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Research Article

Predator detection and a possible dispersal behavior of the invasive New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843)

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Co-Editors' Note: This paper is a contribution to the **Behaviour in Aquatic Invasions** Special Issue of *Aquatic Invasions*. Papers in this Special Issue explore how behaviour contributes to invasion success; native species' behavioural strategies that reduce the impacts of invasions; how knowledge of behaviour can enhance management of invasive species; and potential effects of climate change on the behavioural impacts of aquatic invasive species.

Citation: Levri EP, Luft R, Li X (2019) Predator detection and a possible dispersal behavior of the invasive New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843). *Aquatic Invasions* 14 (in press)

Received: 10 August 2018

Accepted: 30 January 2019

Published: 27 May 2019

Handling editor: Kit Magellan

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Abstract

Behavior can play a large role in invasion success. Of particular importance may be the ability of an invader to detect and respond to unfamiliar potential predators. We examined a behavior related to dispersal in populations of the New Zealand mud snail (*Potamopyrgus antipodarum*) in response to chemical cues from crayfish and piscine predators. Populations of the snail isolated from North America and its native New Zealand were used in two separate experiments. In both experiments, groups of snails were placed in water either with or without predator cues, and the number of snails found floating attached to the surface tension of the water after ten minutes was noted. In the first experiment, a crayfish odor cue was used (from *Procambarus clarkii*), and, in the second, two fish, blacknose dace (*Rhinichthys atratulus*), and separately zebrafish (*Danio rerio*), were used to provide the chemical cues. In both experiments there was significant variation among populations in the proportion of snails that exhibited the floating behavior, with native populations tending to exhibit less floating behavior. The snails generally increased floating behavior in response to both crayfish and blacknose dace but not to zebrafish. In addition, we found no differences between populations of snails in their response to predator cues. Surprisingly, we also found that there were differences in floating proportions between populations of the same invasive clonal genotypes. These results suggest this behavior, which may be related to dispersal within water bodies, may be important in partially explaining the invasion success of the most common invasive clones in North America.

Key words: kairomone, crayfish, dace, zebrafish

Introduction

There is a growing body of evidence demonstrating that behavior can play an important role in invasion success (Holway and Suarez 1999; Pavlov et al. 2006; Dick 2008; Phillips and Suarez 2012; Weis and Sol 2016). Variation in behavior or other traits between populations or individuals of the same species can be important in determining invasion success as different traits may be advantageous at different stages of the invasion process or in different ecosystems (Fogerty et al. 2011; Chapple and Wong 2016). Thus, variation in certain behavioral traits within and between populations may be important in the invasion process. The types of behaviors that influence

invasion success are varied but include predator avoidance (Levri and Clark 2015), intra- and interspecific aggression, boldness, dispersal, changes in activity and changes in social behaviors (reviewed in Rehage et al. 2016). In aquatic systems, invasive crayfish (Bubb et al. 2006) and mosquitofish (Rehage and Sih 2004) exhibit greater tendencies to disperse than similar native species. Invasive signal crayfish demonstrate increased aggressive behavior and boldness compared to native species (Pintor et al. 2008), and invasive mosquitofish that disperse more exhibit greater exploratory behavioral tendencies (increased activity) (Rehage and Sih 2004; Cote et al. 2011).

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843) (NZMS), is a world-wide aquatic invader (Alonso and Castro-Díez 2008, 2012) with well-established populations in Australia (Ponder 1988), Europe (Ponder 1988), Asia (Shamida and Urabe 2003; Son 2008; Nasser and Son 2009), North America (Bowler 1991; Zaranko et al. 1997), and South America (Collado 2014). In its native range in New Zealand, it is composed of mixed populations of sexual and asexual individuals, with multiple clones inhabiting the same area (Winterbourn 1970; Lively 1987). However, in its introduced range, the NZMS is composed entirely of asexual clones (Alonso and Castro-Díez 2008). The North American populations are composed of at least three clones (Proctor et al. 2007): US1, which is distributed widely in the American West as well as in several locations in the Eastern US (Finger et al., *in review*); US2, which is mostly confined to the Laurentian Great Lakes and associated streams (Zaranko et al. 1997; Levri et al. 2008; Levri and Jacoby 2008; Levri et al. 2012a; Finger et al., *in review*); and US3, which does not appear to be invasive and is found in only one location in the Snake River in Idaho near the location where US1 was first discovered in the 1980's (Proctor et al. 2007).

