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ORIGINAL PAPER

Parallel variation among populations in the shell morphology between sympatric native and invasive aquatic snails

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Abstract A phenotypic response, either plastic or evolved, is often required for successful invasion of novel environments. Populations of the invasive snail *Potamopyrgus antipodarum* have colonized a wide range of environments in the western U. S. since 1985, but the extent of plastic adjustment and evolved adaptation to local environments is largely unknown. We examined variation in shell morphology among four sites in the Snake River, Idaho, including both still-water and free-flowing river habitats and compared the variation to that of a native snail (*Pyrgulopsis robusta*) using geometric morphometric techniques. Using Generalized Procrustes analysis, we tested for phenotypic responses by determining (1) whether *Po. antipodarum* from the four locations differed in shell morphology, and (2) whether these snails exhibited corresponding shell shape variation with sympatric populations of a native snail. Both native and invasive snails exhibited similar variation in shell morphology across three of the four sites. The Canonical Variate assignment test grouped 85 % of both snail species to their rightful sample site. In addition, the Principal Component Analysis displayed similar patterns of shell variation across the four sites, indicating parallel variation in shell shape. For three of the four sites, both the native and invasive snails exhibited differences in shell shape consistent with water flow variation (still-water versus fast free-flowing river). Taken together, these results suggest that the shell shape of the invasive snail has changed either through plasticity or evolution, and that both native and invasive snail populations responded to local environmental conditions in a similar manner.

Keywords Biological invasions · Phenotypic plasticity · Adaptive evolution · Shell morphology · *Potamopyrgus antipodarum* · *Pyrgulopsis robusta*

Introduction

A species colonizing a novel range may need to change phenotypically for populations to grow under unfamiliar abiotic and biotic conditions. As a consequence, traits linked to fitness are expected to vary in established invasive species across spatially heterogeneous environments. Variation in these traits may be genetically or epigenetically based, or a plastic response to environmental gradients (Lee 2002; Richards et al. 2006; Prentis et al. 2008). Phenotypic

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plasticity can promote invasion success across environmental gradients through the production of environmentally-induced phenotypes by a given genotype (Baker 1965; Stearns 1989; Agrawal 2001; Yeh and Price 2004). Plasticity itself is a product of evolution that can be selected for during colonization of novel habitats (Richards et al. 2006). However, once a plastic genotype becomes fixed in a population, any phenotypes it expresses are generally considered not to be heritable (Ghalambor et al. 2007), but might be based on epigenetic inheritance (Bonduriansky et al. 2011). Both adaptive evolution and epigenetic transgenerational plasticity allow the rapid production of adapted genotypes specialized for different habitats, thereby allowing establishment and spread of invasives across broad ranges (Lee 2002; Lee and Gelembiuk 2008; Bonduriansky et al. 2011). The capacity to respond adaptively, whether plastic or evolved, to environmental gradients might influence the success of an invasive species in establishing across a wide geographic range.

We examined shell shape responses within invasive populations of a freshwater snail, *Potamopyrgus antipodarum*, the New Zealand mud snail, near the initial focal area of its recent North American invasion (Proctor et al. 2007). This snail is parthenogenetic in its invasive range (Zaranko et al. 1997), and single *Po. antipodarum* clonal genotypes have spread across environmental gradients in the western United States, Europe, and the Great Lakes (Ponder 1988; Hauser et al. 1992; Dybdahl and Drown 2011). Since 1985, *Po. antipodarum* has spread rapidly along the Snake River (Kerans et al. 2005; Hall et al. 2006), which has considerable abiotic spatial variation in water chemistry, temperature, and especially water velocity (Hershler et al. 2010). The pattern of establishment and spread by clonal lineages lacking variation at neutral genetic markers suggests that adaptive phenotypic plasticity might be necessary for invasion success, but some studies suggest there is potential for genetic variation among *Po. antipodarum* population in the Columbia and Snake Rivers (Dybdahl and Kane 2005; Hershler et al. 2010). In this paper we address the following questions: (1) do invasive populations exhibit variation in shell shape, which is known to influence fitness across different environmental conditions, and (2) if so, how does shell shape variation compare to that in a native species that is presumed to be adapted to local environments?

