

Variation in predator-induced behavioral changes in introduced and native populations of the invasive New Zealand mud snail (*Potamopyrgus antipodarum* Gray 1843)

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Received: 29 March 2016 / Accepted: 17 July 2017 / Published online: 1 August 2017

Handling editors: Jennifer Rehage and Kit Magellan

Abstract

Predator detection and avoidance can be important factors determining the success of an introduced species. The New Zealand mud snail (*Potamopyrgus antipodarum*) exhibits avoidance behaviors when chemically detecting native piscine predators in New Zealand, and these behaviors appear specific to sympatric fish populations. Here we utilized three different introduced clonal populations of the New Zealand mud snail from North America, and various clones from New Zealand lakes, to examine the effect of a novel piscine predator, the blacknose dace (*Rhinichthys atratulus*), on behavior. Two of the introduced clones in North America are invasive (US1 and US2) while a third clone (US3) has not exhibited invasive tendencies. In a laboratory setting, we examined geotactic (vertical movement in response to gravity), photokinetic (differential speeds in response to light), and emergence behaviors of each clonal population in the presence and absence of a predator chemical cue. Geotaxis was measured by determining the vertical distance traveled in two minutes. Photokinesis was measured by quantifying the horizontal distance travelled in two minutes in light and dark conditions. Emergence time was determined by removing individuals from the water for five seconds and then replacing them in water and measuring the time to emerge from the shell. We found that the two invasive populations of the New Zealand mud snail (US1 and US2) detected and behaviorally responded to the novel predator utilizing positive photokinesis. The US1 population also showed some evidence of geotactically responding to fish odor. The introduced but likely non-invasive US3 population did not exhibit a detectable response to the odor of fish. Some evidence of photokinetic behavioral responses to the North American fish odor was also found in New Zealand native populations. Fish odor did not appear to influence the time it took for any population of snails to emerge from their shells. These results suggest that the ability to detect and respond to novel predators may be an important trait in the invasion success of New Zealand mud snails by potentially allowing snails to avoid novel predators.

Key words: predator avoidance, chemical detection, behavior, *geotaxis*, *photokinesis*, native predator, invasive prey

Introduction

One of the great difficulties in invasive species research is the inability to find universal traits that can predict invasive capacity (Hayes and Barry 2008). This is likely because invasion success results from the interaction among species traits, environmental conditions and random factors (Sakai et al. 2001). The enemy release hypothesis suggests that when moving to a new environment, introduced species can escape their natural enemies, such as predators and parasites, and succeed as a result (Keane and

Crawley 2002; Torchin et al. 2003). However, when colonizing a new environment, introduced species will likely encounter new potential enemies that may impede their ability to successfully invade (Lodge 1993; Reusch 1998; Maron and Vila 2001; Verhoeven et al. 2008). The ability to detect and respond to potential enemies may be an advantageous trait in introduced species, potentially resulting in a greater probability of invasion success (Rehage et al. 2005). This may be the case with the world-wide invasive mollusk, the New Zealand mud snail (*Potamopyrgus antipodarum* Gray, 1843).

The New Zealand mud snail in North America presents an interesting opportunity to study traits related to invasion success. The snail currently has established invasive populations on five continents: Australia (Ponder 1988), Europe (Ponder 1988), Asia (Shamida and Urabe 2003; Son 2008; Naser and Son 2009), North America (Bowler 1991; Zaranko et al. 1997), and South America (Collado 2014). It thrives in a multitude of freshwater and brackish water habitats, and typically exists in its invaded range in populations of asexual clones (Proctor et al. 2007; Alonso and Castro-Diez 2008, 2012). A comparison of clones of the same species offers a powerful way to assess the traits that may influence invasion success because it allows for the control of genetic variation, which could potentially be a confounding variable in invasion studies. Two of the three genotypes identified in North America (US1 and US2) are invasive and widespread (Proctor et al. 2007; Levri et al. 2008). The US1 clone is found widely in the American west, and it has recently expanded its range to include isolated locations in the eastern US; while the US2 clone is found primarily in the Great Lakes region of North America and has been referred to in other publications as “the Ontario clone” (Levri et al. 2008; Levri et al. 2012a). However, one clone (US3) does not appear to be invasive as it is found in only a small part of the Snake River in Idaho (likely the point of introduction), and has not spread over the past decade since it has been monitored (Proctor et al. 2007; Dybdahl and Drown 2011). There are no known locations where US1 and US2 coexist. US1 and US3 coexist in the one location where US3 is found. This apparent variation in invasive ability in the different North American clones suggests that comparing traits, such as behavior (Levri and Clark 2015), among clones may lead to a better understanding of the characteristics related to invasion success in this species. Recent work has shown that the most invasive North American clone, US1, exhibited different geotactic (vertical movement in response to gravity), photokinetic (difference in speed in response to light), and rheotactic (movement with or against a current) behaviors compared to other introduced North American genotypes. In addition, the US1 clone showed a greater propensity to disperse by attaching to the surface tension of the water than any other genotype investigated (Levri and Clark 2015).