Dispersal ability can play an important role in the invasion process (reviewed in Rehage et al. 2016), and an increasing number of dispersal vectors are expected to lead to greater invasion success (Ozinga et al. 2004). The NZMS is thought to use multiple modes of dispersal to expand its range between and within continents with much of its dispersal aided by human activities. It is thought that the US1 and, probably, the US3 clones originally arrived in the Snake River in Idaho in North America unintentionally via aquaculture products such as trout or trout eggs from Australia or New Zealand (Proctor et al. 2007). The US2 clone likely arrived from Europe (as it is identical to one of the three European clones) via ballast water released from international shipping in Lake Ontario (Zaranko et al. 1997). Humans have also been very important in the dispersal of the snail within continents as it is presumed that recreational water users, such as fishermen, move individuals from one water body to another (Proctor et al. 2007). Within water bodies, humans likely also aid in dispersal, but other modes may also be important. Vinson and Baker (2008) demonstrated that a large proportion of NZMS that were consumed

by trout actually passed through the fish alive. Some reports also suggest that snails, including NZMS, can be easily spread attached to waterfowl (Haynes et al. 1985; van Leeuwen and van der Velde 2012; van Leeuwen et al. 2012). Thus, the rate of spread could be aided by the movement of fish and potentially other vertebrates. The NZMS can also effectively move downstream attached to floating vegetation (Ribi 1986; Richards et al. 2001). Natural upstream movement can occur by crawling, and the NZMS has been found to be positively rheotactic (tends to move into the current) (Levri and Clark 2015).

An additional mode of dispersal may also be important. *Potamopyrgus antipodarum* exhibits a floating behavior in which the snail crawls to the surface of the water and attaches to the underside of the water surface using surface tension. In water with little turbulence, the snail could potentially flow downstream for great distances in this manner. This behavior has been observed in the lab (Levri 1998; Levri and Clark 2015) and in the field (Levri, *unpublished data*). The behavior appears to be influenced by chemical cues as NZMS sensing dead conspecifics were more likely to exhibit floating behavior (Levri 1998). Floating behavior has been found to aid in dispersal of other species. For example, *Hydrobia ulvae* juveniles on the British coast and in the North Sea significantly increased their dispersal distances using floating (Little and Nix 1976; Armonies and Hartke 1995). Thus, it is quite possible that floating may serve a dispersal function for NZMS as well. The most widespread NZMS clone in North America, US1, exhibited floating behavior at a greater rate than other introduced or native clones (Levri and Clark 2015) suggesting that this behavior may play some role in its invasion success.

One reason to disperse is to avoid predators (Poethke et al. 2010), and this may also be important in invasion success (Weis 2016). The avoidance response is often associated with the detection of a chemical cue (kairomone) (Lass and Spaak 2003). Some aquatic gastropods disperse or move out of the water in response to the detection of potential predators including crayfish and fish (Alexander and Covich 1991a, b; Covich et al. 1994; Turner et al. 1995; McCarthy and Fisher 2000; Dalesman et al. 2007, 2009). The NZMS has demonstrated the ability to detect and behaviorally respond to piscine predators in its native range in New Zealand (Levri 1998; Levri et al. 2012b) as well as in its introduced range (Levri et al. 2017). An introduced species, such as the NZMS, may gain an advantage if it can detect and respond to a novel potential predator. In some cases, novel predators are capable of eliciting defense responses (Levri et al. 2017) and in other cases not (Gomez-Mestre and Diaz-Paniagua 2011), but unfamiliar prey can also learn to avoid novel predators (Polo-Cavia and Gomez-Mestre 2014).

