Predator regime influences innate anti-predator behaviour in the freshwater gastropod Lymnaea stagnalis

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Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*

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SUMMARY

1. Predation incurs high fitness costs in aquatic organisms either through direct consumption or through avoidance responses that reduce time for activities such as feeding and reproduction. Hence, avoidance responses of aquatic organisms should vary to match closely the predation threat in their environment.

2. The freshwater gastropod *Lymnaea stagnalis* occurs in a variety of environments which vary in the presence or absence of predatory fish. We used naïve snails reared from six populations of this species experiencing different predator regimes (three co-occurring with molluscivorous fish and three without) to assess whether populations differed in the type and degree of their avoidance behaviours. Innate behavioural responses to four treatments (control, conspecific alarm cues, fish kairomones and fish kairomones paired with alarm cue) were compared in laboratory trials.

3. The primary anti-predator behaviour of *L. stagnalis* in response to fish kairomones was to crawl out of the water rather than seek refuge under water. This response was strongest when fish kairomones were paired with alarm cues, and varied depending on population origin; snails reared from populations co-occurring with predatory fish showed a stronger response than those raised from populations not experiencing such predators. In addition, populations co-occurring with predatory fish responded to the fish kairomones presented alone.

4. Our findings suggest that the degree of innate anti-predator behaviour shown by *L. stagnalis*, in terms of both the level of risk to which it responds and the degree of response, varies depending on the predator regime experienced by field populations. Together with previous work on cue association, this demonstrates that this gastropod is able to match its avoidance behaviour very closely to short and long term predation threats within its habitat.

Keywords: alarm cue, chemical communication, gastropod, induced defences, local adaptation

Introduction

Predators can impose high fitness costs on their prey species, directly through predation events but also indirectly, either by modifying prey behaviour so as to reduce opportunities to feed or reproduce (Lima & Dill, 1990) or by imposing costs through

plastic morphological responses (DeWitt, 1998; Caramujo & Boavida, 2000; Van Buskirk, 2000). Changes in morphology and behaviour in response to one predator may also make prey organisms more vulnerable to alternative predator species (Sih, Englund & Wooster, 1998). A number of studies have demonstrated strong responses to predation cues in freshwater systems (Reviewed in: Dodson *et al.*, 1994; Chivers & Smith, 1998; Lass & Spaak, 2003; Werner & Peacor, 2003). However, many aquatic prey species occupy a wide variety of habitats and experience varying levels of predation

threat, such that a single mode of behavioural response is unlikely to be an effective method of avoiding all potential predators (McPeek, 1990). Hence anti-predator behaviour should vary between populations with different predator regimes to match closely the type and level of predation threat present.

Local adaptation to predation threat is likely to occur in relatively sedentary species with low dispersal abilities, whereas induced responses are more likely in species which have potential for high dispersal between generations, when parents and offspring are more likely to encounter alternate predator regimes (Kawecki & Ebert, 2004). Adaptation may also be favoured where plastic responses are costly if the lag between expressing one phenotype or its alternative is too far behind the changes in the environment (Padilla & Adolph, 1996), where information gathering to produce the correct phenotype is costly (DeWitt, 1998) or where costly responses to non-threatening species are likely (Langerhans & DeWitt, 2002).

Gastropods have been utilized extensively to study the responses to predation threat in aquatic systems. Freshwater gastropods have limited dispersal ability, as they are only able to spend short periods emersed without risking desiccation. As such it may be predicted that populations will be under selection dependent on the predator regime they experience, and this prediction is supported by empirical data (Covich *et al.*, 1994; McCarthy & Fisher, 2000; Bernot & Whittinghill, 2003). However, these studies have not explicitly tested for differences between populations across replicate sites differing in their predator status. Also, this previous work used wild-caught animals, which means that any differences between populations may reflect plastic responses rather than local adaptation. Previous studies using laboratory reared animals suggest that the anti-predator response in freshwater gastropods does have an innate element, but can also vary with experience (Dalesman *et al.*, 2006; Turner, Turner & Lappi, 2006).

