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Cue association and antipredator behaviour
in a pulmonate snail, *Lymnaea stagnalis*

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Associative learning may help to offset costs of unnecessary escape behaviour by providing accurate information about the current risk to potential prey. We investigated innate antipredator behaviour and cue association learning in naïve gastropods. Juvenile laboratory-reared great pond snails, *Lymnaea stagnalis* (L.), were exposed to odour cues from a natural predator, tench, *Tinca tinca* (L.), and crushed conspecifics. The snails showed an innate antipredator behaviour to odour from *T. tinca*, by crawling above the water line (crawl-out response). This crawl-out response was significantly increased in the presence of alarm cues (crushed conspecifics). In a second experiment, juvenile *L. stagnalis* were exposed to tench odour and alarm cues in aquaria before being tested in behavioural assays. The behavioural responses to tench cue alone were similar to those seen in response to tench plus alarm cues presented together during the first experiment. Hence, *L. stagnalis* is apparently capable of relating potential predation risk to recent experience. In a final experiment snails were removed from pre-exposure cues for periods of 1, 4 and 8 days prior to behavioural assays. A raised level of avoidance persisted for at least 8 days, suggesting that this behaviour may be retained over timescales relevant to predation risk in the natural environment. The ability of organisms to modify antipredator behaviour based on recent experience, as found in *L. stagnalis*, would allow costs associated with unnecessary responses to be reduced while still allowing the organisms to avoid active predators.

Failure to respond to a potential predator may be fatal; however, unnecessary escape behaviour may have direct energetic costs as well as costs associated with reduced opportunity to feed or reproduce (Lima & Dill 1990). Sensory information obtained about a predator may assist an organism in assessing the potential risk accurately and therefore reduce these costs (Lima & Dill 1990; Chivers & Smith 1998). A range of sensory inputs can be used in predator detection; however, where other cues are impaired by environmental or physiological constraints, for instance visual cues in an aquatic system, chemical cues may provide an alternative (Dodson et al. 1994). Predator-released kairomones (Dicke & Sabelis 1988) can give information about the presence, proximity, physiological state and diet of potential predators (Kats & Dill 1998). Injury-released chemical cues from conspecifics frequently induce an antipredator response in aquatic taxa (Wisenden 2000). However, although a good indication of potential threat in isolation, these types of cue may not provide accurate information about the nature of current risks, and so may elicit inappropriate antipredator responses.

Several studies have shown that combining alarm cues produced by injured conspecifics with predator kairomones elicits the greatest antipredator response in organisms, suggesting that this represents the greatest perceived risk. In experiments, this combination of cues either takes the form of the predator feeding on conspecifics (Crowl & Covich 1990; Loose & Dawidowicz 1994; Sih & McCarthy 2002; Turner & Montgomery 2003; Jacobsen & Stabell 2004) or predator cues paired with crushed conspecific, thought to simulate a predation event (Turner 1997; De-Witt et al. 1999; Rundle & Brönmark 2001; Cotton et al. 2004). Some prey organisms that respond to predator cues paired with conspecific cues do not respond to predator cues alone (Turner 1996; Jacobsen & Stabell 2004). An important question is whether this is due to an inability to

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recognize the predator or to a lower perceived risk from a nonfeeding predator. The ability of gastropods to differentiate between the types of predator present from cues paired with injured conspecifics suggests that the latter may be the case in some predator–prey systems. For instance, the freshwater snails *Physella gyrina* and *P. integra* are able to differentiate between cues from different predators, crayfish, *Orconectus rusticus*, and pumpkinseed fish, *Lepomis gibbosus*, when presented with crushed conspecific snail, and respond with appropriate antipredator behaviour (Turner et al. 1999; Bernot & Turner 2001).

Experience of predation cues is an important element in the development of antipredator behaviour in a wide range of vertebrates as well as invertebrates. Learning from experience of predation is integral to most fish species' ability to recognize predators (Brown 2003; Kelley & Magurran 2003). For instance, fathead minnows, *Pimephales promales*, learn to recognize a potential predator, northern pike, *Esox lucius*, from chemical cues produced by pike feeding on minnows (Mirza & Chivers 2001). Learning about predators through diet information, or pairing of predator cues with damaged conspecifics, has also been noted in several invertebrates including damselflies, *Enallagma* spp. (Chivers et al. 1996), crayfish (Hazlett et al. 2002), whelks, *Buccinum undatum* (Rochette et al. 1998) and an aquatic flatworm, *Dugesia dorotocephala* (Wisenden & Millard 2001). In these cases, it was unclear whether the prey species were learning to identify a potential predator per se, or whether the association between cues was providing information about the perceived risk from the predator.

