and respiratory responses to stressful stimuli. At the same time, we ask whether individual differences in all of our measures are correlated with each other, i.e., when individuals that

are especially behaviorally responsive to the threat of predation or aggression are also particularly physiologically responsive to a different stressor at a different point in time. Finally, we assess whether the observed behavioral reactions (and correlated physiological response) are likely to represent a stable characteristic of each individual.

Methods

Overview

We measured the stress-induced ventilation rate on individual subadult three-spined sticklebacks from 11 different populations varying in predation pressure by fishes. Individual behavioral responses to an unfamiliar conspecific and to a predator were measured 1 day later.

Sticklebacks were collected in Scotland between 11 August and 25 September 2004 (see Malhi et al. 2006 for geographic information about the sites). The intensity of predation by piscivorous fish (pike, perch, brown trout, rainbow trout, eels and arctic charr) was assessed using historical records and whole-loch seine surveys. We expect that all of the freshwater populations have some shared evolutionary history with pike because pike are widely distributed throughout the region. Eleven different freshwater populations were classified as ‘low predation’ if they did not contain any piscivorous fishes, and were classified as ‘high predation’ if piscivorous fishes were detected (Table 1). Although sticklebacks are also subject to predation by birds and invertebrate larvae, those predators can move between water bodies, making it difficult to determine predation pressure by those predators. Previous work has shown that the populations are genetically differentiated

from each other and are approximately 15,000 years (Malhi et al. 2006).

Experiments were carried out at the Glasgow University Scottish Centre for Ecology and the Natural Environment (SCENE), Rowardennan. Groups of fish ($n = 10\text{--}40$) were maintained in separate flow-through stock tanks (210 L, 90-cm diameter \times 33-cm-deep). All of the fish were allowed to adjust to the laboratory for at least 1 month before their behavior was observed. The water temperature in the stock tanks was $9 \pm 2^\circ\text{C}$. The photoperiod was 14L:10D. Fish were fed with frozen bloodworms ad lib daily except on the day of observation, when they were unfed.

Behavioral observations took place between 2 October and 15 December, 2004, when the fish were approximately 3–5 months of age. None of the fish were sexually mature. Glass aquaria were located inside in a U-shaped flume containing water from Loch Lomond at $10 \pm 1^\circ\text{C}$.

Four aquaria in the flume were used for behavioral observations (‘observation tanks’, 40 L, $48 \times 32 \times 26$ cm). Exterior lines on the tanks were divided into 16 equally sized areas. All the observation tanks were surrounded by opaque screens on three sides and were situated next to a window in the flume so that the behavior of the fish could be observed. The windows were covered by a blind with a small opening which allowed the observer to see through the window with minimal disturbance to the fish. Each observation tank contained a plastic plant and a length of pipe (12 cm diameter, 36-cm-tall) that stood vertically on one side of the tank and allowed fish to be introduced into the tank with a minimum of disturbance.

The observation tanks were situated in different compartments (402 L, $220 \times 63 \times 29$ cm) on either arm of the flume. Each of the two compartments contained one of two live pike (*Esox lucius*, 46, 41 cm standard length) and cloth plants that served as hiding places for the pike. The compartments were fitted with a removable opaque cover that created a dark, shaded area for the pike. The pike rarely left the sheltered area unless the cover and hiding places were removed. The pike were caught by hook and line on 5 February 2004 in the Dubh Lochan near the SCENE Field Station. The two pike were fed dead minnows and dead sticklebacks ad libitum.

Measuring stress-induced ventilation rate

A stickleback was removed from the stock tank and placed in a 1-L beaker filled with water. Opercular beats were immediately counted from an overhead position every 15 s for 1 min producing four repeated measures of stress-induced ventilation. For comparison with baseline ventilation rate, we also report overall stress-induced ventilation per minute. Then, the fish was placed into an observation tank for one night to acclimate to the flume

Table 1 Description of the populations

Population	Location	Confirmed fish predators
Carbeth	Near Glasgow	Brown trout, rainbow trout
Balmaha	Near Glasgow	
Balvormie	Near Edinburgh	
Blackwaterfoot	Isle of Arran	Brown trout
Eliburn	Near Edinburgh	Pike, perch
Greenan	Isle of Bute	
Machrie	Isle of Arran	
Mealt	Isle of Skye	Arctic charr
Mugdock	Near Glasgow	
Quien	Isle of Bute	Brown trout
Storr	Isle of Skye	Brown trout, eel

The ‘location’ refers to the general geographic location of the population in Scotland

and behavioral observations commenced the following morning.

