Influence of rearing and experimental temperatures on predator avoidance behaviour in a freshwater pulmonate snail

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SUMMARY

1. Predicted increases in the temperature of freshwaters is likely to affect how prey species respond to predators. We investigated how the predator avoidance behaviour of the freshwater pulmonate snail *Lymnaea stagnalis* is influenced by the temperature at which it was reared and that at which behavioural trials were carried out.

2. Crawl-out behaviour of juvenile snails from two populations (high predation risk versus low predation risk) reared at either 15 or 20 °C was assessed in response to predation cues (predatory fish kairomones and conspecific alarm cues) in behavioural trials at both 15 and 20 °C.

3. Trial temperature had a significant effect on the time that snails spent in avoidance, regardless of their population of origin. Crawl-out behaviour was greater during behavioural trials at 15 °C, but there was no effect of trial temperature on the speed with which animals showed avoidance behaviour.

4. There was no interactive effect of rearing temperature (RT) and trial temperature, but the effect of RT on avoidance behaviour did differ between populations. For an RT of 15 °C, snails from the South Drain (high risk) population showed a more rapid and longer avoidance response than those from the Chilton Moor (low risk) population. In contrast, for snails reared at 20 °C, there was no difference between populations for the duration of the avoidance response and snails from Chilton Moor crawled out faster than those from South Drain.

5. Hence, whilst (predictable) differences relative to natural predation threat in crawl-out behaviour were apparent at 15 °C, raising the developmental temperature to 20 °C eliminated or, in the case of latency, reversed these differences. This suggests that *L. stagnalis* populations that cohabit with predatory fish and experience high developmental temperatures may have a reduced ability to respond to fish predation risk.

Keywords: antipredator behaviour, chemical communication, developmental plasticity, gastropod, temperature

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Introduction

Water temperature can directly alter the behaviour of freshwater organisms, which in turn can affect the way they interact with their environment, including predator–prey relationships (Mccoull, Swain & Barnes, 1998; Moore & Townsend, 1998; Weetman, Atkinson & Chubb, 1998; Anderson *et al.*, 2001; Kishi *et al.*, 2005). It is also possible that temperature may

affect the behaviour of individuals through an influence during their development. Temperature is known to affect development rate in a wide range of aquatic animals including zooplankton, aquatic insects, amphibians and fish (reviewed in: Gillooly *et al.*, 2002). However, little is known about whether temperature regime experienced during development affects subsequent antipredator behaviour later in the animals' life. An indication that this may be the case comes from work on wood frog tadpoles (*Rana sylvatica*), where both egg development temperature and larval rearing temperature (RT) interact to affect burst swimming speed (Watkins & Vraspir, 2006), therefore the embryonic developmental temperature could potentially affect the ability of this species to escape predators.

Developmental responses to temperature can vary between populations over a small spatial scale. For example, common frog tadpoles (*Rana temporaria*) varied in their developmental response to temperature between populations originating from shaded or exposed ponds separated by between 200 m to 45 km (Van Buskirk & Arioli, 2005). Development and subsequent survival of larval pool frogs (*Rana lessonae*) also differed depending on temperature regime, but the magnitude of the populations response to temperature differed over comparatively small geographical scales (i.e. <5 km; Orizaola & Laurila, 2009). This variability in the developmental response to temperature regime between populations could also alter the way in which they respond to other environmental stimuli. Prey species in the aquatic environment frequently respond to chemical cues from predators by showing short-term avoidance behaviours (reviewed by Kats & Dill, 1998) or induced defences (reviewed by Tollrian & Harvell, 1999). Developmental temperature may also alter the effectiveness of defences in response to the presence of a predator and, if populations differ in their developmental response to temperature, the magnitude of the effect temperature has on defence trait expression is also therefore likely to differ between populations. This effect has been demonstrated in common frog tadpole populations sampled along a latitudinal gradient (Laurila, Lindgren & Laugen, 2008), but the extent to which this temperature-dependant population variability in defence trait expression is applicable to other groups of aquatic organisms is currently unknown.