The giant pond snail, *Lymnaea stagnalis* (L.), is slow moving relative to many molluscivorous predators, and lives in an aquatic environment where visual information may often be hindered by turbidity or dense vegetation. It responds to chemical cues from potential predation risk by either sheltering in crevices or crawling above the water line (Snyder 1967; Rundle & Brönmark 2001). As such *L. stagnalis* is predicted to use chemical cues as an important source of information about predators (Wisenden 2000), which makes it an ideal model organism to study the way in which chemical cues can be used to assess predation risk. Our aims in this study were to use naïve laboratory-reared snails to (1) determine the extent of innate responses to predator cues, (2) assess the ability of *L. stagnalis* to form cue associations, (3) determine the effect of these associations on behavioural responses, and (4) determine the longevity of these associations. We predicted that (1) *L. stagnalis* would respond to higher risk by displaying a stronger antipredator response, (2) the perceived risk from predator cues would be enhanced by cue association with a simulated predation event, and (3) perceived risk would diminish over time from exposure to a simulated predation event.

METHODS

Study Organisms

Adult *L. stagnalis* were collected from Matford Park canal, U.K. (5°42′N, 03°31′W) in April 2004. In the laboratory, adults were kept in 6-litre Savic plastic aquaria (Aquatics online, Bridgend, U.K.) in 4 litres of aerated artificial pond water (ASTM 1980) with 90 mg/litre [Ca^{2+}] (Rundle et al. 2004). They were maintained at $15 \pm 1°C$ under ambient light levels, and fed on Iceberg lettuce. Experimental snails were bred from egg masses laid at least 2 months after collection, to minimize the potential influence of adult experience on juvenile development and behaviour. Juvenile snails were then maintained separately in the same conditions as the adults. Individuals with a standard spire height of 6 ± 0.5 mm were used for behavioural experiments.

Tench, *Tinca tinca*, were taken from a laboratory stock originally obtained from Emperor Tropicals & Water Garden Centre, Plymouth, U.K. They were maintained in aerated and filtered water at $15 \pm 1°C$ under ambient light levels in 25-litre tanks at a stocking density of 12 fish per tank (0.006 kg/litre). Gravel substrate and shelters positioned in the tank were used to mimic natural conditions. The tench were fed Nutri-flake (Hozelock Cyprio, Aylesbury, U.K.), which contains no mollusc extracts, and hence avoided potential dietary cues affecting the trials. No experimental manipulations were carried out on these fish and the welfare of the fish was carefully considered throughout. After the trials, the tench were retained as laboratory stock.

Odour Production

Predator and alarm cues were produced in the same way in all the experiments. We used *T. tinca* to produce the predator cue because they are a known natural predator of freshwater gastropods (Brönmark 1994). We produced predator cue water by placing three *T. tinca* (length 10 ± 1 cm) into 4 litres of aerated artificial pond water for 1 h, alarm cue by mixing three crushed juvenile *L. stagnalis* (6 ± 0.5 mm) into 4 litres of aerated artificial pond water, and predator plus alarm cue by mixing three crushed *L. stagnalis* (6 ± 0.5 mm) into 4 litres of tench cue water. We killed the snails for the alarm cue by instant crushing in a pestle and mortar, as physical methods of killing are thought to be the least distressing for the animal (Close et al. 1996). Owing to the small size of the snails, the ease with which their shells can be crushed and the potential alteration of chemical cues with other methods, we considered this the most appropriate way to kill the snails.

Behavioural Assay

We carried out behavioural trials in the same way for all experiments under laboratory conditions analogous to the conditions in which the snails were maintained. Behavioural chambers consisted of a white plastic dish (165 mm in diameter × 60 mm deep; A.W. Gregory & Co. Ltd., London, U.K.), with a longitudinally sectioned white PVC pipe (36 mm long, 30 mm in diameter) attached open side down to the centre with nontoxic sealant to provide a refuge. Juvenile snails were acclimatized to behavioural chambers for 24 h in 630 ml of artificial pond water prior to behavioural assays. We added 70 ml of cue water to each chamber at the start of the behavioural assay to

give a 10% final concentration of cue water. Preliminary trials with predator cue alone showed that this was an adequate odour strength to initiate an antipredator response in *L. stagnalis*.