To answer these questions we examined variation in *Po. antipodarum* shell morphology using geometric morphometrics. Snail shells are an important component related to a snail's overall fitness and exhibit considerable plasticity (Kemp and Bertness 1984; Vermeij 1995; Minton et al. 2011). Environmental forces such as current velocity, temperature, and predator abundance drive the evolution of shell morphology (Struhsaker 1968; Janson and Sundberg 1983; Vermeij 1995; Rolan-Alvarez et al. 1997; Bourdeau 2009). In its native range, *Po. antipodarum* exhibits considerable morphological variation in response to water flow (Haase 2003), predation risk (Holomuzki and Biggs 2006), and parasitism (Negovetic and Jokela 2001). However, little is known about shell morphology and processes of adaptation in the invaded range of these widespread snails.

Water velocity is often a strong agent of selection for aquatic snails (Vermeij 1995). For example, snail shells in high flow environments often exhibit short spires and large apertures, which in turn reduce lift and drag pressure while maximizing foot size for better attachment (Dussart 1987; Statzner and Holm 1989; Vermeij 1995; Minton et al. 2011). For *Po. antipodarum* in its native range, Haase (2003) found clinal variation in shell morphology in response to water flow to be the product of phenotypic plasticity; snail shell size increased in response to increasing water velocity.

In the western U.S., invasive populations of *Po. antipodarum* inhabit a wide variety of habitats, from still-water reservoirs and estuaries to high velocity flow in rivers (Dybdahl and Drown 2011). There is considerable shell variation among three geographically distinct invasive populations, the result of both plastic and evolved responses (Kistner and Dybdahl 2013). However, it is unclear how this shell shape variation is related to water flow, and whether shell shape is an adaptive response to water flow.

In this study, to determine how shell shape varies with water flow, we first examined variation in shell morphology among populations of *Po. antipodarum* in different river sections, including free-flowing and still-water sites along the Snake River, Idaho. We found significant variation in shell shape that suggested an adaptive response to flow velocity. To further test whether these responses were adaptive in the local environment, we compared shell shape of the invasive snail to that of an ecologically similar native snail, *Pyrgulopsis robusta*. One indication of an

Table 1 Collection information for sympatric *Po. antipodarum* and *Py. robusta* sites along the Snake River

Site name	Collection location	Nearest city	Latitude	Longitude	Current velocity (m^3/s)
Reservoir	Arm of CJ strike reservoir, river mile 495	Grand View	42.9478°N	115.9452°W	17 m^3/s
River 1	Snake river, river mile 545	King Hill	42.9921°N	115.2295°W	3,048 m^3/s
River 2	Snake river, river mile 537	Glenns Ferry	42.9388°N	115.3355°W	1,677 m^3/s
River 3	Snake river, river mile 538	Glenns Ferry	42.9366°N	115.3106°W	1,524 m^3/s

All sites were located along the Snake River in Idaho. Reservoir is a still-water site in the CJ Strike Reservoir, while the other three sites are free-flowing reaches of the Snake River. Current velocity measurements are mean annual water flow rates from 1985 to 2008 matching the time of *Po. antipodarum*'s arrival in the Snake River

adaptive response would be parallel patterns of shell shape variation in the two species (Young et al. 2009). *Po. antipodarum* and *Py. robusta* are in the same family (Hydrobiidae), are ecologically similar (Riley et al. 2008), and coexist along the middle section of the Snake River (Hershler and Liu 2004; Dybdahl and Drown 2011). *Py. robusta* has a long evolutionary history dating back hundreds of thousands of years in pre-historic Lake Idaho and then the Snake River after Lake Idaho drained (Taylor 1985; Hershler and Liu 2004). On the other hand, *Po. antipodarum* has only been in this region for about 25 years but has spread rapidly, sometimes reaching great abundance (Kerans et al. 2005; Hall et al. 2006). In summary, we tested for adaptive responses in invasive *Po. antipodarum* by examining shell variation of sympatric native and invasive snail populations along a water flow gradient.

Materials and methods

Study sites

We obtained field samples of adult snail shells for both *Py. robusta* and *Po. antipodarum* from four sites where the two species are sympatric. The four sites were located along the Snake River, Idaho, in the western United States (Table 1).