Measuring baseline ventilation rate

To verify that the observed differences in ventilation rate reflected a response to stress, we measured baseline ventilation rate on undisturbed sticklebacks from a subset of the populations. During normal holding conditions (10°C), five individuals from each of six of the populations (Balmaha, Balvormie, Blackwaterfoot, Carbeth, Eliburn and Machrie) were measured for ventilation rate (beats/min) by eye.

Measuring behavioral responses to an unfamiliar conspecific intruder

We employed a procedure that was designed to simulate a challenge to the resident fish by an intruding conspecific (Bell et al. 2007). A live conspecific (approximately 10% smaller than the resident fish) was removed from a holding tank (49 L, 61 × 31 × 26 cm) containing the 6–7 different fish that were used as intruders. Each intruder was allowed at least 30 min to recover between tests. Different intruders reacted similarly to this situation, and we observed no effect of repeated testing on the behavior of intruders and no trends in behavioral outcomes were evident in successive trials with the same intruder (Bell, personal observation). The intruder was placed into the observation tank with the resident fish. The number of times the resident nipped at the intruder ('nips'), the total time orienting to the intruder ('time orienting') and the number of times the resident raised its dorsal spines ('spines') was recorded for 5 min after the resident first oriented to the intruder. After the behavioral observation, the intruder was removed from the tank and the resident fish was presented with the pike 4 h later.

Measuring behavioral responses to a predator

This procedure was designed to simulate a potential predatory threat by a live pike. A chamber to contain the pike (61 × 32 × 22 cm) was added to the flume and positioned lengthwise next to the observation aquarium. The chamber had an opaque top and bottom and clear sides. One of the sides had a removable door. A removable opaque divider was situated between the observation aquarium and the predator chamber. Prior to observing an individual stickleback's behavior, the pike was transferred into the chamber by removing the cover over the pike compartment of the flume and opening the door to the chamber. In general, the pike willingly swam into the chamber, seeking cover. After entering the chamber, the door was closed and 500 mL of water from the pike's holding area was added to the aquarium, providing olfactory cues of predation risk.

The divider separating the observation aquarium from the chamber was gently removed allowing the stickleback to have a clear view of the pike. The behavior of the individual stickleback was observed for 5 min after the divider was removed and the following behaviors were recorded: the number of times the focal fish moved into a different area of the tank (moves), the number of times the focal fish raised its dorsal spines (spines), the number of times the focal fish swam up to the mouth of the pike (inspection) and total time orienting toward the pike (time orienting). After the behavioral observation, the opaque divider separating the chamber from the observation aquarium was replaced. To eliminate any olfactory cues that might affect subsequent behavioral observations, the water in each of the observation tanks was replaced after each behavioral observation.

After behavioral testing, the fish were measured for weight and a small amount of tissue from the tail fin was placed in 80% ethanol for DNA extraction for sex determination. DNA was extracted from each fin clip and genetic sex was determined by genotyping each individual for a male-specific genetic marker validated for sticklebacks (Peichel et al. 2004).

Estimating repeatability

To test whether individual differences in behavioral reactions to the pike were stable across time, a small subset of individuals ($n = 4-7$) from one of the populations (Balmaha) was assayed for their reaction to the pike on several occasions (3–7) over 4 months with at least 7 days between observations.

All of the procedures were carried out according to the institutional guidelines and in accordance with the UK Animals (Scientific Procedures) Act of 1986.

Data analysis

To compare the time course of stress-induced ventilation rate, a repeated measures model was used with the following factors: weight, predation pressure (high or low predation) and population (nested within predation pressure). General linear models were used to compare behavior. Many of the behavioral variables were not normally distributed or were heteroscedastic, but were resistant to transformation. Although GLM is relatively robust to violations of normality and homoscedasticity (Zar 1999), we also report the results of the nonparametric Mann–Whitney U test comparing high and low predation populations. Because we did not have a priori predictions about causal relationships between stress-induced ventilation rate and behavior, we used partial correlations (controlling for population and length) to look for relationships between the different

responses across all the individuals, and Spearman correlations to detect relationships among population means. The sequential Bonferroni procedure was applied to correct for multiple comparisons (Rice 1989). Repeatability of each of the behaviors was estimated as in Lessells and Boag (1987). All analyses were carried out in SPSSv16, and all tests are two-tailed.

