Aquatic macroinvertebrate responses to native and non-native predators

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ABSTRACT

Non-native species can profoundly affect native ecosystems through trophic interactions with native species. Native prey may respond differently to non-native versus native predators since they lack prior experience. Here we investigate antipredator responses of two common freshwater macroinvertebrates, *Gammarus pulex* and *Potamopyrgus jenkinsi*, to olfactory cues from three predators; sympatric native fish (*Gasterosteus aculeatus*), sympatric native crayfish (*Austropotamobius pallipes*), and novel invasive crayfish (*Pacifastacus leniusculus*). *G. pulex* responded differently to fish and crayfish; showing enhanced locomotion in response to fish, but a preference for the dark over the light in response to the crayfish. *P. jenkinsi* showed increased vertical migration in response to all three predator cues relative to controls. These different responses to fish and crayfish are hypothesised to reflect the predators' differing predation types; benthic for crayfish and pelagic for fish. However, we found no difference in response to native versus invasive crayfish, indicating that prey naiveté is unlikely to drive the impacts of invasive crayfish. The Predator Recognition Continuum Hypothesis proposes that benefits of generalisable predator recognition outweigh costs when predators are diverse. Generalised responses of prey as observed here will be adaptive in the presence of an invader, and may reduce novel predators' potential impacts.

RÉSUMÉ

Réponses de macro-invertébrés aquatiques aux prédateurs indigènes et non indigènes

Mots-clés :
comportement antiprédateur, non-native, invasive,

Les espèces non indigènes peuvent affecter profondément les écosystèmes par des interactions trophiques avec des espèces indigènes. Les proies indigènes peuvent réagir différemment aux prédateurs indigènes ou non indigènes, car elles manquent d'expérience préalable. Nous étudions ici les réponses antiprédatrices de deux macro-invertébrés d'eau douce communs, *Gammarus pulex* et *Potamopyrgus jenkinsi*, aux signaux olfactifs de trois prédateurs ; poisson sympatrique natif (*Gasterosteus aculeatus*), l'écrevisse indigène sympatrique (*Austropotamobius pallipes*), et une nouvelle écrevisse invasive (*Pacifastacus leniusculus*).

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Austropotamobius pallipes, Pacifastacus leniusculus, alien, écologie des communautés

G. pulex ont réagi différemment aux poissons et aux écrevisses ; montrant une locomotion accrue en réponse aux poissons, mais une préférence pour le noir sur la lumière en réponse à l'écrevisse. *P. jenkinsi* ont montré une augmentation des migrations verticales en réponse à tous les trois signaux des prédateurs par rapport aux témoins. Ces différentes réponses aux poissons et écrevisses sont supposées refléter les différents types de prédation des prédateurs ; benthique pour les écrevisses et pélagiques pour les poissons. Cependant, nous n'avons trouvé aucune différence en réponse aux écrevisses invasives ou natives, ce qui indique que la naïveté de la proie n'intervient pas dans les impacts de l'écrevisse invasive. L'hypothèse du Continuum de Reconnaissance du Prédateur propose que les avantages de la reconnaissance générique du prédateur l'emportent sur les coûts lorsque les prédateurs sont divers. Les réponses des proies comme observées ici seront adaptatives en présence d'un envahisseur, et peuvent réduire les impacts potentiels de nouveaux prédateurs.

INTRODUCTION

Adaptive changes in life history, morphology, and behaviour in response to predator pressure (*e.g.* DeWitt and Scheiner, 2004) are generally costly in terms of a reduction in growth, survival and reproduction (Auld *et al.*, 2010; Trussell and Nicklin, 2002). 'Inducible defences' are adaptive as they allow prey to employ potentially costly predator avoidance strategies only in the presence of a predator (Turner, 2008). The reliability of environmental cues is important for the evolution of stable inducible defences (Harvell and Tollrian, 1999; Reed *et al.*, 2010).