The purpose of this study was to determine if there were variation both between and within clonal genotypes in the floating behavior of the NZMS in response to the odor of novel crayfish and piscine predators. We used

clonal genotypes isolated from New Zealand and genotypes isolated from invasive populations in the United States. For some genotypes, we used snails from more than one location resulting in multiple populations of the same clonal genotype. We predicted that the most successful introduced clones in North America, US1 and US2, would detect and respond to the potential predators more than other clones, including native clones. To test this prediction, we performed two independent experiments where we assessed the proportion of snails exhibiting floating behavior in plain water and water treated with predator odor.

Materials and methods

We conducted two separate experiments to examine the influence of predator chemical cues on the floating behavior of NZMS. We conducted the first experiment in 2013 and used odor from red crayfish (*Procambarus clarkii*) obtained from Carolina Biological Supply. There are two widespread freshwater crayfish species in New Zealand. Their distributions significantly overlap that of NZMS (McDowall 2005), and at least one species is known to consume NZMS (Hollows et al. 2002). The crayfish species used here, *Procambarus clarkii*, is a common native in the southern United States and is introduced and invasive in other North American locations (and other parts of the world) (Hobbs et al. 1989), but it is not known if it interacts with invasive NZMS populations in North America. We housed a single crayfish in a 19 L aquarium with aeration and fed the crayfish small amounts of tuna three times per week in a separate feeding tank. We used a separate feeding tank to minimize the influence of the tuna on the odors in the water, however, it is possible that some tuna odor or post-digestion tuna cues were present.

The data from the control group in the crayfish odor experiment were previously presented in a paper that demonstrated variation between populations in multiple behaviors including floating (Levri and Clark 2015). The crayfish odor treatment was conducted at the same time as the control and was not published in that paper but is reported here. Levri and Clark (2015), showed (among other things) that there were differences between NZMS populations in their propensity to exhibit floating behavior.

In 2017, we conducted a second experiment using cues from two fish, blacknose dace (*Rhinichthys atratulus* Hermann, 1804) and zebrafish (*Danio rerio* Hamilton, 1822). Blacknose dace is a potential predator of NZMS as its distribution overlaps stream populations of NZMS in the eastern US and it has been found to consume gastropods (Tarter 1970). However, not all of the introduced populations used in the experiments may have encountered the fish. There is little evidence, however, that zebrafish, which are not naturally found in North America, consume mollusks (Spence et al. 2007). We used zebrafish in this experiment to determine whether the

Table 1. Population Sources and Experimental Designs. The experimental designs of the crayfish odor (a) and fish odor (b) experiments. The numbers in the boxes indicate the number of replicate trials run for each population in each treatment with all replicates including 20 individual snails from that population ($n = 20$).

a.

Population	Source	Collector & Year	Control (Plain Water)	Crayfish Odor
US1-WY (introduced)	Polecat Creek, WY	M. Dybdahl, 2010	4	4
US2-O1 (introduced)	Unnamed stream, NY	E. Levri, 2012	4	4
US2-O29 (introduced)	Fish Creek, NY	E. Levri, 2012	3	3
US3 (introduced)	Snake River, ID	J. Finger, 2010	2	2
Peorua4 (native)	Lake Peorua, South I., NZ	M. Neiman, 2009	4	4
B52 (native)	Lake Alexandrina, South I., NZ	M. Neiman, 2009	4	4
Gunn2 (native)	Lake Gunn, South I., NZ	M. Neiman, 2009	4	4

b.

Population	Source	Collector	Control (Plain Water)	Blacknose Dace Odor	Zebrafish Odor
US1-WY (introduced)	Polecat Creek, WY	M. Dybdahl, 2010	4	4	4
US1-PA. (introduced)	Spring Creek, Centre Co., PA	E. Levri, 2014	4	4	4
US2-O1 (introduced)	Unnamed stream, Niagara Co., NY	E. Levri, 2012	4	4	4
US2-O29 (introduced)	Fish Creek, Niagara Co., NY	E. Levri, 2012	4	4	4
Gunn2 (native)	Lake Gunn, South I., NZ	M. Neiman, 2009	4	4	4
Map (native)	Lake Mapourika, South I., NZ	M. Neiman, 2009	4	4	4

odor of any species of fish causes behavioral changes in the snails or only the odor of potential predators. We housed three adult blacknose dace in one 37.9 L aquarium, and six adult zebrafish in another. We used different numbers of fish to maintain similar biomass in the two fish tanks. Both fish species were fed fish flakes (TetraMin Tropical Flakes) daily.