The great pond snail, *Lymnaea stagnalis* (Linnaeus, 1758), inhabits a wide range of freshwater habitats, including those with and without predatory fish and might be predicted to exhibit adaptation in response to these differing predator regimes. This species has been demonstrated to have both innate and learnt components in its behavioural response to predator cues (Dalesman *et al.*, 2006). Here, we investigate whether the innate aspects of this behaviour varies between populations experiencing differing predator regimes. F1-generation snails were tested for their responses to cues from tench, *Tinca tinca* (Linnaeus, 1758), a known gastropod predator (Brönmark, 1994) that commonly overlaps in distribution with *L. stagnalis*. Responses to fish kairomones were tested both on their own and concurrently with cues from crushed gastropods to simulate a predation event. Hence, we were able to assess how populations differed in their response to the type of cue presented and in the degree of response. Adaptation through natural selection has been shown to account for divergence between aquatic populations experiencing differing predation pressure (Cousyn *et al.*, 2001; O'Steen, Cullum & Bennett, 2002; Meyer *et al.*, 2006). As such we predicted that populations that co-existed with predatory fish would have adapted to show higher innate responses than those that did not experience such predation risk.

Methods

Field collection sites

Adult *L. stagnalis* used to produce experimental snails were collected between September 2005 and May 2006 from six sites on the Somerset Levels, an extensive wet meadow system in the southwest U.K., covered by a series of interlocking drainage ditches and channels. The three sites with predatory fish present (South Drain – 51.18N 2.88W, Sowy River – 51.07N 2.88W, North Moor – 51.07N 2.96W) were large channels (width: 6–22 m, depth: 0.7–1.1 m). Information from the local angling club, Taunton Angling Association (S. Dalesman, pers. com.), was used to confirm the presence of tench at these sites. The three other sites (Chilton Moor – 51.19N 2.88W, Little Hook – 51.06N 2.87W, Wistaria Farm – 51.07N 2.98W) were small ditches (width: 1.8–2.9 m, depth: 0.45–0.7 m); electro-fishing was used to confirm the absence of molluscivorous fish. Sites with and without predatory fish were located in a pair-wise fashion such that each site lacking predatory fish was closer to a site with predatory fish than to the nearest site without such fish and vice versa to reduce any potential influence of geographic location. The mean distance between sites with and without predatory fish was 960 m (range

from 865–1036 m). Repeated sampling at all the sites between March 2004 and September 2006 indicated that *L. stagnalis* is common at these sites for most of the year.

Study organisms

In the laboratory, adult *L. stagnalis* were maintained in aquaria with 4 L of aerated artificial pond water (ATSM, 1980) with 90 mg L^{-1} [Ca^{2+}] (Rundle *et al.*, 2004) at 15 ± 1 °C under a 12 : 12 light : dark cycle, and fed on Iceberg lettuce and spinach. A minimum of 12 adults were collected from each source population to breed the F1 generation to ensure juveniles used for behavioural trials were representative of the population of origin. To reduce the influence of adult experience on juvenile development and behaviour, the F1 snails (6 ± 0.5 mm spire height) used as responders were bred from egg masses laid a minimum of 2 months post collection. They were then maintained separately in the same conditions as the adults. Tench used to produce predator cue were kept in analogous condition to the snails, and fed Nutri-flake (Hozelock Cyprio, Birmingham, U.K.), which contains no mollusc extracts, and hence avoided potential dietary cues affecting the trials.

Odour production

Predator kairomones and alarm cues were produced in the same way in all the experiments (Dalesman *et al.*, 2006). Four exposure treatments were used: (i) fish kairomone water, produced by placing three tench (length 10 ± 1 cm) into 4 L of aerated artificial pond water for 1 h; (ii) alarm cue, produced by mixing three crushed snails (6 ± 0.5 mm) into 4 L of aerated artificial pond water; (iii) fish kairomone plus alarm cue, produced by mixing three crushed snails (6 ± 0.5 mm) into 4 L of tench cue water; and (iv) the control which was aerated artificial pond water. During a single behavioural trial six individuals were exposed to each of the four treatments and the same odour treatment was used for all six individuals (i.e. tench odour was only produced once for each behavioural trial). To complete each treatment × population combination four behavioural trials were required, and odour treatments were produced separately for each trial.