Environmental cues experienced during development may be important for antipredator responses (Dalesman *et al.*, 2009), particularly in freshwater systems (see Brönmark and Hansson, 2007), but in some instances, antipredator responses may be innate (reviewed by Mery and Burns, 2010). Ferrari *et al.* (2007) refer to this continuum between innate responses and generalised learnt responses as the Predator Recognition Continuum Hypothesis. The Predator Recognition Continuum Hypothesis suggests that generalising antipredator responses to novel potential predators is beneficial when the number of different predator species is high, and that innate, fixed responses are preferential when the number of predators is low (Ferrari *et al.*, 2007). This results from a high degree of predictability in prey responses where only a small number of predator species are encountered. Alternatively, where a large number of different predators may be encountered, flexibility in responses is preferable to innate antipredator behaviours.

Invasive, non-native species are major drivers of biodiversity loss and changes in community structure in freshwater ecosystems (Vitousek *et al.*, 1996; McGeoch *et al.*, 2010). Many problems associated with animal invasive alien species arise from their predatory/consumptive impacts on native species (*e.g.* Albins and Hixon, 2013). Predatory invaders may affect prey densities and may also drive altered behaviour, life history, and morphology, with potential ramifications throughout the ecosystem (reviewed in Simberloff, 2011). In many cases, invasive predators have greater impact on prey than do native predator species (Haddaway *et al.*, 2012). The predatory impact of non-native invasive species may be a result of novel predation strategies of non-native species that differ from those of native predators, or may be because native species of prey cannot recognise non-native species nor respond to them with appropriate predator-avoidance mechanisms (reviewed in Sih and McCarthy, 2002). Indigenous species possess a repertoire of adaptive defences to local pathogens, parasites, competitors, predators and/or herbivores as a result of ecological interactions over the course of evolution, but depending on the specificity of the defensive trait, such defences may not be useful against newly introduced species.

Invasion by the American signal crayfish, *Pacifastacus leniusculus*, in Europe is associated with environmental changes, including habitat degradation (Harvey *et al.*, 2011) and reductions in native macroinvertebrate diversity (*e.g.* Jackson *et al.*, 2014). The invasive crayfish has

stronger predatory impact than the native species *Austropotamobiu pallipes* on several native prey species that has been shown to result from differences in prey choice and predatory functional response (Haddaway *et al.*, 2012), leading to the prediction that native prey show lower antipredator defences to the invasive crayfish predator. Here we examine the antipredator responses of two highly abundant macroinvertebrates (the amphipod *Gammarus pulex* and the gastropod *Potamopyrgus jenkinsi*) in UK rivers to three predators: a native fish (the stickleback *Gasterosteus aculeatus*), a native crayfish (the white-clawed crayfish *A. pallipes*), and an invasive non-native crayfish (the North American crayfish *Pacifastacus leniusculus*). We test the hypothesis that prey should show a greater response to the native crayfish than to the invader. We also compare prey responses to crayfish and fish predators with different predatory strategies; crayfish are nocturnal sit-and-wait predators (Gherardi *et al.*, 2001); whereas the stickleback is a diurnal visual forager (Svenster *et al.*, 1995).

METHODS

> PREY COLLECTION AND STORAGE

G. pulex were collected by kick-sampling from Meanwood Beck (NGR: SE279372, Lat/Long: 53.830319/1.577584) and *P. jenkinsi* were collected from Wyke Beck, UK (NGR: SE341363, Lat/Long: 53.821861/1.483487) in March 2010. Both sites are inhabited by the native crayfish (*A. pallipes*) and the native fish predator (*G. aculeatus*). Animals were naïve to the invasive crayfish (*P. leniusculus*). All animals were maintained at the University of Leeds at 17 degrees C on a fixed light:dark cycle of 17L:7D and were fed *ab libitum* on rotting birch leaves. Experiments were all undertaken in March 2010 during daylight. Prey animals were used as natural food for crayfish following the experiments.