One site, referred to as the Reservoir site, was located in still-water habitat in a reservoir, CJ Strike, on the Snake River. CJ Strike Reservoir is a man-made lake created by the impoundment of the Snake River and Bruneau River near Grand View, Idaho. Adult snails were collected from field sites by sifting aquatic vegetation and substrate using wire sieves. Specimens from CJ Strike were collected in April 2008. Three other sites were located in free-running reaches of the Snake River, referred to as River 1, 2 and 3 (Table 1). The specimens from these sites were collected in June 2007 and loaned to us by Orma J. Smith Museum of Natural History. Current velocity data for these sites was obtained from the U. S. Geological Survey (USGS National Water Information System 2013). If water velocity is an important determinant of shell shape variation, and shell shape is adaptive, we would expect that (1) longer narrower shells would occur in slower water, such as the reservoir site, (2) shorter, stouter shells would be favored in faster flow sites (Haase 2003; Minton et al. 2011), and (3) the two species would exhibit parallel patterns of variation across the four sites. A pattern of variation that does not match a priori predictions, or that is not parallel between species would be inconsistent with an adaptive explanation.

Geometric morphometric analysis

Shells from both native and invasive species were scrubbed clean of algae, dried, and mounted on museum gel to prevent shadows when being photographed. Only adult snails with five whorls were photographed and used in the geometric morphometric analyses. Images were obtained using a digital camera attached to a dissecting microscope. Shells were oriented with the axis of coiling horizontal, and the aperture face up. A millimeter ruler was mounted in the plane of aperture focus. Consistent orientation of the specimen is critical to minimize random error in morphometric analyses (Schilthuizen and Haase 2010). A series of repeated photos of the same shell was taken to quantify errors due to imprecision in orientation. Photos of individual shell specimens were then analyzed using geometic morphometric techniques.

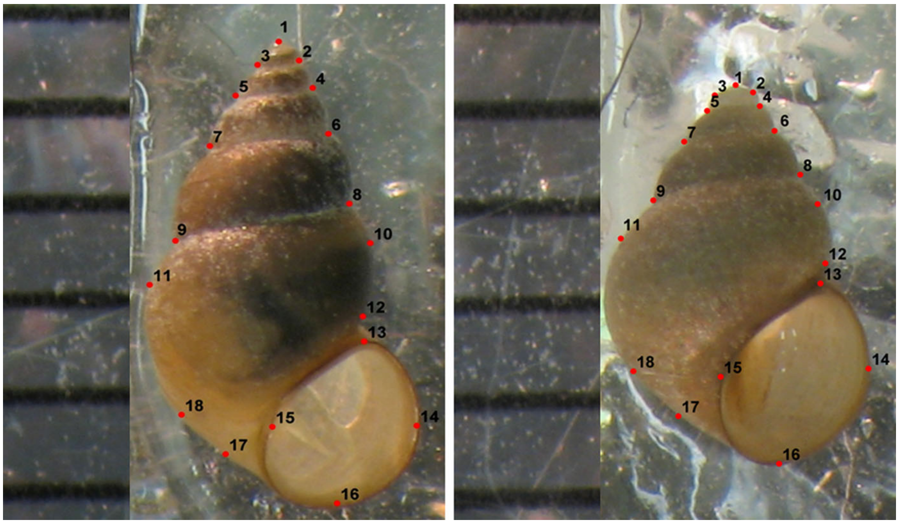

Fig. 1 Digitized landmarks used in the morphometric analysis. Eighteen landmark points shown on *Po. antipodarum* (*Left*) were also used on *Py. robusta* (*Right*). Ruler units are in millimeters

Geometric morphometrics is a common method for analyzing shape. Morphometrics is a quantitative method of addressing shape comparisons using digitized landmark points (Zelditch et al. 2004). Morphometric landmarks were chosen that are likely to represent structurally corresponding points across both species. Structurally corresponding landmarks are defined by two criteria: distinctness from other locations and recognizable in all specimens (Zelditch et al. 2004), and represent correspondence of parts but not evolutionary homology (Lele and Richtsmeier 2010). Thirteen corresponding points were found on *Po. antipodarum* and *Py. robusta* (Fig. 1) including the apex (LM 1), whorl grooves (LM 2–9) and the aperture (LM 13–16). Body whorl correspondence (LM 10–12, 17–18) may be subject to random error, given the lack of basal cords and variation in curvature between the native and invasive snail shell. Nevertheless, our significant results suggest that this error was minimized.