Some of these same behaviors in *P. antipodarum* appear to be important in predator avoidance (Levri et al. 2007) and have been shown to be influenced by the detection of fish predators (Levri et al. 2012b). In lakes in New Zealand, these snails behaviorally respond geotactically and photokinetically to the detection of a predatory fish species in ways that

appear to decrease the probability of predation (Levri 1998; Levri et al. 2012b). The snails typically move downward more strongly in light compared to dark and in the presence of fish odor compared to without. Photokinetically, snails typically move faster in the light than in the dark (Levri and Fisher 2000; Levri et al. 2007) and in the presence of fish chemical cues (Levri et al. 2012b). In addition, these behaviors differ in *P. antipodarum* in different lakes as they respond behaviorally to a predatory fish from their own lake more strongly than they do to the same species of fish from a different lake (Levri et al. 2012b). Thus, the behavioral response to the detection of this fish appears to be the result of local adaptation.

Here we aimed to determine if various introduced and New Zealand native source populations of *P. antipodarum* are capable of adjusting their behavior when presented with a novel piscine predator stimulus as may be found in an invaded habitat. The predator used was the blacknose dace (*Rhinichthys atratulus* Hermann, 1804), which is known to consume gastropods in nature (Tarter 1970) and is native to the eastern United States and Canada (Lee et al. 1980). It is common in streams in New York and Pennsylvania, two states where populations of the New Zealand mud snail currently reside (Levri et al. 2012a). Although the fish has not been documented to co-occur with New Zealand mud snails, based on the distribution of both in the eastern United States, it is likely that they do, or will soon, co-occur. We hypothesized that if fish predation is an important selective force on introduced New Zealand mud snail populations, the most successful introduced clones would exhibit the greatest response to the detection of fish. Specifically, we expected that the US1 and US2 clones would exhibit greater positive geotaxis (move toward the source of gravity) and photokinesis (move faster in light than in dark) in the presence of fish odor, and show longer emergence times when detecting fish than the less invasive US3 clone and New Zealand native clones.

Methods

Snail collections and maintenance

New Zealand native snail samples were originally obtained from endemic populations in multiple New Zealand lakes in 2009 (Table 1). Native New Zealand clones were collected from the South Island except for one genotype, Wak1, which was collected from the North Island (see Table 1). Most of the New Zealand clones were genotyped using mitochondrial haplotype data (Neiman et al. 2010, 2011; Neiman *unpubl. data*). The Gunn2 clone has never been genotyped, although clones from different lakes have

Table 1. Details of the snail populations used in the three experiments.

Population	Invasion Status	Location	Collection site coordinates	Year of collection	Approx. Number of Generations in Captivity	Number Used in Geotaxis Experiment	Number Used in Photokinesis Experiment	Number Used in Emergence Experiment
US1	Invasive	Polecat Creek, WY	44.108974; -110.684666	2010	4–9	34	34	182
US2	Invasive	Niagara County, NY	43.271111; -79.021219	2012	3–5	20	20	
US3	Introduced but not invasive	Snake River, ID	42.913559; -115.070097	2010	4–9	20	20	
Kn4	NZ native	Lake Kaniere, NZ (South Island)	-42.805551; 171.155136	2009	5–10	12	12	
Gunn2	NZ native	Lake Gunn, NZ (South Island)	-44.875886; 168.090282	2009	5–10	20	20	60
B52	NZ native	Lake Alexandrina, NZ (South Island)	-43.940770; 170.452122	2009	5–10	20	20	60
Peorua4	NZ native	Lake Peorua, NZ (South Island)	-42.704813; 171.495172	2009	5–10			60
Wak1	NZ native	Lake Waikaremoana, NZ (North Island)	-38.798053; 177.119933	2009	5–10			60