> PREDATOR COLLECTION AND STORAGE

Predators were collected using a combination of stone-turning and trap-setting for crayfish, and a D-net for fish from the following sites under relevant licenses (Environment Agency/Natural England/CEFAS); *G. aculeatus* from/to Saltfleet in Lincolnshire (NGR: TF453939, Lat/Long: 53.421887/0.185085), *A. pallipes* from/to Wkye Beck (described above), and *P. leniusculus* from Baylis Pools in Shropshire (NGR: SJ733088, Lat/Long: 52.676239/2.396330). Predators were maintained in constant environmental conditions (as for prey, above) at the University of Leeds for two weeks prior to cue production. Predators were fed daily on a mixture of frozen bloodworm and specialist crustacean dried pellets produced from white fish meal (Hikari Tropical Crab Cuisine®). All predators were fed the same food, with excess removed regularly to avoid fouling of the water. Predators were returned to their respective sources immediately following the experiments (with the exception of *P. leniusculus*). *P. leniusculus* were not released but held for further study, in agreement with UK legislation regarding invasive species.

> EXPERIMENTAL DESIGN

Cues

Aquatic macroinvertebrates show antipredator responses to various cues (Dicke and Grostal, 2001). However, aquatic environments are often characterised by reduced visibility and chemoreception is the major method of environmental sensing (Nyström and Åbjörnsson, 2000). Olfactory cues detected by prey may arise from predator kairomones (that benefit the receiver but not the transmitter) or alarm signals (transmitted between conspecifics) (reviewed by Chivers and Smith, 1998). In order to focus on differences in prey response between predators we used only predator kairomones and not alarm signals. Previous research has shown

that prey can respond to olfactory cues from feeding fish irrespective of diet (conspecific versus other prey) (Paterson *et al.*, 2013), and so feeding cues may conflate alarm signals with kairomones. Future research may benefit from including feeding predator olfactory cues to investigate these collective impacts of kairomones and alarm signals.

Olfactory predator cues were produced using water taken from tanks containing one of three predator species (each observed to predate all prey species in preliminary trials); native fish (the three-spined stickleback *G. aculeatus*), invasive crayfish (the North American signal crayfish *P. leniusculus*), and native crayfish (the white-clawed crayfish *A. pallipes*). Individual size-matched (c. 80 mm total length) predators were maintained, unfed, in separate plastic tanks filled with 1.6 l of dechlorinated tap water with one 5 cm pipe for shelter for a period of 24 h. Cues were produced from a total of three individual predators of each species, and individuals' cues were randomised across replicates throughout the experiment. Predators were exchanged every three days during trials. A control cue was produced using dechlorinated tap water.

Experimental procedure

For *G. pulex*, we investigated light/dark preferences (*e.g.* Bethel and Holmes, 1973) and developed a new protocol for an experiment to measure locomotory responses to predator cues. For *P. jenkinsi* we measured vertical migration in response to predator cues (*e.g.* Turner, 1996).

Experiment 1 - Locomotory response in *G. pulex*

In this experiment *G. pulex* were tested for antipredator responses in the form of changes in locomotion pattern. In order to track movement, a silt suspension was made using filtered (0.1 mm mesh) mud from the field site. The suspension (c. 500 ml) was added to a white tray (417 × 315 mm) along with 30 ml of predator or control cue, mixed thoroughly and then allowed to settle for 2 min. Individual *G. pulex* were placed in the centre of the tray and the path of the test animal was followed by photographing the trail it produced in the silt over 180 s. Thirty individual prey were used, with each individual being used a total of four times, once for each predator cue, with individuals assigned to random predator cue sequences. Whilst this is not as preferable as using different individuals, they were exposed to cues on four separate days with two rest days between cues to allow baseline behaviours to re-establish between treatments , and treatments were assigned randomly across time to each prey. Individual animals were maintained in dechlorinated tap water. Shelter and food was provided in the form of washed rotting sycamore leaves (1 leaf per container).

TPS Dig2 (Rohlf, 1997) software was employed to place landmarks on digital images where a substantial change of direction (>20 degrees) occurred. Sketches of track direction were used to order landmarks correctly. In this way the number of changes of direction, individual distances travelled and total distance travelled were recorded.