Behavioural assay

Behavioural trials were carried out in the same way for all experiments under laboratory conditions analogous to those in which the snails were maintained (Dalesman *et al.*, 2006). Twenty-four individuals were used for each treatment combination, giving a total of 96 individuals per population, 576 in total, with no individual snail used more than once during behavioural trials. Behavioural chambers consisted of a white plastic dish, 165 mm diameter × 60 mm depth (A.W. Gregory & Co. Ltd., London, U.K.), with a longitudinally sectioned white PVC pipe (36 mm long, 30 mm diameter) attached open side down to the centre using non-toxic sealant (Wickes Ultimate Sealant and Adhesive; Wickes Building Supplies Ltd., Northampton, U.K.) to provide a refuge. Juvenile snails were acclimated to behavioural chambers for 24 h in 630 mL of artificial pond water prior to behavioural assays. Seventy millilitres of cue water was added to each chamber at the start of the behavioural assay to give a final concentration of 10% cue water. The position of each snail within the behavioural chambers was recorded every 5 min for 2 h following cue addition. Crawl-out behaviour has been found to be the main anti-predator response to tench cue by *L. stagnalis* originating from a population with fish predators present (Dalesman *et al.*, 2006). However, use of a refuge by *L. stagnalis* may occur in response to fish predators (Rundle & Brönmark, 2001), so both types of behaviour were analysed here using two variables: the proportion of total time spent in avoidance behaviour; and the latency to avoidance, calculated as the proportion of total time during the trial before an avoidance behaviour was observed (Cotton, Rundle & Smith, 2004).

Statistical analysis

Data were analysed using ANOVA in GMAV5 (Institute of Marine Ecology, University of Sydney, Sydney, Australia). Initially each population was analysed separately to test for an effect of trial on the response to alarm and tench cues, however as no effect of trial was found in any of the six populations trial was excluded from further analyses. Alarm cue, tench cue, habitat type (predatory fish present or absent) and population (nested within habitat type) were used as factors in the analysis. Both time spent in avoidance

and latency to avoidance were calculated as proportions of total duration, and were arcsine-square root transformed prior to analysis (Sokal & Rohlf, 1995). Student–Newman–Keuls (SNK) tests were used to carry out *post hoc* pair-wise comparisons.

Results

The treatment with a combination of tench kairomones plus alarm cue produced the greatest avoidance response, with the longest time spent crawled out for all populations. This response was significantly higher for individuals bred from adults that originated from populations experiencing fish predation (Fig. 1a, Table 1; $F_{1,4} = 16.66$; $P = 0.015$; SNK; $P < 0.01$). In addition, snails originating from populations experiencing fish predation also showed a significant response to the fish cue treatment, though this was significantly lower than the response to combined cues (SNK; $P < 0.05$); individuals from populations where no fish predators were present showed no significant crawl-out response to fish cues alone. None of the populations showed a significant crawl-out response to alarm cues in isolation.

The latency to crawl-out was significantly shorter in response to tench kairomones and alarm cues combined ($F_{1,4} = 194.13$; $P < 0.001$) compared with either cue presented alone for all the populations tested, and did not differ depending on habitat origin (Fig. 1b; Table 1). However, the response to tench kairomones presented alone differed significantly between habitats, with latency being significantly lower in populations originating from areas with fish predators present (Fig. 1b; Table 1; $F_{1,4} = 76.08$; $P = 0.001$; SNK; $P < 0.01$).

The time spent under the refuge significantly decreased in response to the presence of tench cue (ANOVA: main effect of tench cue; $F_{1,4} = 13.90$; $P = 0.020$) but was not affected by any other factors.