separate origins (Paczesniak et al. 2013). The introduced source populations used in these experiments were US1 (originally collected from Wyoming in 2010), US2 (collected from a stream in New York State in 2012 [Levri and Jacoby 2008; Levri et al. 2012a]), and US3 (originally collected from Idaho in 2010). The North American clones were originally genotyped using allozyme, microsatellite DNA, and mitochondrial DNA genetic markers (Dybdahl and Drown 2011). All clones used in the experiments were lab reared and had spent several generations in a lab environment prior to this study (Table 1), typically undergoing one to two generations per year (*M. Neiman pers. comm.*). All New Zealand clones and US3 were started from isolated females. The snails were then maintained in our laboratory for at least six months prior to the experiments. While there is some variation in the amount of time spent in a lab environment among genotypes (Table 1), the number of generations is relatively few, and the snails, being clonal, are unlikely to generate much genetic variation. Therefore, we expect that there is little chance that selection could create much genetic difference between these lab populations and the wild populations in the time since collection.

All clones were maintained prior to the experiments in one-liter plastic bins in water containing 3 ppt seawater, since the snails have been found to grow faster in 3 ppt seawater compared to freshwater (Drown et al. 2011). Snails were fed *Spirulina* powder, and their water was changed three times per week.

The experiments described here were conducted between 2013 and 2015. Of the introduced populations, US1, US2, and US3 were used in each of the experiments, except for the emergence time experiment where only the US1 clone was utilized, due to a limited number of snails available from the other populations at the time of that experiment. Different native New Zealand clones were used in the three experiments to maximize sample size.

Experiments

We conducted three experiments utilizing New Zealand mud snails collected from both the native range in New Zealand and the invaded range in the United States. Each experiment examined the influence of a chemical predator cue (from a fish) on a specific behavior: Experiment 1 assessed geotaxis, Experiment 2 investigated photokinesis, and Experiment 3 examined emergence time. Geotaxis and photokinesis were measured in a similar way to that described by Levri and Fisher (2000) and Levri and Clark (2015). All trials in these three experiments were conducted between 9 am and 12 pm because previous studies demonstrated that time of day influences behavior (Levri and Lively 1996). The clonal populations of snails used and their sample sizes in each of the experiments are detailed in Table 1.

Two weeks before the experiments began, snail containers were placed in ambient sunlight on a windowsill to acclimate them to a natural light-dark

cycle. Although size varies in nature (Winterbourn 1970) and snail shell size and shape are phenotypically plastic (Kistner and Dybdahl 2013), there is little variation in maximum length among clones reared in the lab (about 5.5 mm) which is similar to the maximum size seen in introduced populations in North America (Levri *unpubl. data*). However, in order to control for any effect of size on behavior, immediately prior to the experiments, the length (shell apex to aperture lip) of each snail was measured, and only snails 4.0–5.0 mm were used in the experiments.

Experiment 1: Geotaxis

To assess geotaxis, 1 L of water containing predator cue, derived from four adult blacknose dace (*Rhinichthys atratulus* Hermann, 1804) housed in a 37.9 L aquarium, was added to one of a pair of 37.9 L aquaria filled with aged tap water. The other aquarium contained only aged tap water. Individual snails were placed in a 20 cm long by 10 mm diameter horizontal glass tube in each aquarium. During each trial, once the snail emerged from the shell, the tube was oriented vertically in ambient light for 2 minutes and the direction (up or down) and distance traveled were noted. Each snail was measured in each of the two conditions; no predator cue and predator cue. The order of the trials was randomized for each snail.

Experiment 2: Photokinesis

To assess photokinesis two 37.9 L aquaria were set up as Experiment 1: one containing only aged tap water and one with 1 L of water containing the predator cue added. Individual snails were placed in a horizontal glass tube within one of the aquaria as above. Once the snail emerged from its shell, the distance the snail travelled was measured after 2 minutes. The same protocol was carried out in light and dark conditions and with and without a predator cue. Dark conditions were achieved by placing an opaque box over top the aquarium. Each snail was thus assessed in each of four conditions; no predator cue in light, no predator cue in dark, predator cue in light and predator cue in dark. The order of the trials was randomized for each snail.