We recorded the position of each snail within the behavioural chambers every 5 min for 2 h after adding the cue. Crawl-out behaviour (where the snail moved above the water line) was the main antipredator response of *L. stagnalis* to the predator, with the refuge not used; hence only results from crawl-out behaviour are presented here. To analyse crawl-out behaviour we used two variables: the proportion of total time spent crawled out of the water; and the latency to crawl-out, calculated as the proportion of total time during the trial before crawl-out behaviour was observed (Cotton et al. 2004). Proportion of total time spent crawled out was used in conjunction with the time at crawl-out because snails occasionally returned beneath the water line. The results of a power analysis on our preliminary study indicate that using 20 or more individuals per treatment gives an 80% or higher probability of detecting an effect of treatment.

Risk Level and Avoidance Behaviour

After acclimatization of the snails to the behavioural chambers, we used four treatments to assess the response of *L. stagnalis* to varying degrees of predation risk: (1) control (untreated artificial pond water); (2) alarm cues alone (crushed conspecifics); (3) predator odour alone; and (4) predator odour plus alarm cues. We predicted that the control would represent the lowest risk level and predator plus alarm cues the highest, with predator or alarm cues presented individually being perceived as an intermediate risk. We randomly assigned individuals to a treatment level such that equal numbers of snails were exposed to each treatment per trial ($N = 24$ snails per treatment combination).

Cue Association

To assess whether a simulated predation event (predator and alarm cues together) increased the perceived risk from a predator, we exposed snails to a conditioning treatment where both cues were presented together, followed by exposure to predator cues alone during the behavioural assay. This would represent experience of a predation event in the environment followed by the predator returning to the environment without subsequent predation occurring.

Juvenile snails were pre-exposed for 48 h, maintained in 1 litre of water in 1.6-litre Savic tanks in one of four conditioning treatments: (1) control (untreated artificial pond water); (2) alarm cues alone (crushed conspecifics); (3) predator odour alone; and (4) predator odour plus alarm cues. Cue water was produced as in the behavioural assays and again added at a concentration of 10% by volume.

After pre-exposure, individual snails were randomly allocated and acclimatized to behavioural arenas in control water for 24 h. Before the behavioural assays, we added either control water or predator cue to the behavioural arena, so that for each trial half of the snails that had been conditioned to each treatment were exposed to the control or predator cue. We used 192 snails; 48 were exposed to each conditioning treatment, and then half of each group was exposed to predator odour during behavioural assays and half to a control, resulting in 24 snails for each treatment combination.

Persistence of Cue Association

To assess the persistence of cue association, we pre-exposed juvenile snails as before for 48 h to either control water (artificial pond water only) or predator odour plus alarm cues. They were then either acclimatized in behavioural arenas for 24 h, placed into control water for 3 days and then acclimatized for 24 h, or placed into control water for 7 days then acclimatized for 24 h. This resulted in total periods of 1, 4 and 8 days since cue exposure before the behavioural assay. Snails were randomly allocated to a behavioural arena, and exposed to either control water or predator odour during the behavioural assay ($N = 23$ snails per treatment combination).

Statistical Analysis

We analysed data as balanced ANOVAs using GMAV5 (Institute of Marine Ecology, University of Sydney, Sydney, Australia) except in the case of the persistence of cue association experiment which was initially analysed with a general linear model procedure in MINITAB13 (Minitab Ltd., Coventry, U.K.) owing to an unbalanced design between data collection trials. As no significant effect of trial was found, we removed trial as a factor and carried out further analysis using a balanced ANOVA in GMAV5. All proportion data were square-root arcsine transformed before analysis (Underwood 1997). Student–Newman–Keuls tests (SNK) were used for post hoc comparisons.

Log-likelihood tests (G tests) were used to assess the number of snails crawling out in response to different treatments; data were $N + 1$ transformed when zeros were present, and adjusted by Williams' correction (Sokal & Rohlf 1995). α was corrected using the Bonferroni correction to $\alpha' < 0.025$ to test cue association, and $\alpha' < 0.0125$ to test the persistence of cue association, as multiple comparisons were required to analyse the data (Sokal & Rohlf 1995).

RESULTS

Risk Level and Avoidance Behaviour

The number of *L. stagnalis* crawling out differed significantly between treatment levels (G test: $G_3 = 66.750$, $P < 0.001$): no snails crawled out in either the control or alarm cue treatments; however, 15 snails crawled out in response to the predator cue alone, and 21 snails crawled out in response to predator plus alarm cues.