Experiment 2 - Light/Dark choice in *G. pulex*

To compare light/dark choices in the different predator treatments, a circular transparent plastic pot (80 mm diameter) was filled to a depth of 20 mm with dechlorinated water (c. 100 ml) and 30 ml of predator cue. One half of the pot was covered in black plastic and a single light source was placed directly above the pot to create a shadowed "dark side" and a "light side". Individual *G. pulex* were placed in the centre of the pot, and over 180 s the time spent in the dark was recorded. Treatments (30 replicates per treatment) were randomised over time and the pot was rotated by 180 degrees between each trial. Trials were carried out on four separate days with two non-experimental days between cues to allow baseline behaviours to re-establish.

Experiment 3 - Vertical migration in *P. jenkinsi*

In this experiment, the snail *P. jenkinsi* was tested for antipredator response in the form of vertical migration and floating. Small plastic containers measuring 80 mm in diameter were filled to a depth of 40 mm (c. 100 ml) with dechlorinated water and 30 ml of predator cue. In each replicate, twenty individual *P. jenkinsi* were placed on the centre of the base of the pot. After 3 h the number of snails in each of four location positions was recorded. The positions were: "base" (remaining in the centre on the base), "climbing" (between the substrate and the surface), "surface" (at or above the surface in contact with the sides), or "floating" (at the surface and not in contact with the sides). Thirty replicates (each replicate containing 20 snails) were used per treatment and each group of snails was used once for each treatment (four times in total; order of treatments randomised). Experiments were run in a total of four days, with six replicates run per day. Two rest days were used between trials to allow for re-establishment of baseline behaviour. Pots were randomised spatially.

> *STATISTICAL ANALYSIS*

All analyses were performed using R (R Development Core Team, 2005). In all cases where significant differences between predator cues were detected, pairwise comparisons were performed without adjustment of *p*-values. Instead, Bonferroni adjustment of alpha (typically $\alpha = 0.05$) based on the number of pairwise comparisons was employed for clarity.

The number of changes of direction and total distance travelled by *G. pulex* were compared between treatments using linear mixed effects models with individual prey identity as a random effect, since residuals were normally distributed (distance travelled (Shapiro-Wilk: $W = 0.992$, $p = 0.583$), changes in direction (Shapiro-Wilk: $W = 0.988$, $p = 0.228$)). All variances were homogeneous ($p < 0.05$). Time spent in the dark was compared between predator cues for *G. pulex* using a linear mixed effects model (LME) using individual prey identity as a random effect. Pairwise comparisons were undertaken using similar linear mixed effects models. A principal components analysis (PCA) was performed on position data from experiments with *P. jenkinsi* and used to produce three principal components. The first and second principal components were tested against treatment with linear mixed effects model (LME (Pinheiro and Bates, 2000)) with the random effect being the snail group used.

RESULTS

> *EXPERIMENT 1 - LOCOMOTORY RESPONSE IN G. PULEX*

We found that total distance travelled by *G. pulex* differed significantly between predator cues (LME: $F_{3,109} = 7.055$, $p < 0.001$) (Figure 1, Table I). *G. pulex* travelled further in the presence of the invasive crayfish than in controls or in the presence of native fish. There was no significant difference in distance travelled in response to native versus invasive crayfish. Native fish cues elicited no detectable change in comparison with controls and all other pairwise comparisons were not significant ($p > 0.008$; threshold of significant, α, conservatively reduced to account for multiplicity of *p*-values in pairwise comparisons). We found the number of changes of direction by *G. pulex* to be unaffected by predator cue (LME: $F_{3,109} = 2.450$, $p = 0.070$).

