Short report

Sticklebacks from streams are more bold than sticklebacks from ponds

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Abstract

Risk-taking behaviour has important consequences for fitness. Here, we show that risk-taking behaviour in sticklebacks consistently varies according to the habitat of origin. We compared the risk-taking behaviour of individual sticklebacks from three pond and three stream populations. Specifically, we measured willingness to forage under predation risk following a simulated attack by a model heron predator. Sticklebacks from stream populations were more willing to forage under predation risk than fish from pond populations. Sticklebacks from streams resumed eating after the simulated attack faster and spent more time eating compared to sticklebacks from ponds. We discuss these findings in terms of differences in life history and predation pressure in the two habitat types.
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1. Introduction

Risk-taking behaviours have important consequences for fitness (Dugatkin, 1992; Biro et al., 2003). Factors affecting the propensity to engage in risk-taking behaviours include age (Miklosi and Csanyi, 1999), hunger (Gotceitas and Godin, 1991), experience (Álvarez and Nicieza, 2003) and genetic background (Coss, 1999). Variable risk-taking behaviours might be an evolved, adaptive response to selection imposed by ecological pressures (Rohr et al., 2003). Three-spined stickleback (*Gasterosteus aculeatus*) is a good candidate to identify and test whether specific ecological factors favour different risk-taking propensities because sticklebacks occur in diverse habitats – oceans, estuaries, rivers, streams, ponds – with contrasting ecological and biological conditions (Bell and Foster, 1994), and the species is amenable to laboratory investigation. In Scotland, this species is common in ponds and streams. These two habitat types vary not only in water flow, but also in food availability, predation

regime, habitat structure and availability of refuges. Sticklebacks from ponds and streams vary in life history (Baker and Foster, 2002), learning (Braithwaite and Girvan, 2003; Odling-Smee and Braithwaite, 2003) and morphology (Hendry and Taylor, 2004; Moore and Hendry, 2005).

In the present study, we test whether sticklebacks from ponds and streams also differ in their willingness to forage under predation risk.

2. Material and methods

2.1. Fish and rearing conditions

The sticklebacks were captured as juveniles between September and December 2004 in six different localities in central and west Scotland: three ponds (Balvormie, Balmaha and Eliburn) and three rivers (Kelvin, Forth and Endrick; see (Álvarez and Metcalfe, 2007) for more details about the particular localities). All the fish were captured with dip nets and transferred to the laboratory on the same day. Sticklebacks were placed in 80 L tanks and maintained in those tanks until the beginning of the experiment. During this period, fish were fed *ad libitum* with frozen bloodworms and temperature was maintained at $12 \pm 0.2\,^{\circ}\mathrm{C}$ while the photoperiod was adjusted weekly to match the natural photoperiod in the studied area. We added

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125 mL of seawater per tank to prevent white spot infection (*Ichthyophthirius multifiliis*).

2.2. Behavioural observations

Fish were placed individually in 20 L experimental tanks to acclimate for one night prior to behavioural observations. The experimental tank contained refuges (artificial plants) and a bloodworm feeder on the front of the tank. Prior to behavioural observations, a great egret (*Casmerodius albus*) skull was attached over the observation tank. The egret skull was situated so that when the skull was released via a lever, the tip of the bill splashed the water surface within 4 cm of the worm dispenser. This stimulus simulated the sudden overhead attack of a heron searching for prey (Giles, 1984).

To measure risk-taking behaviour, five live bloodworms were added to the feeder. When the focal fish approached the food within one body length, a predator attack was simulated twice in quick succession to evoke the anti-predator response (as in: Jonsson et al., 1996; Bell, 2005). If the focal fish did not approach the bloodworm feeder within 10 min after adding the food, the observation was terminated and that individual was excluded from subsequent analyses.

Following the simulated strikes, the focal fish's behaviour was observed for 5 min. The following behaviours were recorded: the latency to the first bite at the food after the egret strike ('latency to resume'), the average time that the fish spends within one body length of the bloodworm feeder ('mean eat'), and the time hiding in a refuge ('time hiding'). Time was measured in seconds.

Fish were observed between 7 and 23 September 2005, until a total of 18 fishes per population responded to the experimental procedure. All observations were "blind", so the observer did not know the population of origin of the fishes. Individuals were measured for standard length immediately following the behavioural observation.

2.3. Statistical analysis

We used principal components analysis (PCA) to generate a new variable that summarized an individual's overall behavioural response. Differences in body size and factor scores were assessed using one-way Anovas which tested for the effect of habitat and population, nested within habitat. The level of significance was $P \leq 0.05$. All analyses were performed using the SPSSv.11.0 statistical package.