We investigated the relative importance of RT and trial temperature on the predator avoidance behaviour of the freshwater pulmonate snail *Lymnaea stagnalis* (Linnaeus, 1758). This species has previously been demonstrated to exhibit local adaptation to predation risk from fish predators, with those populations experiencing high predation risk in the wild exhibiting a greater innate avoidance response than those experiencing low predation risk (Dalesman, Rundle & Cotton, 2007). We aimed to test whether this local adaptation to predators was influenced by temperature regime and so compared the effects of developmental versus behavioural trial temperature in both a low and a high predation risk population. We predicted that because of the high risk and low risk sites also varying in temperature regime, such that the population at the smaller low risk site will naturally experience greater fluctuations in temperature, we would find variation in the response to changes in temperature regime between these populations.

Methods

Adult great pond snails, *L. stagnalis* were collected in April 2008 from two sites on the Somerset Levels, U.K., situated <1 km apart: South Drain (51.18N 2.88W) and Chilton Moor (51.19N 2.88W). These sites differ considerably in predation pressure and other habitat features; the Chilton Moor site (low predation risk) is a small drainage ditch subject to fluctuating water levels during the course of the year and contains no fish predators. The South Drain site (high predation risk) is a large drainage canal with stable water levels and contains predatory fish including tench (*Tinca tinca*, Linnaeus, 1758). The average daytime temperature, based on 12 separate daytime measurements per site, did not differ significantly between sites over the total activity period April to September (*t*-test: $t = 0.84$, $P = 0.411$, d.f. = 21; Chilton Moor: 14.9 ± 1.6 °C; South Drain: 16.7 ± 1.5 °C), with temperature variation of 7.2–27.4 °C at the Chilton Moor site and 10.1–24.6 °C at the South Drain site.

Twenty-four adults from each population were collected, and 12 from each population were randomly allocated to 10- L aquaria containing artificial pond water (ASTM, 1980) with 90 mg L^{-1} [Ca^{2+}] and maintained at either 20 ± 0.5 or 15 ± 0.5 °C under a 12 : 12 light:dark cycle. This temperature range is well

within the natural range experienced by each population over the course of the year and also within the range for optimal survival of *L. stagnalis* (Vaughn, 1953). Animals were fed *ad libitum* on iceberg lettuce and spinach. Egg masses used to rear the F1 generation snails for behavioural trials were obtained at least 1 week following adult collection. On the day, they were laid the egg masses were randomly allocated to sixteen 4 L aquaria such that there were four juvenile rearing aquaria for each of the four treatments, with eight maintained at 15 °C, four for the Chilton (low risk) and four for the South Drain (high risk) population, and eight aquaria maintained at 20 °C, four for each population. Juvenile density was maintained at 50 snails per aquarium by randomly thinning the population once all egg masses had hatched.

Once F1 juvenile *L. stagnalis* had reached 6 ± 0.5 mm standard spire height, they were used for behavioural trials. Twenty snails were randomly selected from each aquarium. Ten of these were tested in the temperature at which they had been reared and 10 were tested at the alternate temperature. From each group of 10 individuals tested at each experimental temperature, five were exposed to control conditions (artificial pond water alone) and five to predation cue water. This resulted in $N = 5$ for each treatment combination during the behavioural trial (population*aquaria*RT*experimental temperature*cue treatment) giving 320 individuals used in total.