> *EXPERIMENT 2 - LIGHT/DARK CHOICE IN G. PULEX*

We found that time spent by *G. pulex* in the dark relative to the light differed significantly between predator cues (LME: $F_{3,115} = 17.408$, $p < 0.001$) (Figure 2, Table II). *G. pulex* showed no difference in phototactic behaviour in response to cues from native or invasive crayfish in comparison with the control, whereas prey spent significantly more time in the dark in response to the native fish relative to controls, invasive crayfish and native crayfish.
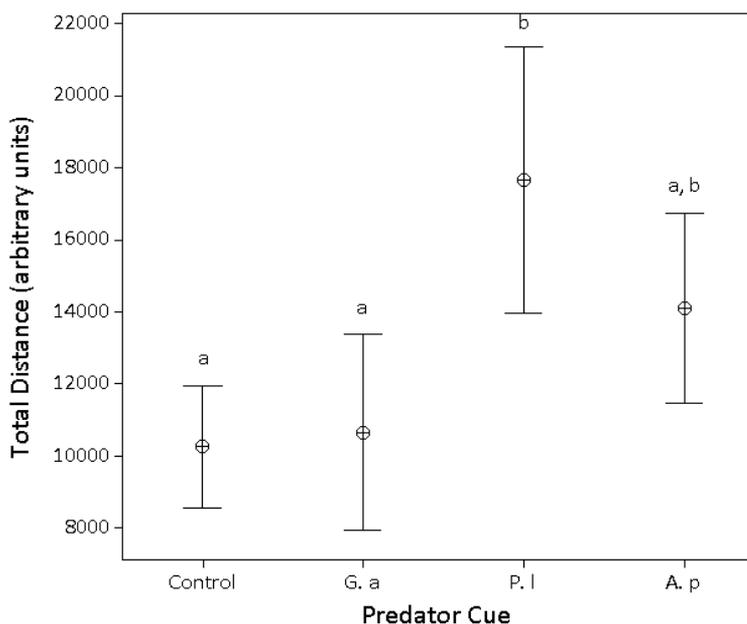
Figure 1
Total distance travelled over 180 seconds for Gammarus pulex presented with different predator cues; "Control" – no predator cue, "Native fish" – Gasterosteus aculeatus, "Non-native crayfish" – Pacifastacus leniusculus, "Native crayfish" Austropotamobius pallipes. Points are means and bars are ± two standard errors. Lines above indicate significant pairwise differences at Bonferoni adjusted α = 0.008.

Table I
Pairwise comparisons for linear mixed effects model of total distance travelled in G. pulex locomotory response experiment. Degrees of freedom for all pairwise comparisons =1,56. *denotes statistical significance relative to a Bonferonni-adjusted level of alpha, α = 0.008.

	Control	Native fish	Native crayfish	Invasive crayfish
Control	–	$F = 0.076$ $p = 0.784$	$F = 5.599$ $P = 0.021$	$F = 14.315$ $P < 0.001$*
Native fish		–	$F = 4.672$ $P = 0.040$	$F = 10.933$ $P = 0.003$*
Native crayfish			–	$F = 2.608$ $P = 0.119$
Invasive crayfish				–