Latency to crawl-out was significantly shorter in response to predator plus alarm cues compared to the

Table 1. Latency to crawl-out and proportion of total time spent crawled out in response to exposure to tench cues and conspecific alarm cues

Source	df	Latency			Proportion of time		
		Mean square	F	P	Mean square	F	P
Trial (Tr)	2	565.79	1.53	0.225	701.01	2.49	0.089
Alarm cue (Al)	1	2600.47	6.99	0.010	6511.94	23.17	<0.001
Tench cue (Te)	1	52 921.62	142.16	<0.001	31 123.61	110.74	<0.001
Tr*Al	2	647.53	1.74	0.182	127.22	0.45	0.638
Tr*Te	2	565.79	1.52	0.225	701.01	2.49	0.089
Al*Te	1	2600.47	6.99	0.010	6511.94	23.17	<0.001
Tr*Al*Te	2	647.53	1.74	0.182	127.22	0.45	0.638
Total	95						

$N = 24$ for all treatment combinations.

predator cue alone, indicating a more rapid response with increased risk (SNK test: $P < 0.05$; Table 1, Fig. 1a). The proportion of time spent crawled out of the water was significantly greater in response to the predator cue paired with alarm cue than to the predator cue alone (SNK test: $P < 0.05$; Table 1, Fig. 1b).

Cue Association

Cue association significantly affected the response of *L. stagnalis* to predator cues (Fig. 2). SNK tests ($P < 0.05$) showed that the effect of preconditioning to predator
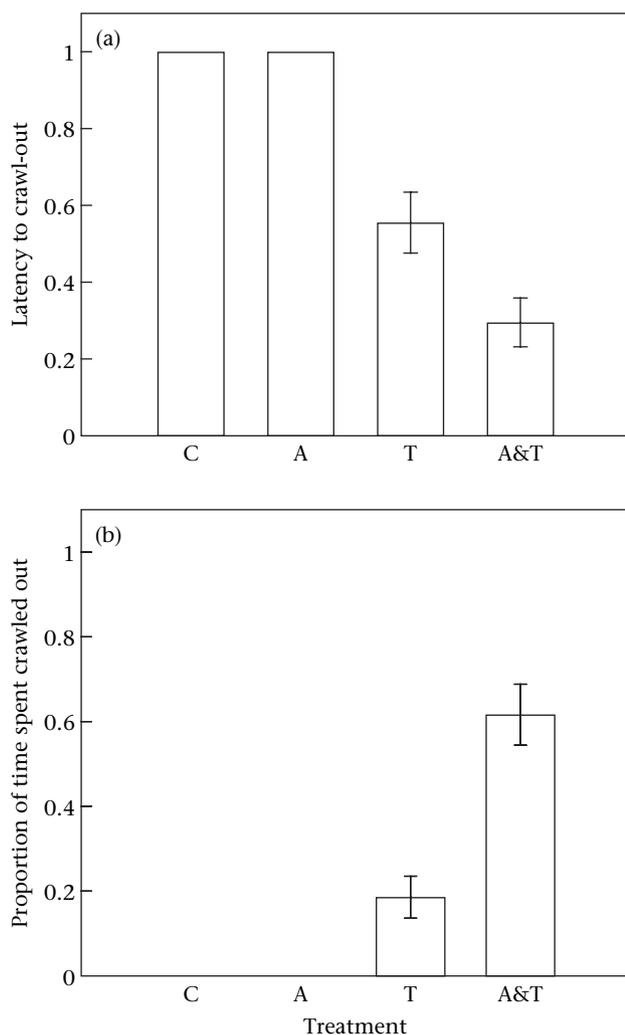