Prior to behavioural assays, a single juvenile snail was placed into each behavioural chamber 165 mm diameter × 60 mm depth (A.W. Gregory & Co. Ltd., London, U.K.) containing a central shelter, 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.) and allowed to acclimate for 24 h at the behavioural trial temperature in 630 mL of artificial pond water. Trials were initiated by adding 70 mL of predation cue or control water to each behavioural assay chamber to give a 10% final concentration of cue water. Predation cue water was a combination of alarm cues and tench kairomones, produced by mixing three crushed juvenile snails (6 ± 0.5 mm spire height) into 4 L of artificial pond water in which three tench (10 ± 1 cm length) had been held for 1 h (Dalesman *et al.*, 2006). Following

cue addition the position of the snail in the behavioural chamber was recorded every 5 min for 2 h. Crawl-out behaviour, where the snail crawls above the water line, has been found to be the main response of *L. stagnalis* from the two populations to tench kairomones combined with alarm cues (Dalesman *et al.*, 2007). This response was therefore used to measure the effect of rearing and experimental temperature using both the proportion of time spent crawled out and latency to crawl out, calculated as the proportion of total time during the trial before crawl-out behaviour was observed (Cotton, Rundle & Smith, 2004).

Statistical analysis

Time spent crawled out and latency to crawl out were calculated as proportions of the total trial duration and were arcsine-square root transformed prior to analysis (Sokal & Rohlf, 1995). Cochran's test was used to check for homogeneity of variance. Data were analysed using ANOVA in GMAV5 (Institute of Marine Ecology, University of Sydney, Sydney, Australia), using Student–Newman–Keuls (SNK) tests to carry out post hoc analyses. Factors used in the analyses were as follows: source population (Po: Chilton vs. South Drain), RT (20 °C vs. 15 °C), aquaria which was nested within population*RT (Aq: 4 levels nested in Po*RT), experimental temperature during the behavioural trial (ET: 20 °C vs. 15 °C) and cue treatment during the behavioural trial (Tr: control vs. alarm cue paired with tench kairomones).

Results

Snails did not demonstrate crawl-out behaviour in control groups (i.e. in the absence of predation cues), and there was no significant effect of any factor on crawl-out behaviour in the control treatments. There was also no significant effect of individual rearing aquaria on any of the results obtained from this study.

Time spent crawled out

In the presence of predation cues, the overall proportion of time that snails spent crawled out was longer at 15 °C (0.52 ± 0.04) compared with 20 °C (0.42 ± 0.04) independent of population or RT (ANOVA: two-way interaction between cue treatment and

behavioural trial temperature: $F_{1,12} = 5.16$, $P = 0.042$; SNK: $P < 0.01$). There was also an influence of RT on behavioural responses to predation cues but this effect varied depending on the source population (ANOVA: three-way interaction between cue treatment, RT and population: $F_{1,12} = 5.55$, $P = 0.036$). Snails from the South Drain population reared at 15 °C crawled out for significantly longer when exposed to predation cues than those from the Chilton population reared at 15 °C (Fig. 1; SNK: $P < 0.01$); however, there was no difference in crawl-out duration between populations reared at 20 °C (Fig. 1; SNK: $P > 0.05$).

Latency to crawl out

In contrast to the time spent crawled out, there was no significant effect of behavioural trial temperature on the latency to crawl out. However, there was again a significant interaction between RT and population when exposed to combined cues during the behavioural trial (ANOVA: three-way interaction between cue treatment, population and RT: $F_{1,12} = 11.41$, $P = 0.006$); snails crawled out of the water significantly faster in the South Drain population when they had been reared at 15 °C (Fig. 2; SNK: $P < 0.01$), but significantly faster in the Chilton population when reared at 20 °C (Fig. 2; SNK: $P < 0.05$).
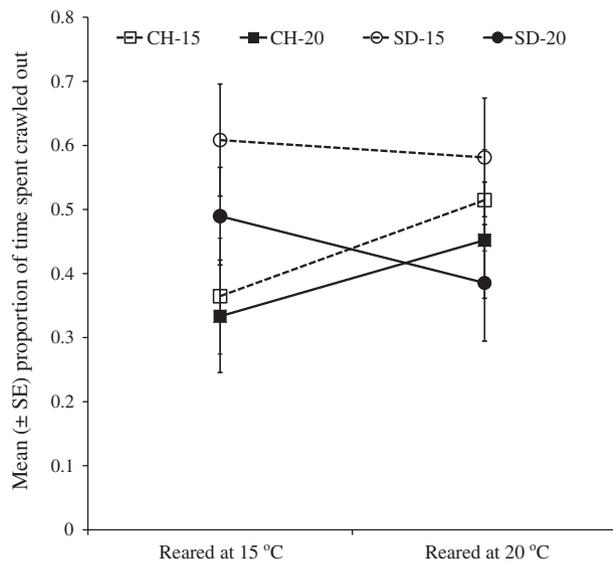