The 18 landmark points were digitized from photos using TPSDIG Version 2 (Rohlf 1997). These landmarks were placed on all 280 snail shells used. Geometric morphometric analyses were conducted using these digitized landmarks. The file of digitized coordinates was opened in CoordGen7 (Sheets 2011), which was then used to scale digitized landmarks to unit centroid size, and rotated to minimize the summed squared distances between landmarks. This standard alignment known as Procrustes alignment removes size differences among specimens while retaining allometric relationships, making it possible to analyze shape independent of size (Zelditch et al. 2004). Thus, the effects of non-shape information (position, orientation, and scale) were mathematically eliminated from these landmark configurations using a Generalized Procrustes analysis (GPA). Since *Py. robusta* has shorter, more globular shells than *Po. antipodarum*, we used Procrustes analysis to ensure that size differences between the two species did not overshadow variation in shell morphology across the four sample sites. In addition, GPA generates landmark plots that display the mean location of each of the 18 landmarks, enabling visual comparison of shell structure among sites and between species. GPA plots were generated in PCAGen7 (Sheets 2011).

To test for among-population variation in shell morphology, a Canoncial Variates Analysis (CVA) was conducted on the 18 digitized landmark points on both species from the four sites (Sheets 2011). This CVA differentiated snails based on among site differences. A CVA mathematically emphasizes the differences that vary most between groups (sites) while minimizing within group variation (species), making it easier to discern which characteristics are unique to each group (Zelditch et al. 2004). The CVA also conducts a group assessment test in which specimens are assigned groups based on morphological

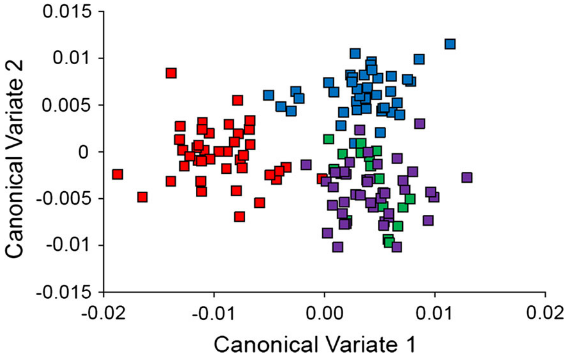

Fig. 2 Canonical Variates Analysis plot of *Po. antipodarum* across four sites (Reservoir—*blue*, River 1—*red*, River 2—*purple*, River 3—*green*). Canonical Variates 1 and 2 were significant ($P < 0.001$ in both cases) and comprised 54.4 and 16.4 % of the total variation, respectively

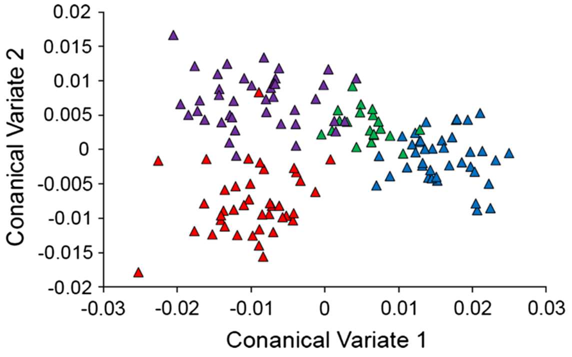

Fig. 3 Canonical Variates Analysis plot of *Py. robusta* across four sites (Reservoir—*blue*, River 1—*red*, River 2—*purple*, River 3—*green*). Canonical Variates 1 and 2 were significant ($P < 0.001$ in both cases) and comprised 61.7 and 30.3 % of the total variation, respectively