Figure 1. The mean ± SE (a) latency to crawl-out as a proportion of the 2-h assay and (b) proportion of time spent out of the water by *L. stagnalis* in response to different treatments: control (C), conspecific alarm cues alone (A), predator odour alone (T) and alarm plus predator cues (A&T).
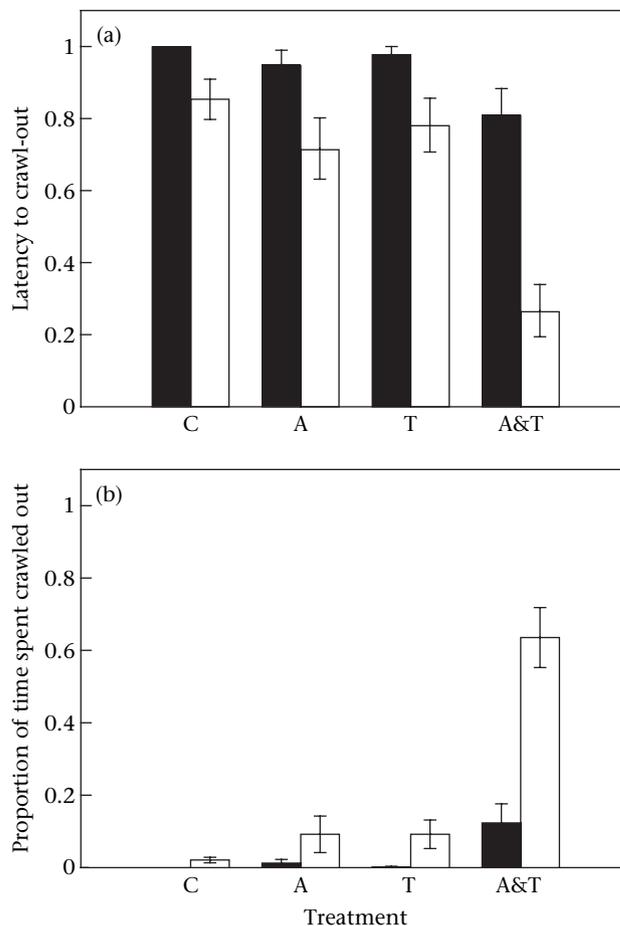

Figure 2. The mean ± SE (a) latency to crawl-out as a proportion of the 2-h assay and (b) proportion of time spent out of the water by *L. stagnalis* responding to control (■) and predator cues (□) after exposure to: control (C), conspecific alarm cues alone (A), predator odour alone (T) and alarm plus predator cues (A&T).

plus alarm cue significantly decreased the latency to crawl-out compared to the control or either cue presented alone (Table 2, Fig. 2a). There was a significant effect of trial on latency to crawl-out (Table 2). However, this was significant only in two pairwise tests between trials, so is not considered to have an overall impact on the results. The proportion of time spent crawled out was significantly greater in response to the predator cue during behavioural assays when *L. stagnalis* had been preconditioned to the predator paired with alarm cue than when individuals had been preconditioned to the control or either cue alone (SNK test: $P < 0.05$; Table 2, Fig. 2b). There was a significant effect of trial, although this was caused by a change in the magnitude of response on only one of the eight trials on individuals that had been preconditioned to alarm cues, so is not considered to have an overall effect on the results (SNK test: $P < 0.05$; Table 2).

Overall, significantly more snails crawled out when exposed to the predator cue during behavioural assays than when exposed to the control during behavioural assays (G test: $G_1 = 30.974$, $P < 0.001$). The number of snails crawling out in response to both the control and predator cue during behavioural assays was significantly greater when they had been pre-exposed to the alarm plus predator cue compared to the control or either cue presented alone (G test: control: $G_3 = 10.307$, $P = 0.016$: predator: $G_3 = 30.324$, $P < 0.001$).

Persistence of Cue Association

Latency was always decreased when predator cues were present during the behavioural assay compared to the control, regardless of the conditioning treatment (SNK test: $P < 0.05$; Table 3, Fig. 3a). However, pre-exposure to predator plus alarm cues significantly decreased the latency to crawl-out in response to the predator cue during behavioural assays up to, and including the 8 days duration compared to individuals pre-exposed to the control (SNK test: $P < 0.05$; Table 3, Fig. 3a). Pre-exposure treatment had no significant effect when snails were exposed to the control during the behavioural assay, and duration since pre-exposure to cues did not significantly affect the latency to crawl-out (Table 3).

The proportion of time spent crawled out was significantly greater in response to the predator cue when *L. stagnalis* had been exposed to predator plus alarm cues compared to any of the other treatments (SNK test: $P < 0.05$; Table 3, Fig. 3b). This significant increase in crawl-out response to predator cues persisted for 8 days after exposure to alarm and predator cues compared to the other treatments. However, the magnitude of the response decreased with duration from pre-exposure, with the proportion of time spent crawled out being significantly lower 8 days after pre-exposure compared to only 24 h after pre-exposure (SNK test: $P < 0.05$; Table 3). The number of individuals showing crawl-out behaviour in both control and predator groups showed no significant decline between 24 h and 8 days (control followed by control; G test: $G_2 = 5.565$, $P = 0.062$; control followed by predator cue: $G_2 = 0.208$, $P = 0.901$; alarm plus predator followed by control: $G_2 = 1.013$, $P = 0.602$: alarm plus predator followed by predator: $G_2 = 2.672$, $P = 0.263$).