Snail collection and maintenance

Native NZMS samples were originally obtained from populations in multiple New Zealand lakes in 2009 (sources shown in Table 1). Because the two experiments were conducted a few years apart the clones available differed between the experiments except for Gunn2. The introduced source populations used in these experiments were US1-WY, US1-PA (this population was only utilized in the fish odor experiment), US2-O1 (collected from a stream in western Niagara County, New York in 2012, US2-O29, and US3 (this clone was only used in the crayfish odor experiment). All native clones and US3 were started from isolated females in the lab. Most of the New Zealand clones were genotyped using mitochondrial haplotype data to make sure that the individual isolates were truly different genotypes (Neiman et al. 2010, 2011; Neiman, *unpublished data*). The snails of the Gunn2 clone have never been genotyped but they all descended from the same original captured clonal female. They are not likely to be the same clonal genotype as other snails used in these experiments because clones from different lakes have separate origins (Paczesniak et al. 2013). 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 before this study;

typically, in the lab the snails will go through one to two generations per year (Neiman, *pers. comm.*). We maintained the individual snails in our lab for at least six months before the experiments.

Before each experiment, we maintained all populations in one-liter plastic bins in water containing 3 ppt seawater, since NZMS have been found to grow faster in 3 ppt seawater than in freshwater (Drown et al. 2011). We fed the snails *Spirulina* powder and changed their water three times per week. Two weeks before the experiments began, we placed the snail containers in ambient sunlight on a windowsill to acclimate them to a natural light-dark cycle. Immediately before the experiments, we measured the length (shell apex to aperture lip) of each snail, and only snails 4.0–5.0 mm were used in the experiments in order to control for any effect of size on behavior. Previous studies have found that the amount of time spent foraging was size dependent with larger snails foraging less than smaller (Levri and Lively 1996).

Experiments

We assessed floating by placing snails of the same population, 20 per trial, in a 12 cm square plastic container with a depth of 6 cm filled with 0.8 L of solution (aged tap water for controls and aged tap water with odor cues for treatments). Initially snails were retracted into their shells at the bottom of the container. After ten minutes we noted how many snails were attached to the surface of the water (other snails were found on the bottom and attached to the sides of the container). In the crayfish experiment, we assessed snails of each population under two conditions: 0.8 L of aged tap water and 0.7 L of aged tap water plus 0.1 L of water taken from the tank that held the crayfish. We used the same ratio of aged tap water to predator water in the fish experiment, but with three treatments: aged tap water alone (control); aged tap water with blacknose dace water; and aged tap water with zebrafish water. In each trial of each experiment, we used a group of snails only once, thus, the snails used between treatments and trials were independent from each other. We used separate containers for each treatment which were cleaned between trials. We used 40–80 snails from each clonal lineage resulting in 2–4 replicates of each population (Table 1). We conducted the trials for each experiment between 09:00 and 12:00 each day over 5 days. Trials utilizing each treatment were conducted each day of the experiments. The snails and the water used in the experiment were maintained in the same climate-controlled room which was held at a temperature of 17 °C.

In the crayfish experiment, four introduced and three native clonal populations were used (Table 1a). We ran four trials of each treatment for US1, US2-O1, Peorua4, B52, and Gunn2. Three trials were run of each treatment for US2-O29 and two of each treatment for US3 because fewer

snails were available from these populations. In the fish odor experiment, we used four introduced and two native populations with four replicated trials for each population (Table 1b).