Experiment 3: Emergence time

Emergence time was estimated by taking individual snails out of the water for five seconds and then placing them into a glass petri dish filled with either aged tap water (control) or aged tap water from a 37.9 L aquarium housing four blacknose dace. The emergence time was measured as the time elapsed from being placed into the petri dish to the time the

snail's head and foot emerged from the shell and an attempt was made to upright the shell. The petri dishes were emptied and cleaned between trials to reduce the possibility of previous snails affecting behavior. Thirty snails from each population were used in the control treatment and a separate thirty snails from the same populations were used in the predator cue treatment.

Statistical analyses

In the geotaxis and the photokinesis experiments, repeated-measures ANOVAs were used to compare the effect of predator odor on the behavior of the six clones used in each experiment as well as compare the clones to each other (Table 1). In the geotaxis experiment, the dependent variable was the distance and direction moved (positive numbers indicated distance moved up and negative numbers indicated distance moved down) and clone and fish exposure were independent variables. In the photokinesis experiment we subtracted the distance moved by each snail in the dark from the distance the snail moved in the light in both the control and predator cue conditions. This created a photokinetic response variable for each snail; a positive value indicated that the snail moved further in light than in dark. We then utilized a repeated measured ANOVA to compare the photokinetic response (dependent variable) of different clones (independent variable) between the control and predator conditions (independent variable). In the repeated measures ANOVA for both experiments, clone was a between subject factor and fish treatment was within subjects. As we were mainly interested in differences between only introduced genotypes, for both experiments we performed an additional repeated-measures ANOVA on just the introduced clones (US1, US2, and US3) utilizing post-hoc sequential Sidak tests to specifically compare the introduced clones to each other to assess whether some clones are better invaders than others. In all analyses the normality assumption was determined to be satisfied by examining Q-Q plots. In cases with significant interactions between population and predator treatment, we utilized paired t-tests to determine if individual populations responded differently to the predator cue compared to the control.

In the emergence time experiment, an ANOVA was used to determine if the time to emergence (dependent variable) differed among clones (independent variable) and was influenced by the odor of fish (independent variable). Emergence time data were log-transformed to meet the homogeneity of variance assumption (Levene's test). Post-hoc Tukey's tests were performed to compare the emergence times

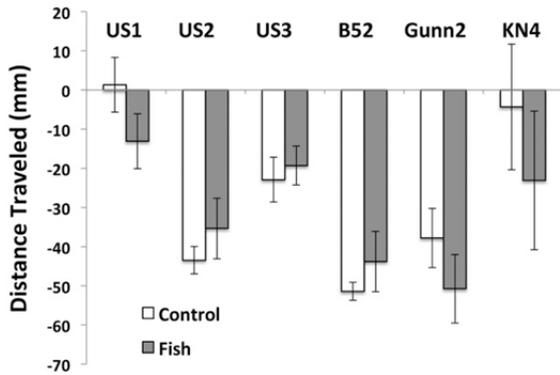


Figure 1. Mean distance traveled in the geotaxis experiment by snails in the presence and absence of a fish predator cue. Movement in the control (absence of predator cue) is in the open bars and movement in the presence of the predator cue is in shaded bars. Positive values indicate average movement in the upward direction. Error bars are standard errors.

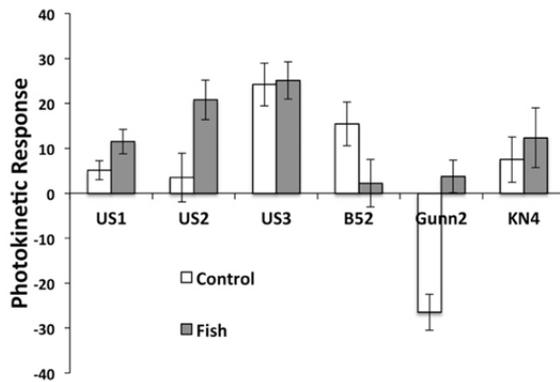


Figure 2. The photokinetic responses of the different populations in the presence and absence of a fish predator cue. The photokinetic response is the difference between the distance travelled (mm) in the light versus the dark conditions. Positive values indicate that the snails moved farther in the light than in the dark and exhibit positive photokinesis. Photokinesis in the absence of the predator cue is in the open bars and photokinesis in the presence of the predator cue is in shaded bars. Error bars are standard errors.

of the invasive US1 population to each of the NZ native populations. The normality assumption was determined to be satisfied by examining Q-Q plots. All analyses were performed using IBM SPSS Statistics statistical software v. 24.