Results

On an average, 16 individuals (range = 3–20) per population were measured for stress-induced ventilation frequency, and 19 individuals (range = 18–20) per population were measured for behavior. Fish from low predation populations were larger than fish from high predation populations (weight: 0.50 ± 0.19 g, $n = 98$ vs. 0.38 ± 0.15 g, $n = 120$; length: 33.76 ± 5.76 mm vs. 30.44 ± 3.83 mm). There were no detectable sex differences in stress-induced ventilation rate, behavior or body size, therefore sex was not considered in subsequent analyses. Stress-induced ventilation rate was not related to body size (Table 2).

Do high and low predation populations differ in baseline ventilation rate?

Baseline ventilation rate was 72 beats/min (standard deviation = 6.21, $n = 30$). We did not detect population variation in baseline ventilation rate (population nested within predation pressure: $F_{1,24} = 2.055$, $P > 0.05$), or a significant difference between high and low predation populations ($F_{1,4} = 0.265$, $P > 0.05$).

Does stress-induced ventilation rate differ between high and low predation populations?

Stress-induced ventilation rate was highest during the first 15 s following transfer to the beaker, and then decreased over the next three 15-s intervals (Fig. 1). The average number of opercular beats during the 1-min observation period was 108 beats, with a maximum of 174 beats/min,

Table 2 Between-subjects effects on repeated measures of stress-induced ventilation rate

Source	df	F	Sig.
Intercept	1	476.01	0.000
Weight	1	0.59	0.442
Population (predation pressure)	9	7.21	0.000
Predation pressure	1	6.84	0.010
Error	159		

The effect of population was tested by nesting it within high or low predation pressure

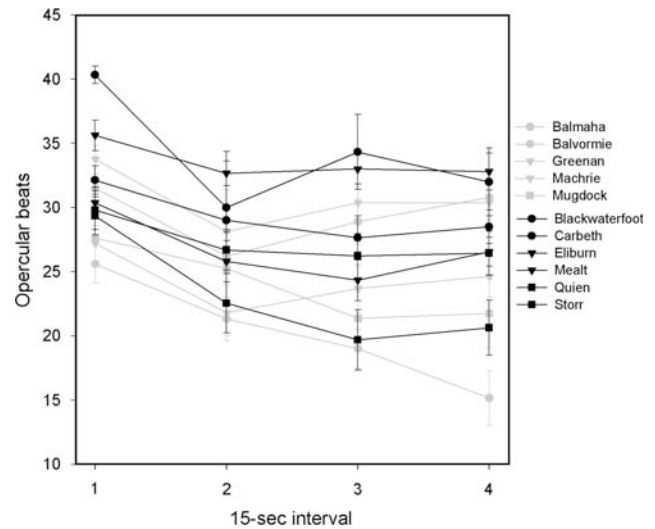


Fig. 1 The number of opercular beats in the four different 15-s intervals (op1-4) as a function of population type. Low predation populations are in gray, high predation populations are in black. Values show mean \pm SE

which is comparable to stress-induced ventilation rates in other fishes (see Brown et al. 2005a).

There was substantial among-population variation in stress-induced ventilation rate, some of which could be attributed to predation pressure: fish from populations with fish predators breathed faster than fish from low predation populations (Fig. 1; Table 2). There was not a significant between-population difference in the time course of the response (sphericity assumed, time \times predation pressure $F_{3,477} = 0.114$, $P = 0.952$). Therefore, for simplicity, we focus on the number of opercular beats during the first 15-s interval (peak rate).

Does behavior vary between high and low predation populations?

When presented with an unfamiliar conspecific, some individuals nipped at the intruder as many as 39 times in 5 min, while other individuals did not nip at all. Similarly, individuals differed in the frequency with which they raised their spines and oriented toward the intruder. Overall, there were significant between-population differences in the aggressive responses toward the intruder, independent of body size (Table 3). However, there was not a detectable systematic difference in the one aggressive behavior of fish from populations with or without piscivorous fishes according to either the GLM or Mann–Whitney U test (Tables 3, 5).