> EXPERIMENT 3 - VERTICAL MIGRATION IN P. JENKINSI

Two grouping variables were identified in the PCA for the vertical migration results; *relocated* (all responses where snails had moved from the bottom of the arena; climbing, at or above the surface, or floating) and *static* (snails remained on the bottom of the arena). This relocated-vs-static variable was the main factor structuring variability along the first principal component. Although we use the terms *relocated* and *static* it is possible that the "static" snails had moved but returned to the base of the pot. The *relocated*-vs.-*static* variable accounted for substantial variation in the data (eigenvalue = 2.498, 62.4 percent of total variation). Table III shows the magnitude and direction of the contribution of each variable to this first principal component. Distinct differences between the three predator cues and the control along this axis are evident in Figure 3 (LME: $F_{3,115} = 13.994$, $p < 0.001$), showing that snails responded significantly to all predators but failed to leave the base in controls. Native and invasive crayfish cues are grouped centrally around zero, corresponding to equal degrees of *static* and *relocated* behaviours (mean snail number of 8.0 relocated and 12.0 static for both native and invasive crayfish). Native fish cues are clustered high on the axis, corresponding to high levels of *relocated* behaviours, with snails responding greatest to native fish, then
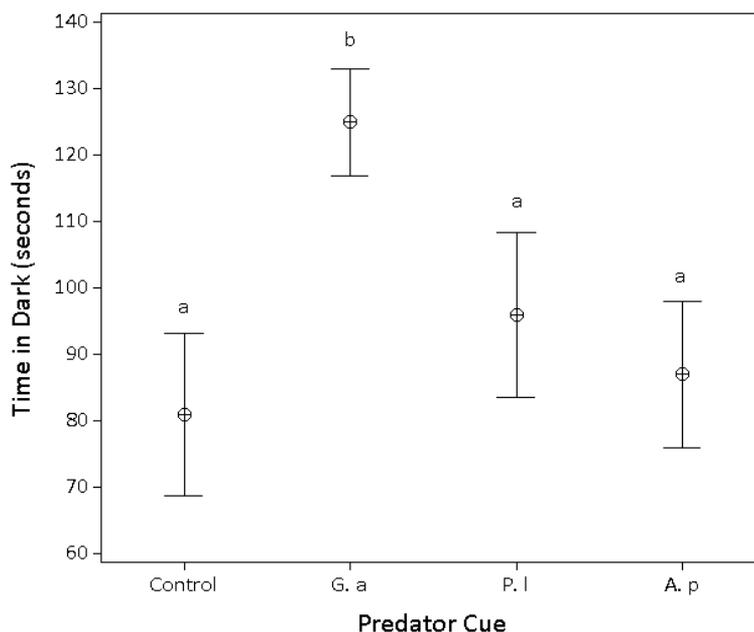
Figure 2
Time spent in dark for Gammarus pulex presented with different predator cues; "Control" – no predator cue, "Native fish" – Gasterosteus aculeatus, "Non-native crayfish" – Pacifastacus leniusculus, "Native crayfish" Austropotamobius pallipes. Points are means and bars are ± two standard errors. Lines above indicate significant pairwise differences at Bonferoni adjusted α = 0.008.

Table II
*Pairwise comparisons for linear mixed effects model of time spent in the light in G. pulex light/dark response experiment. Degrees of freedom for all pairwise comparisons = 1.56. *denotes statistical significance relative to a Bonferonni-adjusted level of alpha, α = 0.008.*

	Control	Native fish	Native crayfish	Invasive crayfish
Control	–	$F = 53.787$ $P < 0.001^*$	$F = 0.594$ $P = 0.447$	$F = 5.520$ $P = 0.026$
Native fish		–	$F = 36.776$ $P < 0.001^*$	$F = 21.708$ $P < 0.001^*$
Native crayfish			–	$F = 1.786$ $P = 0.192$
Invasive crayfish				–

Table III
Contributions of activity measures towards the first principal component (PC1) of a PCA for Potamopyrgus jenkinsi following exposure to predator cues.

Activity Measure	Contribution to PC1
Surfacing (at or out of surface and in contact with sides)	0.484
Floating	0.465
Climbing	0.390
Base (remaining at site of release)	−0.630

native and invasive crayfish together. Pairwise comparisons identified significant differences between controls, invasive crayfish, and fish predator cues, but indicated no differences between crayfish groups (see Table IV). The second principal component (eigenvalue = 0.886 22 percent of variation) was not significantly affected by predator cue (LME: $F_{3,115} = 0.531$, $p = 0.472$).
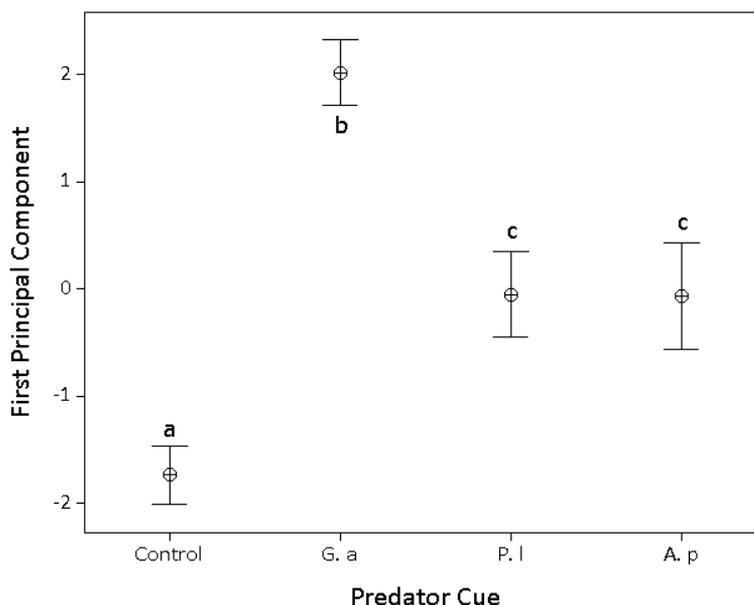
Figure 3
First principal component from PCA of vertical migration activity in Potamopyrgus jenkinsi between predator cues. "Control" – no predator cue, "Native fish" – Gasterosteus aculeatus, "Non-native crayfish" – Pacifastacus leniusculus, "Native crayfish" Austropotamobius pallipes. Points are means and bars are ± two standard errors. Different letters indicate significant pairwise differences at Bonferoni adjusted $\alpha = 0.008$.