variability (see Kistner and Dybdahl 2013). First, a CVA was conducted on 140 *Po. antipodarum* individuals from the four sites and a similar analysis was conducted on 140 *Py. robusta* individuals to determine the extent of morphological variation in each of the species. A CVA and a Principal Component Analysis (PCA) were then conducted on the combined *Po. antipodarum* and *Py. robusta* (280 individuals) to determine if there were any parallel patterns in variation between the two species. The PCA maintains Euclidian distances, generated by the GPA, between specimens, so that patterns of variation among the four sites may be displayed. All Canonical Variates Analyses were performed in CVAGen7 while the Principal Component Analysis was conducted in PCAGen7 (Sheets 2011). Morphological differences detected by the CVA among *Po. antipodarum* populations were analyzed using a univariate ANOVA. A second univarite ANOVA was used to assess morphological differences detected by the CVA among *Py. robusta* populations. If *Po. antipodarum* and *Py. robusta* populations differ in shell shape across the sample sites, there should be significant site effects. Univariate ANOVAs were also used to compare morphological differences detected by the CVA and PCA among sites containing both invasive *Po. antipodarum* and native *Py. robusta* populations.

To further test for patterns in shell morphology among populations, traditional length measurements were calculated using TmorphGen7 (Sheets 2011). This program generates a set of traditional length measurements from a geometric landmark data set of paired coordinate measurements. These calculations maintain the Euclidean distances between specimens generated by the GPA. The following length measurements were calculated: shell height between landmarks 1 and 16, upper body whorl width between landmarks 10 and 11, lower body whorl width between landmarks 12 and 18, aperture width between landmarks 14 and 15, and aperture height between landmarks 13 and 16 (Fig. 1). We employed univariate ANOVAs to compare traditional length measurements among populations. All statistical analyses were performed using the Proc GLM procedure in the SAS language (SAS Institute 2004).

Results

Invasive *Po. antipodarum* and native *Py. robusta* populations exhibited shell shape variation among sites. The CVAs of native and invasive snail populations across the four sites identified two significant canonical axes (Figs. 2 and 3; respectively). In the jack-knife assignment tests with 1,000 replicates using 14 individuals as 'unknowns' (10 % of specimens), 80 % of invasive individuals were correctly classified (77.4 % significantly) while 75.7 % of native individuals were correctly classified (74.9 % significantly). The assignment tests grouped 93 % of the invasive snails to the correct site (Table 2) and 96 % of the native snails to the correct site (Table 3). The effect of site on shell morphology of invasive and native snail populations was significant along both CV1

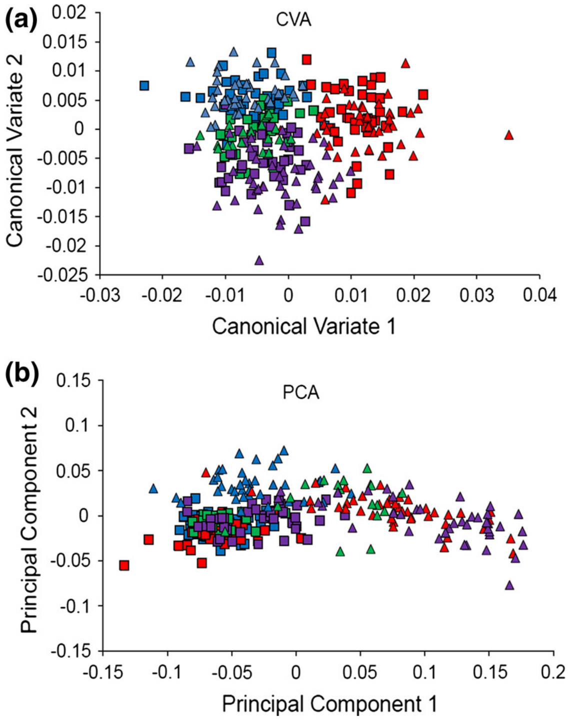

Fig. 4 Canonical Variates Analysis plot **a** and Principal Component Analysis plot **b** showing four sites (Reservoir—*blue*, River 1—*red*, River 2—*purple*, River 3—*green*) and both *Po. antipodarum* (*square*) and *Py. robusta* (*triangle*)

Table 2 Group assignment from CVA-Mahalanobis distances for *Po. antipodarum* populations

	Reservoir	River 1	River 2	River 3
Reservoir	38	1	0	1
River 1	0	40	0	0
River 2	2	0	35	3
River 3	0	0	0	20