Results

Geotaxis

We found that population source influenced the geotactic behavior of the snails, but neither predator stimulus effect nor genotype response to the predator significantly differed (Figure 1; Table 2). However,

Table 2. Results of repeated-measures ANOVA comparing the geotactic behavior of clones in response to fish odor. The degrees of freedom is signified by df and the significance value by p.

Fully factorial model.			
Source	df	Wald Chi-Square	p
Clone	5	76.57	< 0.001
Predator	1	0.87	0.350
Clone x Predator	5	8.84	0.111

Table 3. Results of repeated-measures ANOVA comparing the geotactic behavior of only introduced clones in response to fish odor as well as the results of a sequential Sidak post hoc test comparing the introduced clones to each other. The degrees of freedom is signified by df, the significance value by p, mean difference is MD, and SE denotes standard error.

Fully factorial model.			
Source	df	Wald Chi-Square	p
Clone	2	34.50	< 0.001
Predator	1	0.49	0.825
Clone x Predator	2	5.99	0.050

Post-hoc comparison between clones.

Clone	US2	US3
US1	MD = 15.21 SE = 5.72 P = 0.008	MD = 33.52 SE = 5.71 P < 0.001
US2		MD = 18.30 SE = 5.86 P = 0.004

Table 4. Results of repeated-measures ANOVA comparing the photokinetic behavior of clones in response to fish odor. The degrees of freedom is signified by df and the significance value by p.

Fully factorial model.			
Source	df	Wald Chi-Square	p
Clone	5	88.19	< 0.001
Predator	1	10.39	0.001
Clone x Predator	5	28.98	< 0.001

the US1 population did trend toward moving down more when the predator cue was present (Figure 1). In the post-hoc comparisons among the introduced populations, we found that the US1 population moved down significantly less than the US2 and US3 populations (Table 3) and US2 moved down significantly more than US3 (Table 3).

Photokinesis

Most populations exhibited positive photokinesis in the presence and absence of the predator cue (Figure 2). The populations demonstrated differences in their photokinetic response (Figure 2; Table 4). There were also significant effects of the predator cue and differences between populations in their response to the

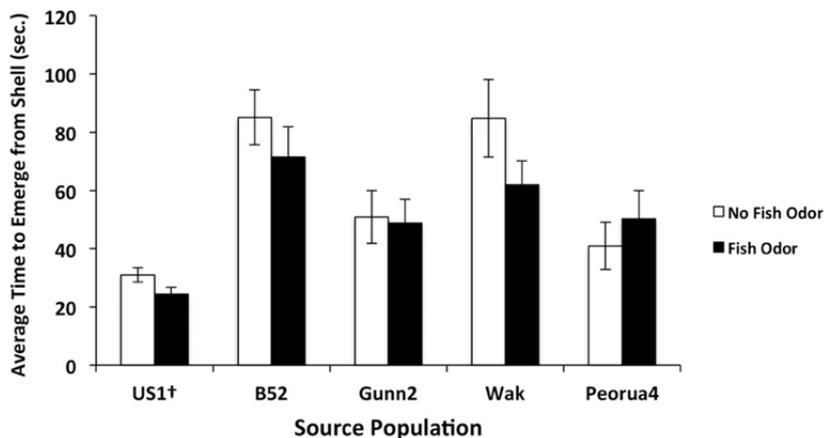


Figure 3. Mean emergence times of different clones of the New Zealand mud snail. Fish odor did not significantly influence emergence time. Error bars are standard errors.

predator cue (Table 4). In the post-hoc comparisons among introduced populations, we found that US3 exhibited a stronger photokinetic response than both US1 and US2, which did not significantly differ from each other (Table 5). There was a significant interaction between population and predator treatment in the analysis of all of the populations demonstrating that the populations differed in their response to the predator cue (Table 4). Paired t-tests on US1, US2, and US3 showed that the US1 and US2 populations both showed marginally significantly stronger photokinetic responses to fish cues (US1 – $t_{33} = -1.995$, $P = 0.054$; US2 – $t_{19} = -2.015$, $P = 0.058$), while the US3 population did not respond differently with or without the predator cue (US3 – $t_{19} = -0.156$, $P = 0.877$).