Table IV
*Pairwise comparisons of P. jenkinsi vertical migration responses to different predator cues. Degrees of freedom for all pairwise comparisons = 1.57. * denotes statistical significance relative to a Bonferonni-adjusted level of alpha, $\alpha = 0.008$.*

	Control	Native fish	Native crayfish	Invasive crayfish
Control	–	$F = 501.469$ $p < 0.001^*$	$F = 72.147$ $p < 0.001^*$	$F = 79.553$ $p < 0.001^*$
Native fish		–	$F = 66.273$ $p < 0.001^*$	$F = 94.727$ $p < 0.001^*$
Native crayfish			–	$F = 0.006$ $p = 0.938$
Invasive crayfish				–

DISCUSSION

We found that *G. pulex* responded differently to fish and crayfish predators, which probably reflects the different selective pressures imposed by these predators. In accord with previous studies (Perrot-Minnot *et al.*, 2007), they responded to olfactory cues from native fish by increasing the time spent in the dark. *G. aculeatus* is a visual predator (Ohguchi, 1978) and is diurnal (Sevenster *et al.*, 1995), and this antipredator behaviour displayed by *G. pulex* should reduce the likelihood of predation. We found no evidence of a locomotory response to the fish predator, in contrast with other studies that have reported decreased locomotion in response to olfactory fish cues (Dunn *et al.*, 2008; Åbjörnsson *et al.*, 2000). These differences may reflect the selective pressures of the habitat. In our study, *G. pulex* were sourced from a shallow muddy stream, where habitat features such as a low water depth and dense benthic detritus may reduce the risk of visual detection whilst favoring refuge seeking responses to fish predators. Antipredator responses can be costly (*e.g.* Daly *et al.*, 2012), affecting reproduction and distribution patterns (*e.g.* Dunn *et al.*, 2008) and hence there is likely to be strong selection for habitat specific responses that optimize the tradeoff between predator avoidance and other life history costs.

In contrast with the response to fish, *G. pulex* showed no change in phototactic behaviour but responded to crayfish by moving further over the 180-second experimental period. This behaviour may reduce the likelihood of predation (Ohman, 1988) by these nocturnal, sit-and-wait predators (Gherardi *et al.*, 2001), since darkness is not necessarily a haven from predation by crayfish and yet increased movement during daylight, when crayfish are less likely to be feeding, may well result in reduced predation.