Original groups based on populations are placed along rows, while CVA groups based on morphological variability are placed along columns. Reservoir, River 1, River 2 populations consisted of 40 specimens while River 3 consisted of 20 specimens. The group assignment test placed 95 % of Reservoir snails, 100 % of River 1 snails, 86 % of River 2 snails, and 100 % of River 3 snails with the correct population. Only 7 % of the specimens were assigned incorrectly

($F_{3,136} = 176.617$, $P < 0.001$; $F_{3,136} = 214.882$, $P < 0.001$; respectively) and CV2 ($F_{3,136} = 103.277$, $P < 0.001$; $F_{3,136} = 105.423$, $P < 0.001$; respectively). GPA revealed that *Py. robusta* exhibited greater among-site variation than *Po. antipodarum*. Invasive and native reservoir snails had long spires, small body whorls and small apertures. River 2 snails were short and stout with short spires, large body whorls and large apertures. River 3 snails were intermediate in all shape features. However, invasive River 1 snails had long spires, small body whorls and small apertures. In contrast, native River 1 snails had short spires, large body whorls and large apertures, indicating differential shape variation between the two species at this site.

Table 3 Group assignment from CVA-Mahalanobis distances for *Py. robusta* populations

	Reservoir	River 1	River 2	River 3
Reservoir	39	0	0	1
River 1	0	38	1	1
River 2	0	1	37	2
River 3	0	0	0	20

Original groups based on populations are placed along rows, while CVA groups based on morphological variability are placed along columns. Reservoir, River 1, River 2 populations consisted of 40 specimens while River 3 consisted of 20 specimens. The group assignment test placed 97 % of Reservoir snails, 95 % of River 1 snails, 92 % of River 2 snails, and 100 % of River 3 snails with the correct population. Only 4 % of the specimens were assigned incorrectly

Native and invasive populations exhibited parallel variation at three of the four sites where they are sympatric. The CVA conducted on both species across the four sites identified two significant canonical axes (Fig. 4a). Canonical Variate 1 and 2 were significant ($P < 0.001$), comprising 55.6 % and 34.8 % of the total variation, respectively. Despite distinct morphological differences between species (Fig. 1), the assignment test grouped 85 % of the snails from both species to the correct site (Table 4). In a jack-knife assignment test with 1,000 replicates using 28 individuals as 'unknowns' (10 % of specimens), 75.7 % of individuals were correctly classified, 74.9 % significantly. The effect of site on shell morphology was significant along both CV1 ($F_{3,276} = 200.67$, $P < 0.001$) and CV2 ($F_{3,276} = 125.43$, $P < 0.001$). The combined species GPA maintained similar shape trends exhibited by both species at Reservoir, River 2 and River 3 sites. In all three GPA analyses, Reservoir snails were long and narrow with small apertures, River 2 snails were short and stout with large apertures, and River 3 snails were intermediate in all features. In contrast, River 1 snails displayed the globular body whorls and large apertures seen in *Py.*

Table 4 Group assignment from CVA-Mahalanobis distances on combined *Potamopyrgus* and *Py. robusta* populations across four sites

	Reservoir	River 1	River 2	River 3
Reservoir	73	1	0	6
River 1	1	75	3	1
River 2	5	2	58	15
River 3	5	1	1	33

Original groups based on populations are placed along rows, while CVA groups based on morphological variability are placed along columns. Reservoir, River 1, River 2 populations consisted of 80 specimens while River 3 consisted of 40 specimens. The group assignment test placed 91 % of Reservoir snails, 94 % of River 1 snails, 73 % of River 2, and 83 % of River 3 snails with the correct population. Only 15 % of the specimens were assigned incorrectly

robusta snails and the long narrow spires exhibited by the *Po. antipodarum* snails at this site.