Statistical Analyses

The data from both experiments were analyzed using logistic regression to determine the ratio of probability (probability of success : probability of failure) of a snail floating at the surface as predicted by treatment (plain or predator water) and snail population. A logit function was used over the proportion because the proportion ranges between 0 and 1 while the logit function ranges between negative and positive infinity and is thus more suitable for a regression because there is no restriction on the values. As the log of zero is undefined, and multiple populations yielded results where no snails were found to float, one was added to the number of floating snails in each trial. The analysis required that one treatment and one population were chosen in each experiment as a reference for comparison of all other treatments and populations. The control group was chosen when examining the effect of treatment, and the US1-WY population was selected when examining the influence of population. The logistic regression analysis was initially performed including the main effects plus the interaction term. If the interaction term was not close to statistically significant, the analyses were redone using only main effects to increase statistical power. The analyses estimate the ratio of probability of the snails floating on the surface, thus, a positive value indicates that a particular variable increased the probability of floating, and a negative value indicates that the variable decreased the probability of floating. If a significant main effect of population was found in any experiment, post hoc tests were performed using the same logistic regression procedure and changing the reference population to determine if populations differed from each other, such that each population was compared to every other. Since we found no significant interaction between treatment and population in either experiment, we did not test for differences between treatments within each population. R Version 3.2.3 (R Development Core Team 2015) was utilized for all statistical analyses.

Results

Crayfish odor

When examining the effect of crayfish odor on floating behavior, we found significant effects of both crayfish treatment and snail population on the ratio of probability (henceforth probability) of floating on the surface (Table 2). The presence of crayfish odor resulted in significantly greater probability of individuals floating in all populations compared to the control (Table 2; Figure 1). Compared to the US1-WY population, the probability

Table 2. Results from logistic regression analysis of the crayfish odor experiment. Populations are compared to US1-WY.

Variable	Estimate	Standard Error	z	P
Intercept	-1.3445	0.2166	-6.208	< 0.0001
Treatment	0.4648	0.2182	2.130	0.033
US3	-1.6715	0.4954	-3.374	0.0007
US2-01	-1.2323	0.3250	-3.791	0.0002
US2-O29	-1.7449	0.4288	-4.070	< 0.0001
Peorua4	-1.7835	0.3871	-4.607	< 0.0001
B52	-1.9079	0.4046	-4.715	< 0.0001
Gunn2	-1.1609	0.3186	-3.644	0.0002

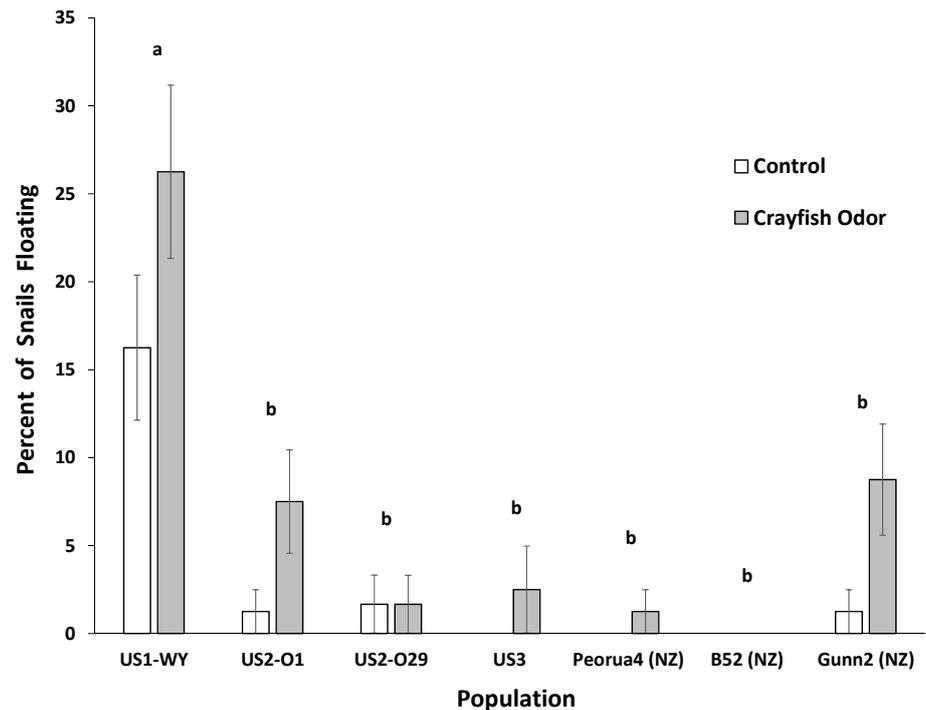


Figure 1. The percentage of snails exhibiting floating behavior in control conditions and in response to crayfish odor. NZ indicates that the population is native to New Zealand. Shared letters above the bars indicated that the populations are not significantly different in pairwise contrasts. Crayfish odor significantly increased the odds of floating, but a significant interaction between crayfish odor and population was not found (Table 2). Error bars are 95% confidence intervals of the overall percentage of snails found floating across all replicates.