3. Results

Individuals differed in their willingness to forage under predation risk. While some individuals resumed foraging within 5 s of the simulated egret attack, other individuals never resumed foraging at all. Anti-predator behaviour could be explained with two principal components (PC1 and PC2) that accounted for 88.68% of the total variance. PC1 explained 72.3% of the variance with high loadings for time hiding and latency to resume foraging (Table 1). Mean time eating loaded negatively on this

Table 1
Results of principal component analysis (PCA) of three behavioural responses from ponds and streams after a simulated attack

Parameter	PC1	PC2
Eigenvalue	2.169	0.492
Explained variance (%)	72.30	16.38
Component loadings		
Latency to resume	0.845	−0.436
Time hiding	0.833	−0.086
Mean time eating	−0.822	−0.541

component, while time hiding and latency to eat loaded positively. Therefore, individuals with low values on this axis were relatively bold and willing to forage under predation risk; they quickly resumed foraging, did not hide under predation risk and ate a lot. Since PC2 explained only the 16.3% of the variance and the interpretation was less clear, only PC1 was analyzed for behavioural comparisons.

Individual variation in behaviour can be attributed to the habitat type from which the sticklebacks originated. Pond fishes were consistently more fearful, or less willing to forage under predation risk, than stream fish (habitat $F(1,102) = 5.019$, $p = 0.027$, Fig. 1). Importantly, there was no effect of population, independent of habitat type, on behaviour (population (habitat)) $F(4,102) = 0.306$, $p = 0.874$). This indicates that habitat type is more important in influencing risk-taking behaviour than the particular population from which the sticklebacks originated.

Although sticklebacks from the different populations differed in standard length (population (habitat)) $F(4,102) = 12.611$, $p < 0.00001$), there was not an overall effect of habitat type (pond or stream) on body size ($F(1,102) = 0.508$, $p = 0.48$). Different-
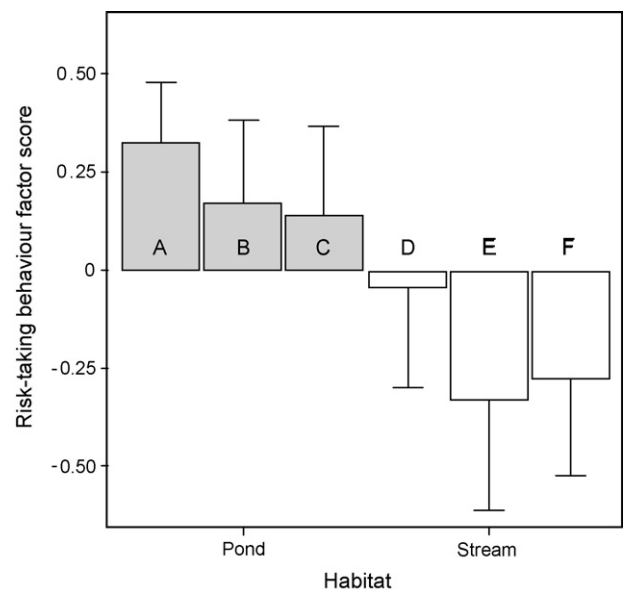

Fig. 1. Behavioural responses (PC1) of the three-spined sticklebacks from the three ponds and three streams studied populations. A: Balvormie; B: Balmaha; C: Eliburn; D: River Kelvin; E: River Forth and F: River Endrick. Ponds: solid bars; streams: empty bars.

sized individuals did not differ in risk-taking behaviour ($r = -0.059$, $n = 108$, $p = 0.547$), even when accounting for the effect of population on body size (partial correlation coefficient $= -0.058$, $n = 105$, $p = 0.553$).

4. Discussion

We found that sticklebacks from ponds were less willing to forage under predation risk than sticklebacks from streams, and this pattern was replicated across several different ponds and streams. The fact that fish from all three ponds were less bold than fishes from all three streams supports the hypothesis that evolution may have driven divergent behavioural strategies in the two habitat types. There are several ecological and life history factors that could contribute to this pattern. For example, while streams are relatively homogeneous structurally at microhabitat scale, ponds are structured environments in which a fish needs to be able to dart quickly into a refuge and then remain still. In addition, ponds are dominated by bird predators such as herons, which stand motionless. When a potential prey approaches close enough, a heron slowly folds its neck back and suddenly strikes. Sticklebacks from ponds might be especially attuned to overhead attacks because the still water in ponds means that the surface will be still, thereby allowing fish underneath to perceive an overhead attack. Therefore, cautious foraging might be favoured in ponds.

In contrast, rivers and streams are dominated by strong currents and the predators are mainly fishes. Therefore, sticklebacks in streams might be less familiar with the predatory tactics of sit-and-wait predatory birds.

Other studies (Baker et al., 1998; Baker and Foster, 2002) have found that sticklebacks in streams and rivers reproduce earlier than sticklebacks in lakes and ponds (at one year of age rather than two). Life history theory predicts that when residual reproductive value is low, individuals should be more willing to take risks, essentially because they have less to lose (Lima and Dill, 1990; Roff, 1992). Therefore, the earlier age at first reproduction of sticklebacks from streams might explain why they are more willing to take risks.

It remains to be seen whether the differences in risk-taking behaviours observed between pond and stream fish are genetically based. Other studies have shown that anti-predator behaviours in this species have a heritable basis (Giles and Huntingford, 1984; Bell, 2005). Also, other differences between stream and pond fish are heritable (Hendry and Taylor, 2004). As this experiment was carried out on wild-caught fish, it is not known the extent to which the behavioural differences reflect genetic and/or environmental influences. Further work, that is, common garden experiments, are needed to address this possibility.

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