The PCA conducted on both species across the four sites identified two significant eiganvalues (Fig. 4b). Principal Component 1 and 2 were significant ($P < 0.001$), comprising 70.84 % and 7.4 % of the total variation, respectively. The effect of site on shell morphology was significant along both PC1 ($F_{3,276} = 23.7$, $P < 0.001$) and PC2 ($F_{3,276} = 12.31$, $P < 0.001$). PC1 represents increases in the shell spire, body whorl, and aperture as the axis becomes more positive (Fig. 5a). Therefore, reservoir snails have long spires with narrow body whorls and small apertures, while river 2 and 3 snails have short spires with large body whorls and large apertures. A pair-wise comparison of PC1 means among sites containing both snail species revealed that all sites were significantly different ($P < 0.01$) except for River 1 and River 3 ($P = 0.469$). PC2 represents decreases in spire length and width, increases in body whorl height, and a leftward shift the position of the aperture as the axis becomes more positive (Fig. 5b). A pair-wise comparison of PC2 means among sites indicated that only Reservoir snails were significantly different from River 1 and 2 snails ($P < 0.001$) with no other significant differences along PC2 ($P > 0.6$).

All five traditional length measurements exhibited significant differences across sites (Fig. 4). The effect of site on shell height was significant ($F_{3,276} = 101.2$, $P < 0.001$) with reservoir snails being 23 % longer than river snails. A pair-wise comparison of shell

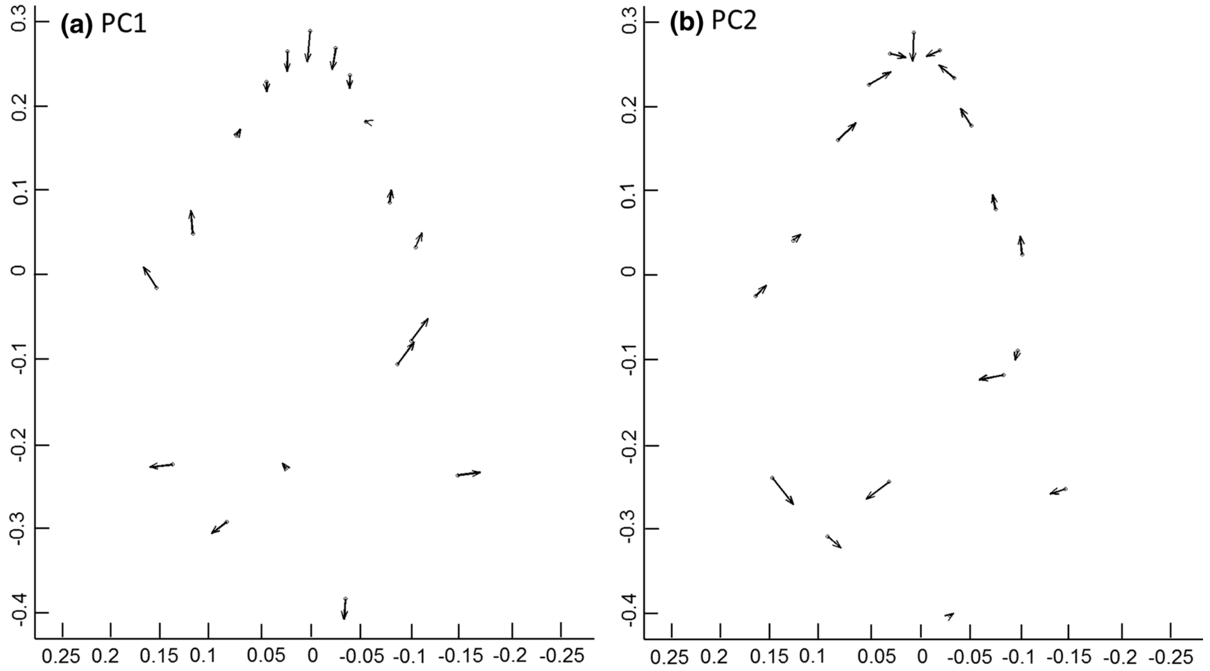

Fig. 5 Landmark vector plots along Principal Component 1 (**a**) and 2 (**b**). The origins of the *arrows* indicate the location of the 18 landmarks in the mean shape of the entire data set along PC1 and PC2. The *line vectors* indicate the changes in the relative position of the landmarks as the score on PC1 (**a**) and PC2 (**b**) increases