Discussion

All the populations used in this study showed a degree of innate anti-predator response to predator kairomones presented in combination with alarm cues by crawling above the water line. This indicates that the populations not experiencing fish predation are still able to recognize the predation threat from tench and respond with appropriate behaviour. The
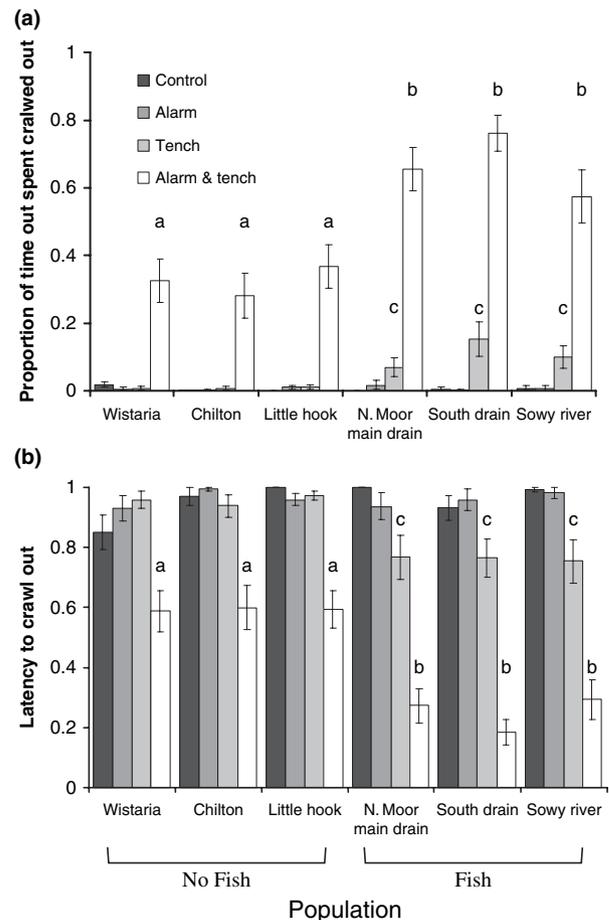

Fig. 1 The mean (±SE) crawl out response of F1 *L. stagnalis* from populations with low (Wistaria; Chilton; Little Hook) and high (N. Moor Main Drain; South Drain; Sowy River) predatory fish regimes, showing (a) proportion of time spent crawled out and (b) latency to crawl out under different treatments ($n = 24$ for each treatment combination). Columns without letters do not differ significantly from one-another but differ significantly from all columns with letters. Columns with the same letter do not differ significantly, but do differ from columns bearing alternative letters.

response from fishless populations was significantly lower than from populations exposed to predatory fish, however, suggesting that the innate perceived threat is lower in the fishless populations. The populations from habitats with predatory fish present also demonstrated an innate response to tench cues alone, not seen in the populations without any predatory fish in their natural habitat. The observed decrease in use of the refuge in response to tench cues is most likely to be an artefact of the increase in crawl out response to this cue, confirming that crawl out behaviour is the main response to tench kairomones

Table 1 Results from four-way nested ANOVA on time spent crawled out of the water and latency to crawl out. Factors used are: habitat type (predatory fish/no predatory fish), population (nested in habitat type), alarm cue (present/absent) and tench cue (present/absent). $n = 24$ for all treatment combinations

Source	d.f.	Proportion of time			Latency		
		Mean square	F	P-value	Mean square	F	P-value
Habitat type (Ha)	1	3.41	27.76	0.006	4.10	30.61	0.005
Population nested in habitat (Po)	4	0.12	1.94	0.103	0.13	1.27	0.282
Alarm cue (A)	1	14.61	266.87	<0.001	13.91	248.47	<0.001
Tench cue (T)	1	24.62	222.23	<0.001	31.16	416.26	<0.001
Ha*A	1	0.73	13.27	0.022	0.35	6.25	0.067
Ha*T	1	3.72	33.57	0.004	5.70	76.08	0.001
A*Po	4	0.05	0.86	0.486	0.06	0.53	0.714
T*Po	4	0.11	1.75	0.138	0.07	0.71	0.586
A*T	1	14.24	355.51	<0.001	14.05	194.13	<0.001
Ha*A*T	1	0.67	16.66	0.015	0.13	1.80	0.251
A*T*Po	4	0.04	0.63	0.640	0.07	0.69	0.602
Total	575						

shown by *L. stagnalis*, as found in a previous study (Dalesman *et al.*, 2006).