Emergence time

Emergence times significantly varied between populations, but the trait was not influenced by fish odor (Table 6; Figure 3). The predator cue also did not affect the populations of snails differently (no clone by predator interaction) (Table 6). Post-hoc tests revealed that the US1 population had a faster emergence time than all New Zealand native populations (Table 6).

Discussion

These results provide evidence that invasive populations of the New Zealand mud snail may utilize altered behavior in response to the detection of potential predators. In these experiments, since only single lineages of each clone were utilized, it is not possible to definitively attribute differences found to clone genotypes. However, the results are consistent with evidence for clone-dependent variation in behavior.

Table 5. Results of repeated-measures ANOVA comparing the photokinetic behavior of only introduced clones in response to fish odor as well as the results of a sequential Sidak post hoc test comparing the introduced clones to each other. The degrees of freedom is signified by df, the significance value by p, mean difference is MD, and SE denotes standard error.

Fully factorial model.

Source	df	Wald Chi-Square	p
Clone	2	18.62	< 0.001
Predator	1	5.42	0.020
Clone x Predator	2	2.66	0.265

Post-hoc comparison between introduced clones.

Clone	US2	US3
US1	MD = -3.84	MD = -16.34
	SE = 3.00	SE = 3.79
	P = 0.200	P < 0.001
US2		MD = -12.50
		SE = 4.09
		P = 0.005

We hypothesized that if these behaviors are advantageous for invasion, positive geotaxis and positive photokinesis should be stronger in invasive populations when exposed to fish stimulus, and the emergence time should increase when fish odor is present in invasive populations. The geotaxis experiment demonstrated that there are differences among populations in their geotactic response, but there was little evidence of an effect of the predator cue on behavior or differences among populations in their responses to the predator cue (Figure 1). However, the invasive US1 population demonstrated a trend towards increased movement downward when detecting the predator cue (Figure 1), suggesting that the US1 population may respond to the fish odor cue more strongly than other populations. The photokinesis experiment revealed that both invasive populations (US1 and US2) moved faster in light when

Table 6. Results of ANOVA comparing the log-transformed emergence times of clones in response to fish odor and the results of a post hoc Tukey test to compare each clone to each other. The US1 population emerged more quickly than any New Zealand native population, but no effect of predator cue was found.

Fully factorial model.

Source	df	F	p
Clone	4	23.89	< 0.001
Predator	1	0.79	0.375
Clone * Predator	4	0.68	0.608
Error	412		
Total	422		

Post-hoc comparison between clones.

Source Population	B52	Gunn2	Wak	Peorua
US1	MD = -0.413 SE = 0.052 p < 0.0005	MD = -0.201 SE = 0.052 p = 0.001	MD = -0.388 SE = 0.052 p < 0.0005	MD = -0.154 SE = 0.052 p = 0.027
B52		MD = 0.211 SE = 0.064 p = 0.009	MD = 0.025 SE = 0.064 p = 0.995	MD = 0.259 SE = 0.064 p = 0.001
Gunn2			MD = -0.186 SE = 0.064 p = 0.030	MD = 0.047 SE = 0.064 p = 0.947
Wak				MD = 0.234 SE = 0.064 p = 0.003

detecting fish than when not detecting fish; most other populations, including US3, did not. These results partially support our hypotheses. We failed to support our hypothesis concerning emergence time, as we found no effect of fish odor on behavior in the invasive population.

Introduced species can be less susceptible to predation than similar native species (Dick and Platvoet 2000; Gonzales and Burkhart 2004; Palmer and Ricciardi 2004; Barton et al. 2005; Kinzler and Maier 2006), and in some cases, the ability of an introduced species to detect and respond to predators may increase invasion success (Pennuto and Keppler 2008; Naddafi and Rudstam 2013). In addition, chemical cues can mediate interactions between native and invasive species (Raw et al. 2015). Responses to kairomones can differ between invasives and natives, and such differences have been suggested to contribute to invasion success in crayfish (Hazlett et al. 2003). In some instances, invasives have been found to be able to detect and respond behaviorally to unfamiliar predators (Grason and Miner 2012; Castorani and Hovel 2016). The work presented here is consistent with these findings. These experiments suggest that some populations of *P. antipodarum* may use plastic behavioral responses to kairomones to avoid predation by an unfamiliar predator.