Individuals also showed variable responses to the pike predator. Although some ($n = 63$) individuals went up to the mouth of the pike and inspected it, other individuals hardly moved at all in the presence of the predator behind glass. Some of the variation among individuals could be

Table 3 Effects of population (predation pressure), predation pressure and body size on aggressive behaviors

Source	Dependent variable	df	F	Sig.
Intercept	Spines	1	8.32	0.004
	Nips	1	2.31	0.131
	Time orienting	1	60.44	0.000
Weight	Spines	1	0.57	0.453
	Nips	1	0.20	0.659
	Time orienting	1	5.24	0.023
Population (predation pressure)	Spines	9	2.95	0.003
	Nips	9	4.91	0.000
	Time orienting	9	4.82	0.000
Predation pressure	Spines	1	1.57	0.212
	Nips	1	0.16	0.686
	Time orienting	1	0.06	0.812
Error	Spines	204		
	Nips	204		
	Time orienting	204		

The effect of population was tested by nesting it within high or low predation pressure

attributed to body size (among all populations, bigger fish moved less: $r = -0.27$, $P < 0.0001$) and some to the population of origin of the fish, independent of body size (Table 4). Unlike aggressive behavior, there was a statistically significant difference in the antipredator behavior of fish from high versus low predation populations: overall, fish from high predation populations moved more, raised their spines more, and may have inspected more than fish from low predation populations (Fig. 2; Tables 4, 5).

Are differences in behavior related to stress-induced ventilation rate?

Individual differences in behavior were related to differences in stress-induced ventilation rate measured in the same fish on the previous day. In general, fish that breathed faster in response to confinement stress were also more aggressive toward the conspecific and bold toward the pike 1 day later, an effect that persisted when differences in body size and between populations were accounted for (Table 6).

A similar pattern was detected at the between-population level: fish from populations with higher average stress-induced ventilation rates were also more aggressive and bold in the presence of a predator (Fig. 3; Table 6).

Are individual differences in behavior stable over time?

Individual behavioral reactions to the pike were repeatable over a period of several months. For example, the repeat-

Table 4 Effects of population (predation pressure), predation pressure and body size on antipredator behaviors

Source	Dependent Variable	df	F	Sig.
Intercept	Moves	1	42.56	0.000
	Spines	1	20.29	0.000
	Predator inspection	1	14.14	0.000
	Time orienting	1	54.20	0.000
Weight	Moves	1	4.75	0.030
	Spines	1	1.46	0.228
	Predator inspection	1	2.35	0.126
Population (predation pressure)	Time orienting	1	0.22	0.643
	Moves	9	3.82	0.000
	Spines	9	5.27	0.000
Predation pressure	Predator inspection	9	1.88	0.056
	Time orienting	9	1.90	0.053
	Moves	1	6.83	0.010
Error	Spines	1	7.92	0.005
	Predator inspection	1	3.22	0.074
	Time orienting	1	0.56	0.457
Error	Moves	204		
	Spines	204		
	Predator inspection	204		
	Time orienting	204		

The effect of population was tested by nesting it within high or low predation pressure

ability estimate of moves in the presence of the pike was 0.29 ($F_{6,36} = 3.26$, $P = 0.01$) and the repeatability of the number of predator inspections was 0.23 ($F_{6,36} = 2.69$, $P = 0.03$). These estimates are comparable to other estimates of the repeatability of behavior (Bell et al. 2009).

Discussion

This study shows that an acute stressor (capture and confinement) causes a rapid, dramatic, but short-lived increase in stress-induced ventilation rate in sticklebacks. While the typical ventilation rate of undisturbed sticklebacks was 72 beats per min [see also (Meakins 1975)], we found that stress-induced ventilation rate rapidly increased to as high as 174 beats per min in response to confinement stress before rapidly decreasing. This result corroborates other studies on fish that have found that ventilation rate is an indicator of acute stress (Barreto et al. 2003; Cooke et al. 2003; Hawkins et al. 2004; Hojesjo et al. 1999; Johnsson et al. 2001; Metcalfe et al. 1995; Sundstrom et al. 2005).

Remarkably, peak ventilation rate during the confinement procedure was correlated with both aggressive and antipredator behavior the following day (at both the

Fig. 2 Variation among types of populations in behavioral responses to a live predator. Bars show means \pm SE. Sample sizes are low $n = 97$, high $n = 118$