of floating in all other populations were found to be significantly lower (Table 2; Figure 1). Utilizing specific comparisons between populations, we found that none of the introduced populations other than US1-WY had probabilities of floating significantly different from each other (Figure 1; $P > 0.28$ in all cases). A test for an interaction between crayfish odor treatment and populations showed no evidence that crayfish odor influences the probability of floating differently among populations ($P > 0.34$ in all cases).

Fish odor

In the fish odor experiment, chemical cues from blacknose dace resulted in significantly greater probability of floating compared to the control and zebrafish cue (Table 3; Figure 2), and the zebrafish cue did not result in

Table 3. Results from logistic regression analysis of the fish odor experiment. Populations are compared to US1-WY.

Variable	Estimate	Standard Error	z	P
Intercept	-1.7125	0.1975	-8.669	< 0.0001
Dace	0.4677	0.1724	2.713	0.0067
Zebrafish	0.0489	0.1806	0.271	0.79
US1-PA	1.0670	0.2103	5.074	< 0.0001
US2-O1	-0.7292	0.2711	-2.690	0.0072
US2-O29	0.6538	0.2158	3.029	0.0025
Gunn2	-0.9297	0.2859	-3.250	0.0012
Map	-1.4760	0.3391	-4.353	< 0.0001

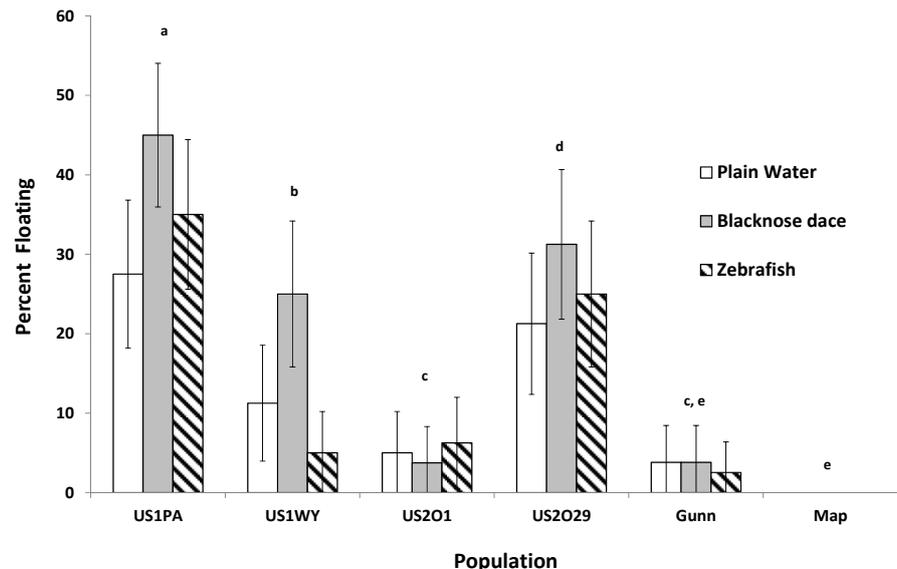


Figure 2. The percentage of snails exhibiting floating behavior in control conditions and in response to two fish odors, blacknose dace and zebrafish. NZ indicates that the population is native to New Zealand. Shared letters above the bars indicated that the populations are not significantly different in pairwise contrasts. Odor from blacknose dace was found to significantly increase the odds of floating compared to zebrafish odor and control water, but a significant interaction between fish odor and population was not found (Table 3). Error bars are 95% confidence intervals of the overall percentage of snails found floating across all replicates.

increased probability of floating compared to the control. As in the crayfish experiment, the US1-WY population showed a significantly different probability of floating behavior compared to all other populations, with the US1-WY having a greater probability of floating than US2-O1 and lower probability of floating than US1-PA and US2-O29 (Table 3, Figure 2). Specific comparisons of introduced populations demonstrated that US1-PA had a significantly greater probability of floating than US1-WY ($P < 0.0001$), US2-O1 ($P < 0.0001$), and US2-O29 ($P = 0.03$). US2-O1 had a significantly lower probability of floating compared to US1-WY ($P = 0.0072$) and US2-O29 ($P < 0.0001$). A test for an interaction between fish odor treatment and population showed no evidence that fish odor influences the probability of floating differently among populations ($P > 0.16$ in all cases).