Fig. 1 Mean time spent by *Lymnaea stagnalis* crawled out in response to combined cues (conspecific alarm cue and tench kairomone). Snails were from two populations (Chilton Moor – CH and South Drain – SD) tested at either 15 °C (15) or 20 °C (20). $N = 20$ for each treatment combination.
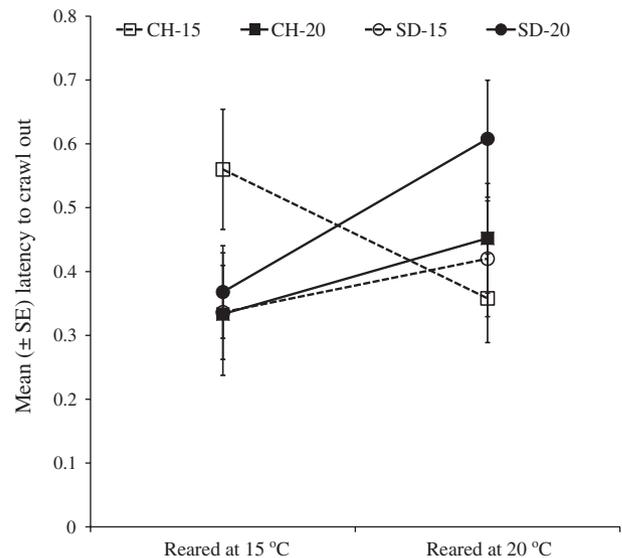

Fig. 2 Mean latency to crawling out by *Lymnaea stagnalis* in response to combined cues (conspecific alarm cue and tench kairomone). Snails were from two populations (Chilton Moor – CH and South Drain – SD) tested at either 15 °C (15) or 20 °C (20). $N = 20$ for each treatment combination.

Discussion

Our study aimed to investigate whether the temperature at which *L. stagnalis* was reared or the ambient temperature during behavioural trials was of greatest importance in influencing the degree to which they responded to a predation threat and whether the effect of temperature differed between geographically close populations. Overall crawl-out behaviour of *L. stagnalis* was significantly increased in response to predation cues relative to controls, concurring with our previous studies on crawl-out behaviour (Dalesman *et al.*, 2006, 2007). Whilst temperature had no effect on crawl-out behaviour in control groups, there was a complex influence of developmental and behavioural trial temperature on crawl-out behaviour in the presence of predation cues, which in the case of developmental temperature differed depending on population of origin. Temperature during behavioural trials influenced the time that snails spent crawled out of the water, with animals showing a greater degree of avoidance at the lower temperature regardless of population, but there was no effect of trial temperature on the speed with which animals showed avoidance behaviour. Although there was no interactive effect of RT and trial temperature, there was an interaction between RT and population. For a RT of

15 °C, snails from the South Drain population (high risk) demonstrated a more rapid and longer avoidance response than those from the Chilton Moor population (low risk), whereas for snails reared at 20 °C there was no difference between populations for the duration of the avoidance response and snails from Chilton Moor crawled out faster than those from South Drain.

The effect of developmental temperature on reducing crawl-out behaviour in the high risk South Drain population suggests that if the climate warms this population could experience increased vulnerability to fish predation. Tench are substrate feeders favouring gastropod molluscs in their diet (Weatherley, 1959; Fitter & Manuel, 1986); therefore, crawling above the water line will be an effective defence for aquatic snails against encounters with this predator. In addition, tench tend to be most active during the warmer summer months (Maitland & Campbell, 1992; Altindag *et al.*, 1998) and, in laboratory conditions, have been shown to have a preference for temperatures around 20–21 °C (Perez-Regadera *et al.*, 1994). The reduction in crawl-out behaviour following development at 20 °C in the high risk South Drain population, which is only marginally higher than the average daytime temperature experienced by this population, therefore seems maladaptive.