DISCUSSION

Risk Level and Avoidance Behaviour

The antipredator response of naïve laboratory-reared F1 *L. stagnalis* to the predator cue alone shows that this snail species has an innate ability to recognize *T. tinca* kairomones and responds with antipredator behaviour. Innate responses to predator threat can be the result of genetic adaptation to recognize potential predators (Åbjörnsson et al. 2004) or a transgenerational influence on offspring

Table 2. Latency to crawl-out and proportion of total time spent crawled out in response to exposure to tench cues after exposure to tench and/or conspecific alarm cues 24 h prior to behavioural assays

Source	df	Latency			Proportion of time		
		Mean square	F	P	Mean square	F	P
Trial (Tr)	7	1594.82	2.91	0.007	817.16	2.90	0.008
Pre-exposure to alarm cue (PrA)	1	14 437.83	26.37	<0.001	12 500.61	44.44	<0.001
Pre-exposure to tench cue (PrT)	1	8885.48	16.23	<0.001	11 009.90	39.14	<0.001
Tench cue during behavioural assay (Te)	1	25 262.17	46.15	<0.001	13 111.73	46.61	<0.001
Tr*PrA	7	1048.81	1.92	0.072	774.09	2.75	0.011
Tr*PrT	7	441.83	0.81	0.583	263.74	0.94	0.480
Tr*Te	7	447.94	0.82	0.574	298.71	1.06	0.392
PrA*PrT	1	5206.34	9.51	0.003	7548.35	26.83	<0.001
PrA*Te	1	3148.68	5.75	0.018	4556.87	16.20	<0.001
PrT*Te	1	2242.74	4.10	0.045	4800.66	17.06	<0.001
Tr*PrA*PrT	7	245.29	0.45	0.870	143.28	0.51	0.826
Tr*PrA*Te	7	504.09	0.92	0.493	327.93	1.17	0.327
Tr*PrT*Te	7	306.20	0.56	0.788	218.58	0.78	0.608
PrA*PrT*Te	1	1487.72	2.72	0.102	2985.55	10.61	0.001
Tr*PrA*PrT*Te	7	237.55	0.43	0.879	170.45	0.61	0.750
Total	191						

$N = 24$ for all treatment combinations.

Table 3. Latency to crawl-out and proportion of time spent crawled out in response to exposure to tench cues after pre-exposure to tench plus conspecific alarm cues at varying durations, 24 h, 4 days and 8 days prior to behavioural assay

		Latency			Proportion of time		
Source	df	Mean square	F	P	Mean square	F	P
Duration (Du)	2	1691.19	2.97	0.053	1403.60	4.07	0.018
Pre-exposure to alarm plus tench cue (Pr)	1	12 189.19	21.40	<0.001	7067.22	20.51	<0.001
Tench cue during behavioural assay (Te)	1	57 709.32	101.33	<0.001	25 736.90	74.71	<0.001
Du*Pr	2	247.89	0.44	0.648	950.91	2.76	0.065
Du*Te	2	653.22	1.15	0.319	1353.53	3.93	0.021
Pr*Te	1	8583.39	15.07	<0.001	6589.99	19.13	<0.001
Du*Pr*Te	2	225.54	0.40	0.674	1452.64	4.22	0.016
Total	275						

Trial is excluded from the model as no significant effect was found using a general linear model. $N = 23$ for all treatment combinations.

(Agrawal et al. 1999). The long time for which adults were held in the laboratory prior to production of F1 juveniles for experimentation in our study potentially reduced the effect of maternal experience on offspring behaviour.
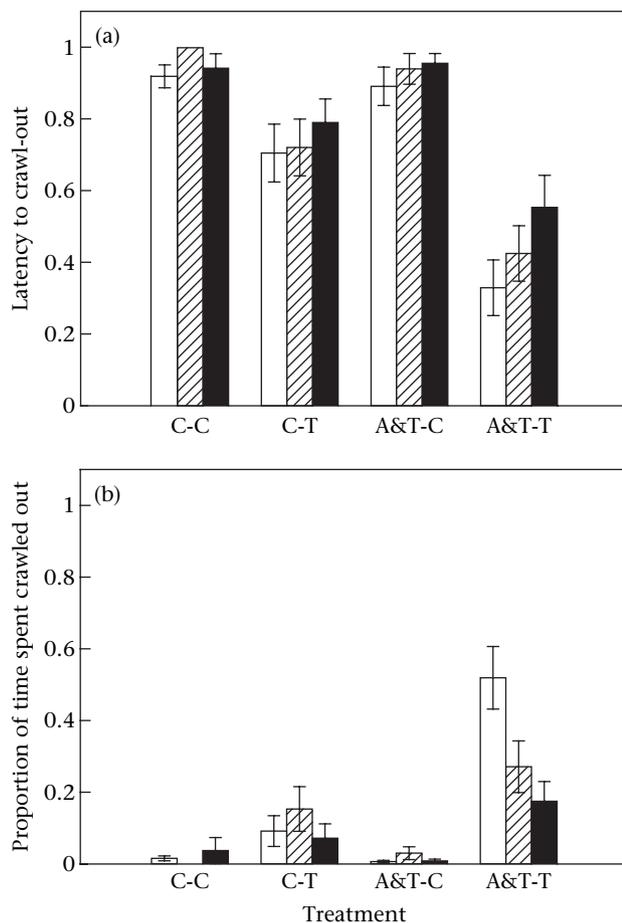