Discussion

The results suggest that floating behavior in NZMS is prevalent in invasive populations and that at least one invasive clone (US1) increases their

floating response in response to potential predators. Our approach here was to compare multiple populations of the same species, some invasive and others not, to determine if the invasive populations exhibited a greater propensity to disperse in response to predators than the non-invasive populations. We found that for both crayfish (Figure 1) and blacknose dace (Figure 2), NZMS did exhibit more floating behavior when exposed to predator-tank water than when exposed to the control. We also found significant differences among populations in the frequency of floating behavior. However, with both crayfish and fish, there were no significant differences between NZMS populations in floating behavior in response to predator cues; i.e. there was no evidence that the populations used in these experiments responded to predator cues by floating differently compared to each other (no significant treatment by population interaction). We infer that increased floating behavior in this species results in greater probability of dispersal, and that increased dispersal would be beneficial for invasion success. We have noticed this behavior both in the lab and in the field (Levri 1998), and other gastropod species have been demonstrated to disperse in this manner (Little and Nix 1976; Armonies and Hartke 1995; Johnson et al. 2001). Thus, we believe that it is highly likely that this behavior does result in significant dispersal for the species, especially in flowing waters.

The US1 clone exhibited a higher frequency of floating behavior than most other clonal genotypes. This result is consistent with our hypothesis that dispersal behaviors should be more represented in highly invasive genotypes. The US2-O29 population also showed a greater propensity to float compared to most other populations in the fish predator cue experiment (Figure 2). Like US1, the US2 clone is a highly successful invader in North America and Europe (Zaranko et al. 1997; Levri et al. 2008). Native genotypes show some evidence, at least in the fish experiment, of a lower frequency of floating behavior. The US3 clone in the crayfish odor experiment demonstrated little propensity to float. This is also consistent with our hypothesis as the US3 clone does not appear to be invasive. Unexpectedly, we also found significant differences in floating behavior between populations of the same clone. Both US1 and US2 clones showed variation between populations in the fish odor experiment. This difference could be explained by at least two possibilities. First, it could be that differences between populations resulted from differences in their laboratory environment. However, we attempted to make the environmental conditions as similar as possible among the populations in the lab where the snails used in these experiments experienced multiple generations by keeping all of the containers in the same location and changing the water at the same times. It is also possible that variation within clonal genotypes could be the result of evolution in the behaviors between the populations. This variation could result from selection acting differently on populations

in different locations or due to neutral evolution. While genetic differences between populations remains a possibility, because NZMS reproduce parthenogenetically, their ability to create genetic variation between individuals is limited to mutation. Thus, it is expected that their rates of evolution should be slow. However, it has been found that the mutation accumulation rate within the parthenogenetic lineages of NZMS is greater than sexual populations of the same species (Neiman et al. 2010). The hypothesis that the populations are adapting to their new environments within their invaded range is intriguing and warrants additional investigation.

The ability to detect and respond to novel predators could be of great benefit to an introduced species. Some invasives have the ability to detect and respond to unfamiliar predators (e.g. Grason and Miner 2012; Castorani and Hovel 2016). Some evidence suggests that some invasives respond more to alarm odors than native species of the same genus (Hazlett 2000; Hazlett et al. 2003). Crayfish odor resulted in an overall increased probability of floating for the NZMS populations (Figure 1). Crayfish tend to crush their prey as they forage in the benthos. Other studies have found that common behavioral responses of gastropods to crayfish include moving upward and, in some cases, out of the water (Alexander and Covich 1991a, b; Covich et al. 1994). Movement to, and attaching to, the surface as seen in this experiment would seem to be an effective antipredator behavior against crayfish. While NZMS did respond to crayfish by floating more, we did not detect any differences between populations in their response to the crayfish odor cue. This could be because all populations have a similar ability to detect and respond to the predator. However, it could be that the response to crayfish is not especially strong, and larger sample sizes are needed to detect the differences between them. In this experiment, several populations showed floating percentages in the presence and absence of the crayfish cue of less than 5% (Figure 1). These rates make detecting differences difficult in those populations. It should be pointed out that in other experiments, population-specific behavioral responses to predators have been found in this species (Levri et al. 2017). In those experiments, invasive US populations displayed different photokinetic and geotactic responses to other populations when presented with blacknose dace, and the responses were consistent with a pattern of more effective predator avoidance.