Population variability over small scale geographic distances in survival, morphology and growth rate in response to developmental temperature has been found in larval anurans (Van Buskirk & Arioli, 2005; Orizaola & Laurila, 2009). Variation in the reaction norm to temperature between geographically close populations in their response to developmental temperature may be because of selective differences in habitat in response to the local temperature regime experienced by each population (Kingsolver *et al.*, 2007). Whilst the average temperature experienced by both populations does not appear to differ significantly, it is clear that the variability in temperature experienced by the low risk Chilton Moor population is greater than that experienced by the high risk South Drain population. The South Drain population comes from a large stable drainage canal, where water depth remains relatively constant throughout the year, so at higher temperatures this population will not experience drying of the water body in which they live. The risk of desiccation under warm conditions in natural complex habitats

that provide some degree of refuge from predators may be greater for a juvenile snail than that associated with fish predation; hence, selection for reduced crawl-out behaviour at higher developmental temperatures may have occurred in the high risk South Drain population. The Chilton population comes from a considerably smaller drainage ditch. During summer, water depth in such ditches on the Somerset levels can be reduced by evaporation and by infill of the ditch from rotting vegetation. Increased temperature and eutrophication of the water will also considerably reduce the oxygen saturation at the Chilton Moor site. This population may therefore have adapted behaviour to spend more time at or above the water surface following development in warmer conditions, therefore increasing the probability of crawl-out behaviour.

Further evidence for the potential for selection to have reduced the tendency for crawl-out behaviour in high temperatures comes from the data on overall time spent crawled out by both populations. We found a significant increase in the time spent crawled out at 15 °C during the behavioural trials compared to 20 °C. Considering the oxygen saturation will be lower at 20 °C, it is very unlikely that in this instance crawl-out behaviour was influenced by oxygen concentration in the water. An alternative explanation might be the higher probability of desiccation at high temperatures, when the snail must balance this desiccation risk with the risk of predation. Desiccation risk is a factor that may limit crawl-out behaviour, even following prolonged exposure to predator cues in high-response populations (Dalesman, Rundle & Cotton, 2009).

Dalesman *et al.* (2007) showed variation at 15 °C in the crawl-out response to predation cues between the two populations used in the current study, which related to the predator regime experienced, with those at South Drain (high risk) showing a greater response than those from Chilton Moor (low risk). This pattern was consistent across four other populations experiencing similar variability in predator regime (Dalesman *et al.*, 2007). Our data suggest that any local adaptation in antipredator behaviour between high risk and low risk populations may be influenced by developmental temperature. Whilst the temperature at which *L. stagnalis* are reared may have an influence on the avoidance behaviour shown in juvenile animals, the lack of an interactive effect with temperature

during behavioural trials suggests that any effects of RT are not 'pre-conditioning' snails to respond more effectively at a certain environmental temperature. The behavioural avoidance exhibited by *L. stagnalis* is therefore influenced by a complex interaction between the temperature that they experience during development and the population from which they come, with a more generalised response to the temperature at which the response is shown. This finding suggests that predicting how such species might respond to warming of freshwaters through future climate change may be difficult and that any such attempt should take population differences and developmental plasticity into account.

Acknowledgments

References

Altindag A., Yigit S., Ahiska S. & Ozkurt S. (1998) The growth features of tench (*Tinca tinca* L., 1758) in Bayindir Dam Lake, Ankara, Turkey. *Turkish Journal of Zoology*, **22**, 311–318.

Anderson M.T., Kiesecker J.M., Chivers D.P. & Blaustein A.R. (2001) The direct and indirect effects of temperature on a predator-prey relationship. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **79**, 1834–1841.