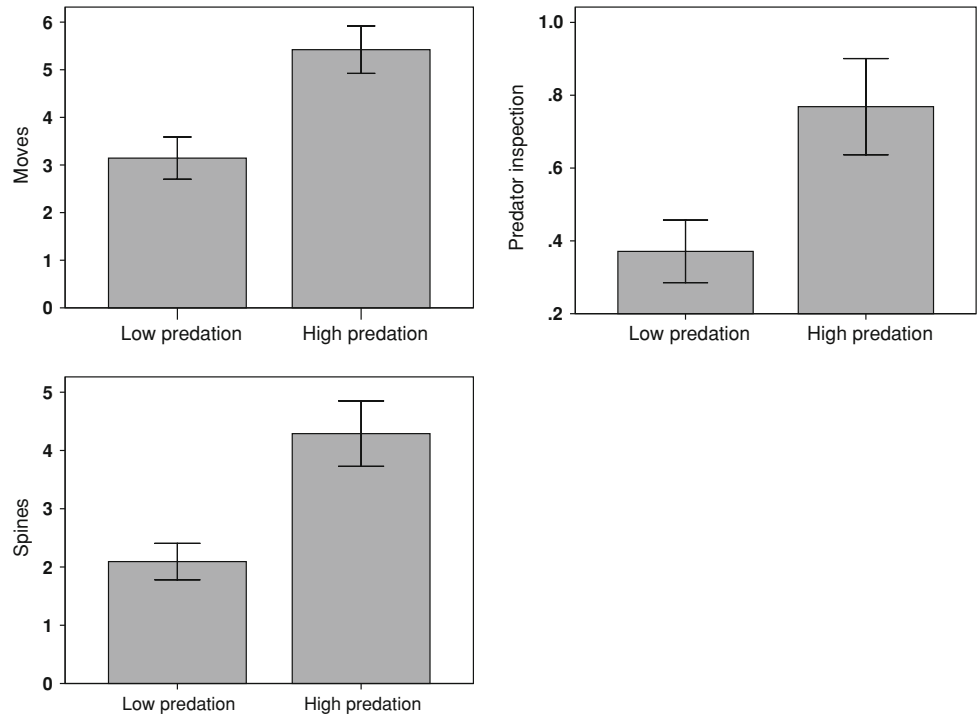


Table 5 Nonparametric tests of behavioral differences between fish from high and low predation populations

	Aggression: spines	Aggression: nips	Aggression: orienting	Antipredator: moves	Antipredator: spines	Antipredator: inspection	Antipredator: orienting
Mann–Whitney <i>U</i>	5,194.5	5,713.5	5,618.5	4,559.5	4,779.5	4,966.5	5,584.0
<i>Z</i>	−1.53	−0.46	−0.57	−2.87	−2.40	−2.44	−0.62
<i>P</i>	0.127	0.648	0.571	0.004	0.016	0.015	0.538

$n = 97$ individuals from low predation populations, $n = 121$ individuals from high predation populations

individual and the population levels). In practical terms, these results suggest that for sticklebacks, ventilation rate is an explanatory, noninvasive and sensitive measure of response to disturbance with significant predictive content. The interrelatedness of this apparently diverse set of measures may not be so surprising when the neuroendocrine basis of both behavior and the stress response are considered.

These results add to the growing body of evidence suggesting that individuals differ in how they respond to mild environmental challenges, with behavioral differences associated with underlying differences in stress physiology (Koolhaas et al. 1999) and metabolism (Careau et al. 2008), an interpretation that is given further weight by the stability observed in individuals’ behavioral responses over time.

The present study also confirms the existence of differences in behavior between fish from populations differing in predation pressure by piscivorous fishes with individuals from high predation populations showing more movement, spine raising and inspection in response to a predator, although no differences in aggression. Studies on other fish

species including bishops (*Brachyraphis episcopi*, Brown and Braithwaite 2004), minnows (*Phoxinus phoxinus*, Magurran 1990) and sticklebacks (Walling et al. 2004) have also found increased levels of ‘bold’ behaviors such as decreased latency to emerge from safety in high predation populations. Although risky behavior in a dangerous environment might seem counter-intuitive, this result is predicted by life history theory. If small individuals are especially vulnerable to predation, individuals that grow fast will be favored because they will grow out of the small vulnerable stage more quickly. Therefore, risk-taking behaviors such as active foraging and aggression that improves access to resources and therefore growth rate should be favored when predation pressure is high (Mangel and Stamps 2001).

This study also shows that an ecological context (level of predation pressure by fishes) has implications for individual physiological responses to challenge. This is reflected in the observed differences in stress-induced ventilation rate between fish from high and low predation populations, with

Table 6 Correlations between stress-induced ventilation rate and behavior

	Aggressive behaviors				Antipredator behaviors			
	Peak rate	Spines	Nips	Time orienting	Moves	Spines	Inspections	Time orienting
Aggressive behaviors								
Peak rate	–	0.175*	0.195*	0.213**	0.101	0.143	0.186*	0.155*
Spines	0.483	–	0.205**	0.226**	0.300***	0.228**	0.230**	–0.019
Nips	0.297	0.444	–	0.555***	0.222**	0.067	0.192*	0.074
Time orienting	0.551	0.614*	0.843**	–	0.286***	0.051	0.191*	0.150*
Antipredator behaviors								
Moves	0.161	–0.143	–0.003	0.284	–	0.719***	0.383***	0.232**
Spines	0.173	–0.188	–0.147	0.177	0.950**	–	0.107	0.088
Inspections	0.786**	0.257	0.331	0.505	0.101	–0.011	–	0.253***
Time orienting	0.276	0.529	–0.378	–0.019	–0.029	0.021	0.059	–