The difference between high and low risk populations in a laboratory-reared F1 generation suggests that adaptation to local predator regimes has occurred in the six populations studied. This result concurs with results on responses from other freshwater organisms with limited dispersal such as *Gammarus pulex* (Åbjörnsson, Hansson & Brönmark, 2004) and the Trinidadian guppy, *Poecilia reticula* (O'Steen *et al.*, 2002), which also showed divergence in the degree of innate response to predatory fish cues relative to the predators present in their environment. As all three of these species have relatively low dispersal abilities these results support the theory that species with low dispersal capabilities adapt to local predator regimes rather than relying purely on plasticity to alter phenotype (Kawecki & Ebert, 2004).

The reduced response to fish cues from populations not normally exposed to predatory fish suggests that *L. stagnalis* does not have a general anti-predator response to all the potential predator species it could encounter, as these populations showing low responses to predatory fish cues are exposed to large numbers of invertebrate predators (S. Dalesman, pers. obs.) whose numbers would be reduced by predatory fish elsewhere (Åbjörnsson, Brönmark & Hansson, 2002). Indeed, it has been previously demonstrated that aquatic gastropods are able to vary their response depending on predator identity (Snyder, 1967; Turner, Bernot & Boes, 2000), so potentially populations may lose their response to one type of predator without reducing their response to another (Mikolajewski *et al.*,

2006). This may eventually result in the complete loss of the ability to recognize potential predator by a prey species if they no-longer come into contact (Stoks, McPeek & Mitchell, 2003). The reduced avoidance response in low risk populations suggests that there may be costs associated with retaining anti-predator behaviour towards fish. Traits linked with an increased tendency to respond to fish cues may incur costs when expressed in fishless environments, for example the loss of sexual ornamentation (Magurran, 1999; Basolo & Wagner, 2004) or the costs associated with maintaining and producing plastic responses (DeWitt, 1998).

Assuming that costs are associated with maintaining the high degree of anti-predator behaviour in response to fish cues when predatory fish are not present, it seems perplexing that the response is retained at all in the low risk populations studied here. One explanation may be that fishless environments can be invaded by predatory fish during times of flooding, as the fish and fishless populations used here are in relatively close proximity (<1.1 km) and on a flood plain. The high and low response populations are connected by a series of drainage ditches so there is also potential for gene flow between populations, preventing complete loss of anti-predator behaviour from the fishless populations. As the fitness consequences of not responding to fish correctly are likely to be much higher than occasional inappropriate behaviour when fish are not present, it is possible that even infrequent encounters with predatory fish may result in the behaviour being retained. An alternative explanation would be that at least part of the observed response to predatory fish cues is due to a general

sensitivity to predators rather than a predator specific response, or linked within a suite of behavioural syndromes (Sih, Bell & Johnson, 2004). In this case the 'low risk' populations are still exposed to an assortment of invertebrate predators including beetles, bugs and leeches (S. Dalesman, pers. obs.), and elements of the response seen to fish kairomones may be included in the anti-predator response to these other taxa.

The nature of the environment in which the snail populations used in this study live, with the close proximity and potential for migration along linked water systems suggests that the differences seen will be under strong selection, both for high response under threat of predation, but also to lose responses when that threat is no longer present. The degree of variation seen in both population groups, with a few individuals from the populations lacking predatory fish showing a high response to fish and alarm cues, and vice versa, indicates potential within the population to react rapidly to a change in predatory threat. Rapid microevolution causing divergence between conspecific populations (Hendry & Kinnison, 1999) has been demonstrated elsewhere as a result of natural selection caused by predators (Cousyn *et al.*, 2001; O'Steen *et al.*, 2002; Losos, Schoener & Spiller, 2004; Meyer *et al.*, 2006; Nosil & Crespi, 2006).

In conclusion, our study suggests that innate differences in behaviour and morphology found in other aquatic species (O'Steen *et al.*, 2002; Åbjörnsson *et al.*, 2004) may be generalized across freshwater taxa with poor dispersal abilities. The inability to move from areas of high predation risk to low predation risk may be promoting adaptive responses to local predator regimes. We have previously demonstrated that *L. stagnalis* is able to alter risk perception of a known predator based on recent experience (Dalesman *et al.*, 2006). Such abilities to associate predation cues with perceived risk to fine-tune the innate recognition of predation threat in the short term, alongside the long term adaptive response to predators demonstrated in the present study, suggest that freshwater snails such as *L. stagnalis* are able to match their anti-predator behaviour closely to their environment.

Acknowledgments

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