ASTM (1980) Standard practise for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. In: *American Society for Testing and Materials, Annual Book of ASTM Standards*, p. 406. American Standard for Testing and Materials, Philidelphia PA.

Cotton P.A., Rundle S.D. & Smith K.E. (2004) Trait compensation in marine gastropods: shell shape, avoidance behavior, and susceptibility to predation. *Ecology*, **85**, 1581–1584.

Dalesman S., Rundle S.D., Coleman R.A. & Cotton P.A. (2006) Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Animal Behaviour*, **71**, 789–797.

Dalesman S., Rundle S.D. & Cotton P.A. (2007) Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Freshwater Biology*, **52**, 2134–2140.

Dalesman S., Rundle S.D. & Cotton P.A. (2009) Developmental plasticity compensates for selected low levels of behavioural avoidance in a freshwater snail. *Animal Behaviour*, **78**, 987–991.

Fitter R. & Manuel R. (1986) *Field Guide to the Freshwater Life of Britain and North-West Europe*. Harper Collins, London.

Gillooly J.F., Charnov E.L., West G.B., Savage V.M. & Brown J.H. (2002) Effects of size and temperature on developmental time. *Nature*, **417**, 70–73.

Kats L.B. & Dill L.M. (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361–394.

Kingsolver J.G., Massie K.R., Shlichta J.G., Smith M.H., Ragland G.J. & Gomulkiewicz R. (2007) Relating environmental variation to selection on reaction norms: an experimental test. *The American Naturalist*, **169**, 163–174.

Kishi D., Murakami M., Nakano S. & Maekawa K. (2005) Water temperature determines strength of top-down control in a stream food web. *Freshwater Biology*, **50**, 1315–1322.

Laurila A., Lindgren B. & Laugen A.T. (2008) Antipredator defenses along a latitudinal gradient in *Rana temporaria*. *Ecology*, **89**, 1399–1413.

Maitland P.S. & Campbell R.N. (1992) *Freshwater Fishes of the British Isles*. Harper & Collins Publishers, London.

Mccoull C., Swain R. & Barnes R.W. (1998) Effect of temperature on the functional response and components of attack rate in *Naucoris congrex* Stal (Hemiptera : Naucoridae). *Australian Journal of Entomology*, **37**, 323–327.

Moore M.K. & Townsend V.R. Jr (1998) The interaction of temperature, dissolved oxygen and predation pressure in an aquatic predator-prey system. *Oikos*, **81**, 329–336.

Orizaola G. & Laurila A. (2009) Microgeographic variation in temperature-induced plasticity in an isolated amphibian metapopulation. *Evolutionary Ecology*, **23**, 979–991.

Perez-Regadera J.J., Gallardo J.M., Ceballos E.G. & Garcia J.C.E. (1994) Model development for the determination of final preferenda in freshwater species application in tench (*Tinca tinca* (L.). *Polish Archives of Hydrobiology*, **42**, 27–34.

Sokal R.R. & Rohlf F.J. (1995) *Biometry: The Principles and Practise of Statistics in Biological Research*. W.H. Freeman and Company, New York.

Tollrian R. & Harvell C.D. (1999) *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton.

Van Buskirk J. & Arioli M. (2005) Habitat specialization and adaptive phenotypic divergence of anuran populations. *Journal of Evolutionary Biology*, **18**, 596–608.

Vaughn C.M. (1953) Effects of temperature on hatching and growth of *Lymnaea stagnalis*. *American Midland Naturalist*, **49**, 214–228.

Watkins T.B. & Vraspir J. (2006) Both incubation temperature and posthatching temperature affect swimming performance and morphology of wood frog tadpoles (*Rana sylvatica*). *Physiological and Biochemical Zoology*, **79**, 140–149.

Weatherley A.H. (1959) Some features of the biology of the tench *Tinca tinca* (Linnaeus) in Tasmania. *The Journal of Animal Ecology*, **28**, 73–87.

Weetman D., Atkinson D. & Chubb J.C. (1998) Effects of temperature on anti-predator behaviour in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **55**, 1361–1372.