Figure 3. The mean ± SE (a) latency to crawl-out as a proportion of the 2-h assay and (b) proportion of time spent crawled out by *L. stagnalis* 24 h (□), 4 days (▨) and 8 days (■) after exposure to control (C) or conspecific alarm plus predator cues (A&T) in response to either control (C) or predator odour alone (T) during behavioural assays.

However, transgenerational effects of maternal exposure to a dipteran phantom midge predator, *Chaoborus flavicans*, on offspring morphology have been found to persist in the F2 generation of *Daphnia cucullata* (Agrawal et al. 1999).

There was no antipredator response to alarm signals from crushed conspecifics alone (Fig. 1), as found in previous studies on gastropod molluscs (McCarthy & Dickey 2002; Ichinose et al. 2003). Alarm cues may not provide enough information about the type of risk to *L. stagnalis* to induce a response; alternatively they may not be perceived as being associated with a risk of predation at all. However, there was an increase in the proportion of individuals crawling out, as well as in the time spent out of the water when alarm cues were presented in combination with predator cues compared to the predator cue alone. This shows that alarm cues can be identified by *L. stagnalis*, and they probably increase the perceived risk in combination with predator cues compared to predator cues alone. An increase in antipredator response with perceived risk has been found in a number of aquatic species in relation to a predator threat, such as predator density (Ramcharan et al. 1992; Wiackowski & Staronska 1999), distance from prey (Turner & Montgomery 2003), number of prey consumed in the immediate environment (Van Buskirk & Arioli 2002), size of the predator (Kusch et al. 2004) and prey vulnerability (Alexander & Covich 1991; Ramcharan et al. 1992; DeWitt et al. 1999; Rundle & Brönmark 2001; Cotton et al. 2004). These factors may combine to increase the accuracy of the antipredator response in relation to the risk to the prey.

Cue Association

Experience of crushed conspecifics (alarm cues) plus predator odour from *T. tinca* induced an increase in antipredator behaviour on subsequent presentations of the *T. tinca* cue alone, equivalent to that seen in response to the predator plus alarm cues combined during the first experiment (Figs 1, 2). No significant increase in antipredator behaviour was seen in response to pre-exposure to alarm cues or predator cues alone prior to the behavioural assays. This suggests that *L. stagnalis* forms an association between the predator cue and an alarm substance, which then

increases the perceived risk associated with *T. tinca* kairomones on subsequent encounter. Cue association between predator kairomones and damaged conspecifics may provide a more accurate assessment of potential risk from a predator, because it gives information about the current diet of that predator. For instance, whelks, *B. undatum*, increased their escape response over a period of 4 days exposure to a starfish, *Leptasterias polaris*, feeding on whelks, compared to exposure to a control or to *L. polaris* cues alone (Rochette et al. 1998). The juvenile *B. undatum* used were wild caught, so in this case, it is impossible to determine whether there was an innate response to the predator cue. It is likely that they had encountered *L. polaris* in the field; hence the laboratory experiment was potentially reinforcing previous experience.

Cue association may be necessary for a prey species to recognize the threat of predation accurately. The flatworm *D. dorotocephala* shows antipredator behaviour in response to injured conspecifics, but not to a potential predatory fish *Lepomis macrochirus* (Wisenden & Millard 2001). However, after exposure to damaged conspecifics plus *L. macrochirus* odour, *D. dorotocephala* showed antipredator behaviour in response to *L. macrochirus* odour alone 2 days later. As *L. stagnalis* responded to predator cues to a lesser extent without cue association being necessary, it seems likely that this population relies on cue association to give current information on the risk of predation rather than to identify the predator per se.