The snail, *P. jenkinsi*, responded to all three predator cues. It showed the greatest movement in response to native fish olfactory cues, displaying more surfacing, climbing, and floating responses than remaining on the base. A lower level of these relocating behaviours occurred in response to the two crayfish predator cues. The difference in magnitude of response elicited between native fish and both crayfish cues may result from the different modes of predation of these predators. Fish feed throughout the water column and snails on all surfaces are susceptible to predation , although those that escape the water column by exiting the water or moving to refuge in habitat complexity may escape, accounting for the elevated activity in response to fish. Crayfish, however, can only feed from the benthos and so snails need only migrate above the level accessible to crayfish chelae to avoid predation. In a simple environment, such as our experimental containers and many natural environments, this corresponds to vertical migration. This observation of vertical migration in response to crayfish predation is in accord with observations in the field (*e.g.* Lewis, 2001), where snail distribution in North American lakes was negatively spatially correlated with crayfish presence. Turner *et al.* (2000) and Bernot and Turner (2001) found the snail *Physa integra* to respond differently to fish and crayfish, but they noted that snails sought shelter in the presence of fish and vertically migrate in the presence of crayfish, whereas shelter was not provided in current study. Our results contrast with studies of *Lymnaea*, however, who found no response to non-native crayfish (Orr and Lukowiak, 2009; Orr *et al.*, 2009).

G. pulex did not demonstrate different antipredator behaviours in the presence of native relative to invasive predator cues to which the study population was naïve. Similarly, no difference in activity was evident in snail behaviours between native and invasive crayfish cues. Covich *et al.* (1994) found that two species of snail were unable to differentiate between native and invasive crayfish predators, responding similarly to both *P. leniusculus* and *Procambarus acutus*. Similar work by Dalesman *et al.* (2006) shows that naïve snails (*Lymnaea stagnalis*) respond to olfactory cues from native fish predators with vertical migration, despite having no prior experience of the predator. Our results indicate that both *G. pulex* and *P. jenkinsi* are able to detect invasive crayfish despite a similar lack of prior experience, responding with appropriate antipredator behaviours.

The Predator Recognition Continuum Hypothesis predicts that prey may be more likely to generalise antipredator responses to novel predators where the number of predator species is high (Ferrari *et al.*, 2007). It is interesting that both the snail *P. jenkinsi* and the amphipod *G. pulex* showed similar responses to the native crayfish with which they are sympatric and the invasive crayfish predator. The number of predatory species in Wyke and Meanwood Becks may be sufficiently high that novel potential predators are responded to in the same manner as known predators as predicted by the Predator Recognition Continuum Hypothesis. This high predator diversity may favour generalised responses to crustacean predators, for example, since innate responses would be unfavourably inflexible. A more parsimonious explanation may be, that kairomones released by the invasive crayfish are sufficiently similar to those of the native crayfish that prey simply respond as they would to native predators.

Ferrari *et al.* (2007) proposed that selection will favour generalisation of antipredator responses to phylogenetically related prey that are likely to share similar predatory tactics. In addition, related predators are more likely to provide similar cues. The native (*A. pallipes*) and invasive (*P. leniusculus*) crayfish belong to the same Family and hence kairomones released by the invasive crayfish may be sufficiently similar to those of the native crayfish that prey respond as they would to native predators. In contrast, Gomez-Mestre and Díaz-Paniagua (2011) found that *Rana perezi* tadpoles exhibited changed activity in response to native dragon fly (*Anax imperator*) predators but not to the phylogenetically distant invader the red

swamp crayfish (*Procambarus clarkii*). Invasive predators drive changes in biodiversity and community structure through their interactions with native prey species. We have recently reported a higher predatory impact by the invasive signal crayfish than the native white-clawed crayfish (Haddaway *et al.*, 2012). However, our data do not support the hypothesis that naiveté underlies this increased rate of predation, with such differences more likely to reflect differences in predator behaviour. The innate responses of both amphipod and mollusc prey observed in our study indicate a generalised antipredator response that is likely to be adaptive in the context of invasion by novel crayfish predators.

Although reductions in invert diversity and abundance have been reported following invasion by signal crayfish, our results do not support the hypothesis that prey naiveté is a factor for snails and amphipods. Further work is needed to investigate whether other species of prey, particularly those that are already threatened by existing factors such as habitat degradation, also recognise novel crayfish.

AUTHOR CONTRIBUTIONS

NH and AD conceived and designed the experiments. NH and DV performed the experiments. NH analysed the data. NH and AD wrote the manuscript; other authors provided editorial advice.

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