Fish odor also resulted in an increased percentage of floating individuals across populations, but only for blacknose dace (Figure 2). Again, no significant population by predator treatment interaction was found indicating that no differences in response to fish were found among populations. Thus, while the snails in the dace treatment did float more, this was consistent across populations. Zebrafish odor failed to elicit increased floating behavior compared to the control. It is possible that the

zebrafish in the tank did not produce enough of the chemical cue to elicit the response or that zebrafish do not produce a cue that the snails can detect. We did attempt to keep the fish biomass similar between the blacknose dace and zebrafish tanks, and they were fed an identical diet. Both blacknose dace and zebrafish are novel potential predators to most of the populations used in this experiment. It is interesting that the snails responded to blacknose dace, a species known to consume molluscs, and not zebrafish, which do not feed on snails. An adaptive explanation would suggest that the NZMS is more capable of detecting fish that are more likely to be predators. This could happen if potential predators produce a different set of chemical cues than non-predators. More work needs to be done to determine if this is the case. In New Zealand the snail appears to be well-adapted to detect and respond to a native fish, the common bully (*Gobiomorphus cotidianus*). NZMS from one lake detect and behaviorally respond geotactically and photokinetically to bullies from their own lake more than they do to the same fish species from a different lake (Levri et al. 2012b). Thus, it appears that piscine predators can be an important selective force on behavior. Numerous other studies have demonstrated that prey are capable of chemically detecting and responding to predators (Alexander and Covich 1991a, b; Dodson et al. 1994; Covich et al. 1994; Levri 1998; Siepielski et al. 2016; Gancedo and Ituarte 2018), and in some cases these behaviors are dependent upon the magnitude of the threat posed by the predator (Helfman 1989).

There is a growing body of evidence indicating that invasive species may exhibit different behavioral traits in their introduced range compared to their native range that likely play a role in invasion success (Candler and Bernal 2015; Levri and Clark 2015; Blight et al. 2017; Gruber et al. 2017; Levri et al. 2017; Reisinger et al. 2017). Here we provide evidence of variation within and among clones for a behavior that appears to be associated with dispersal in a world-wide invasive species. The snail has the ability to detect and respond to multiple potential predators in its introduced range. This antipredator behavior may provide an important advantage to this species and improve its invasive ability. Increased response to predators in the invaded range could result from several processes: 1. Increased floating behavior could evolve in the introduced range due to selection occurring in each population; 2. Variation in floating behavior exists in the native range of New Zealand, and those clones that float more may be more likely to be introduced; 3. Variation in floating behavior exists in New Zealand, and, after introduction of multiple clones, clones that float more are selected for and are more likely to become widespread; or 4. One or more of the above work together. The fact that there is some floating response in some natives (e.g. Gunn2 in the crayfish experiment) suggests that the ability to detect and respond to novel predators by floating exists in the native range.

Acknowledgements

We thank T. J. Clark, Frank Menaquale, Bryce Brindle, Maureen Levri, James Levri, Joseph Levri, Matthew Levri, and Adrianna Levri for assistance in the lab, Mark Oswalt for logistical support, and Maurine Neiman and Mark Dybdahl for donating the New Zealand and western US clones respectively. We also thank Kit Magellan, Maureen Levri, and anonymous reviewers for comments on earlier versions of the manuscript.

Funding

This work was funded by grants from the Mid-Atlantic Panel on Aquatic Invasive Species (<http://www.midatlanticpanel.org>), Penn State – Altoona, and the Penn State – Altoona Division of Mathematics and Natural Sciences. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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