Cue association is an integral part of predator defence in most fish species studied (Utne & Bacchi 1997; Mirza & Chivers 2001; Brown 2003; Kelley & Magurran 2003; Larson & McCormick 2005). However, Arctic charr, *Salvelinus alpinus*, show innate as well as learnt recognition of potential predators (Vilhunen & Hirvonen 2003). Innate recognition of predators has also been found in a gastropod, *Physella virgata*, which changed its morphology in response to cues from six sunfish species (Langerhans & DeWitt 2002). These changes in morphology included a reduction in growth and a more rotund shape in the snails exposed to predator cues, which were thought to be in part the result of changes in behaviour. However, the snails were unable to respond differentially to predators in relation to their potential risk, as they were unable to differentiate between molluscivorous and nonmolluscivorous species.

Other studies that have found a response to predator cues alone usually concern wild-caught animals, where the history of chemical exposure is unknown, or the animals respond to a predator known to be present in their environment (Hopper 2001; Dahl & Peckarsky 2002; Åbjörnsson et al. 2004; Keppel & Scrosati 2004). As many species are capable of learning about potential predators, observed responses may be the result of experience.

Persistence of Cue Association

Cue association appeared strongest at 24 h postexposure, and showed a slight decline over time from 24 h to 8 days postexposure. At 8 days postexposure, the proportion of time spent crawled out was still significantly greater in the group previously exposed to predator plus alarm cues, suggesting that memory of the learned cue association

persists for at least 8 days after exposure. Although the encounter rate between *T. tinca* and *L. stagnalis* in the source population is unknown, it seems feasible that 8 days is an ecologically relevant timescale in the field. The response to pumpkinseed fish, *L. gibbosus*, by the snail *Physa acuta* declined with both distance from the predator and age of the cue (Turner & Montgomery 2003). However, it is not known whether the decline with age of the cue, with a mean behavioural lifetime of 41 h, was due to an increase in perceived time since a predation event or to a decline in cue concentration per se. The decline over time in antipredator response by *L. stagnalis* implies a reduction in the perceived risk from *T. tinca* with time since experience of predation cues. This is potentially caused by the simulated rate of predation on conspecifics affecting the risk perceived by *L. stagnalis* (Van Buskirk 2002).

The ability to learn about potential predators may be important for a species responding to invasive predators or to a new predator regime if the prey species is expanding its range or simply when a predator has not been previously encountered. The crayfish *Orconectus virilis*, *Procambrus clarkii*, *O. rusticus* and *Austropatmobius pallipes* were able to form cue association between a pseudopredator, the common goldfish, *Carassius auratus*, and damaged conspecifics (Hazlett et al. 2002). The duration of this cue association varied between species, but in *P. clarkii* was still present after 3 weeks. Individuals that had not been exposed to the *C. auratus* cue in combination with damaged conspecifics showed no antipredator response to the *C. auratus* cue on subsequent exposure.

A response caused by a random pairing of unrelated cues inducing antipredator behaviour could prove very costly to prey species. Potentially, this is why an antipredator response declines over time, when the cue association is not reinforced. There is also the potential to prevent cue association through learned irrelevance, where cues are presented in a random order so that the organism learns that they are not associated before cues are presented together (Baker et al. 2003; Hazlett 2003), although so far there is no evidence that this would occur in response to a natural predator.

Lymnaea stagnalis has been used in studies to show the neuronal basis of cue association learning (Ito et al. 1999; Benjamin et al. 2000), although with highly artificial stimuli rather than predator cues occurring in the natural environment, and to examine the effects of predator cues on antipredator behaviour and phenotypic plasticity (Rundle & Brönmark 2001; Rundle et al. 2004); however, ours is the first study to show the ability of a freshwater gastropod to learn about predation risk. Our results concur with those of Rochette et al. (1998) who examined the response of a marine gastropod to cue association, although their study did not consider the effect of prior exposure to alarm cues alone or the experience of their wild-caught snails. Our data therefore indicate that the mechanism of cue association learning may be similar in these diverse gastropod taxa.

Cue association learning, combined with variation in response to different types of cue, as shown in this study, could allow individuals to assess real and immediate risks from potential predator species in their environment. The observed decline in response with lack of reinforcement

would also allow organisms to show avoidance behaviours only in the face of active predators and high predation risk, and to reduce costs associated with unnecessary escape behaviour. Such fine tuning of behavioural responses to environmental cues may be highly adaptive, and the precise mechanisms involved represent an intriguing area for future research.

Acknowledgments

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