The ability to detect predators can be very advantageous for introduced species. In New Zealand,

previous work showed that *P. antipodarum* detects and responds to a sympatric fish (Levri et al. 2012b) that is an active predator on the snail (Levri 1998). Previous experiments also demonstrated variation among populations in the snail's ability to detect and respond to sympatric and allopatric predators (Levri et al. 2012b). In the present experiments, all populations of snails utilized (except for possibly the US2 population) had no previous history with the fish used in these experiments, blacknose dace. In addition, the snails were lab reared, so none of the individuals had experienced fish odor prior to the experiment. The fact that some populations of *P. antipodarum* responded behaviorally to the unfamiliar predator while others did not, indicates variation in the ability to detect and/or in the decision to respond to this predator between populations. This variation was found in both introduced and New Zealand native populations of *P. antipodarum*. The result that some New Zealand native genotypes were able to detect and respond to this novel predator indicates that genetic variation exists within New Zealand populations for this trait. Therefore, the possibility exists that introduced populations that detect and respond to this predator may have arrived in their new environment with this trait already in place. It is also possible that the introduced populations evolved the trait independently or that some aspect of the trait was in the founding populations, and it was refined

by selection in its new environment. It is interesting that the two introduced populations that did behaviorally respond to the odor of unfamiliar fish represent the two most invasive clones of the New Zealand mud snail in North America. The non-invasive US3 population did not appear to behaviorally respond to the predator. Thus, it is possible that the ability to detect and respond to novel predators may be an important trait influencing the invasion success of this species.

In previous experiments examining the effect of fish predators in New Zealand on behavior, populations of the New Zealand mud snail from two different lakes were found to detect and respond behaviorally to a fish from their home lake more strongly than the same species of fish from a different lake (Levri et al. 2012b). Thus snails appear to be locally adapted to their home predator. However, snails from Lake Alexandrina responded to fish by altering their geotactic response while snails from Lake Peorua responded by changing their photokinetic response, suggesting that selection in the two different lakes resulted in different behaviors being utilized to avoid predators. Here we found a somewhat similar result in that there may have been variation in the types of behaviors influenced by a predator. Two of the introduced populations showed changes in behavior in response to fish. Both the US1 and US2 populations seemed to increase their positive photokinetic response in response to the predator cue, while the US1 population showed some evidence of positive geotaxis when exposed to the cue.

Levri and Clark (2015) demonstrated that there was variation in geotactic and photokinetic behaviors (as well as other behaviors) between North American introduced populations of *P. antipodarum* and some native New Zealand genotypes. Although this previous work did not consider a predator cue, most of the results from those experiments were consistent with the work described here. However, there were some differences. In the previous work, the US1 population exhibited strong positive geotactic responses in both light and dark conditions. In the present experiment, the US1 population did not exhibit positive geotaxis in control water but did in water with fish odor (Figure 1). An important difference between the two sets of experiments is that Levri and Clark (2015), used water taken directly from a stream that houses a population of blacknose dace in the behavioral trials. Thus, the water from the previous experiment likely had a predator chemical cue (kairomone), making the difference in results between the two experiments for the US1 population more consistent with the present findings.

The US1 population exhibited a significantly shorter emergence time than the New Zealand native genotypes used in this experiment (Figure 3). It is not entirely clear whether a faster emergence time would be beneficial, detrimental, or neutral with regard to invasion success. We had originally hypothesized that a slower emergence time when detecting fish would be beneficial for the snail to avoid movement and the detection by the predator. Since the behavior was not influenced by fish odor, this hypothesis was not supported. An overall faster emergence time (like that exhibited by the US1 population) could result in a greater amount of time foraging compared to other genotypes, especially if detection by predators is unlikely.

The results here provide evidence that at least some populations of the New Zealand mud snail possess the ability to detect and behaviorally respond to the presence of a novel piscine predator. The results are also consistent with the hypothesis that detection of a novel predator and avoidance behaviors may play an important role in explaining the invasion success of different genotypes of the New Zealand mud snail.

Acknowledgements

We thank T.J. Clark, Megan Radyk, Frank Menaquale, and Christina Lehman for assistance in the lab, Mark Oswalt for logistical support, and Maurine Neiman (New Zealand) and Mark Dybdahl (Idaho, Wyoming) for donating the New Zealand and western US clones. We also thank Maureen Levri, Jennifer Rehage, Kit Magellan, and two anonymous reviewers for comments on earlier versions of the manuscript. This work was funded by grants from Penn State – Altoona and the Penn State – Altoona Division of Mathematics and Natural Sciences.

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