Partial correlations across individuals controlling for weight and population are on the top diagonal, $n = 164$ individuals

Correlations between mean values for the different populations are on the bottom, $n = 11$ populations

Correlations that are statistically significant at the $P < 0.01$ level are still significant after the sequential Bonferroni correction

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

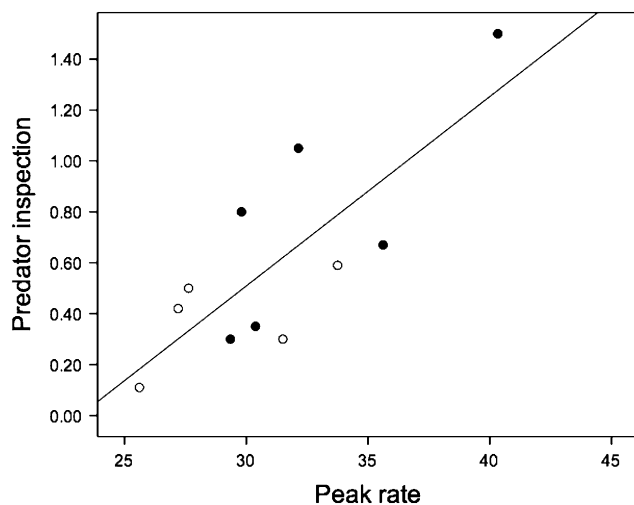


Fig. 3 Populations that were bold toward a predator had higher stress-induced ventilation rate. Each data point represents the mean of a population. Low predation populations are in *open circles*, high predation in *filled circles*. R^2 is 0.618

those from high predation populations having faster rates. We did not have a priori predictions about the effects of coexisting with repeated exposure to predators on physiology because both facilitation and acclimation have been observed in other studies. For example, whereas a comparable study found that bishop fish from areas of high predation showed lower stress-induced ventilation rate (Brown et al. 2007, 2005b), a pattern consistent with acclimation (Romero 2004), we observed the opposite. This suggests a need for further work to develop a conceptual framework for understanding the proximate and ultimate

factors affecting variation in stress responsiveness over developmental and evolutionary time.

We also found that although some of the observed variation in behavior and physiology could be attributed to differences in predation pressure by fishes between the populations, in all of our analyses, there was still significant population-level variation, even within a habitat type. For example, as shown in Fig. 3, the population means do not cluster in the top right and bottom left corners of the graph. In other words, the variation among populations is continuous. Other important factors that varied among populations but which were not controlled for in this study include food availability, density, parasites and gene flow with neighboring populations, etc. Although it is tempting to compare just two different populations varying in the magnitude and predictability of stressors in the environment, our results highlight the importance of comparing multiple, independent replicate populations to detect an overall effect of predation regime among all the variation.

We do not know to what extent the observed population differences in behavior and stress physiology reflect genetic and environmental influences. On the one hand, the animals in this experiment had been held in the laboratory under standard conditions for at least 1 month. This is a relatively long time considering their young age at capture and likely lifespan of 12 months, hence, it is unlikely that the observed differences were due to recent experience. On the other hand, early exposure to stressors can have long-term consequences for stress reactivity and behavior later in life (Auperin and Geslin 2008; Meaney 2001), so it is possible that early experience in the field (including with their

father, Tulley and Huntingford 1987) might have contributed to the observed patterns. In addition, genetic differences between the populations cannot be excluded. Studies on other fish have shown that stress responsiveness can respond to selection (Pottinger and Carrick 1999), and behavioral reactions to both predators (Bell 2005) and conspecifics (Bakker 1986) have a heritable component in sticklebacks. Moreover, our study populations are genetically differentiated (Malhi et al. 2006) and other studies have shown that sticklebacks are capable of rapid (within 10 years) morphological evolution (Bell et al. 2004), so it is possible that the population differences reflect evolutionary responses to divergent selective pressures by predators.

Altogether, these results highlight the important role that local, habitat-specific environmental pressures play in setting up interdependent behavioral and physiological responses to challenges in the environment.

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