height means found significant differences among all sites ($P < 0.001$). For upper and lower body whorl measurements, the effect of site was significant ($F_{3,276} = 84.13$, $P < 0.001$; $F_{3,276} = 49.8$, $P < 0.001$; respectively) and pair-wise comparisons found significant differences among all sites ($P < 0.0001$ and $P < 0.01$, respectively). For aperture height and width, the effect of site was significant ($F_{3,276} = 40.62$, $P < 0.001$; $F_{3,276} = 47.4$, $P < 0.001$; respectively) and pair-wise comparisons found significant differences among all sites ($P < 0.01$ and $P < 0.01$; respectively). As in the GPA and PCA, Reservoir snails had the longest shell height, shortest body whorl lengths, and smallest aperture while River 2 snails exhibited the opposite trend in all five traditional length measurements. River 1 snails displayed shape characteristics of both species with the second longest shell height, the same as the long spired *Po. antipodarum*. In addition, River 1 snails exhibited the second widest body whorl and second largest aperture; the same trend exhibited by *Py. robusta* snails at this site. Once again, River 3 snails were intermediate in all five traditional length measurements.

In all Geometric Morphometric analyses, River 2 populations exhibited short spires, large body whorls, and large apertures. Reservoir populations always displayed long spires, small body whorls, and small apertures. Furthermore, River 3 snails were consistently intermediate in all shape features. Therefore, *Py. robusta* and *Po. antipodarum* displayed similar trends in shell shape variation across three of the four sites. However, River 1 populations displayed opposite shell shapes with *Po. antipodarum* exhibiting long and narrow shells and *Pr. robusta* exhibiting short and globular shells.

Discussion

A central focus in invasion ecology is to determine how invasive species become locally abundant and geographically widespread and the role of adaptive responses to environmental variation. This study sought to determine the importance of adaptive responses in shell morphology of invasive *Po. antipodarum*. We found significant variation among four *Po. antipodarum* populations in the Snake River, Idaho. We compared shell shape variation between the invader and a sympatric, long-established, ecologically similar native snail, and found evidence of parallel shell shape variation in the two species across three of the four sites. Both species exhibited shell variation that was consistent with the water velocity of three of the four sites (reservoir versus free-flowing river), suggesting that shell variation is adaptive (Vermeij 1995; Haase 2003; Minton et al. 2011). Parallel shell responses between the two species to environmental conditions across the majority of sites further suggest adaptive responses (Young et al. 2009).

Adaptive responses by the invasive species could be rejected if (1) invasive snails did not exhibit variation in shell shape among sites, (2) if that variation was not similar to that of a long-established native species, and (3) if shape variation in both species did not match a priori expectations based on flow conditions. In contrast, we found that *Po. antipodarum* exhibited significant variation in shell shape across the four sites, having arisen by plastic adjustments, transgenerational plasticity, or evolution since its recent colonization of the Snake River in 1985. Snails of this species differed among sites in body whorl size, spire length, and aperture shape. Native *Py. robusta* populations exhibited greater variation in shell morphology than sympatric invasive *Po. antipodarum* populations (Figs. 2, 3). Despite the greater among-site variation in *Py. robusta*, both snails consistently differed by the same three shape traits and exhibited similar shape differences. When both species were analyzed together, the Canonical Variate assignment test placed 85 % of individuals to the correct site (Table 4), suggesting similar responses to environmental variation. While River 1 snails did not exhibit the same shell characteristics, 94 % of River 1 invasive and native snails were categorized correctly when analyzed together (Table 4), indicating that snails at this site do share some morphological characteristics. The PCA revealed more within-site variation compared to the CVA. This is probably due to shape differences between the two species. The GPA aligned all specimens within a population to an iteratively estimated mean specimen and these distances between specimens are preserved in the PCA not the CVA (Fig. 4). Given the differences in shape between the two species, the mean specimen was between the two extremes resulting in the clustering of invasive specimens on the right side of PC1 and native specimens more on the left side of this axis (Fig. 4b). Despite this, similar patterns in shell shape did emerge. PC1 placed long narrow reservoir forms on the negative left side and placed the short globular snails from the river sites on the more positive right side. For both species, shell shape variation between both native and invasive snails sampled from Reservoir and the River 2 and 3 sites exhibited the same trends in body whorl size, spire length and aperture shape as indicated by the GPA, PCA, and traditional length measurements. These corresponding patterns in shell shape suggest that the invasive snail exhibited a similar response to environmental variation as its long established native counterpart.

These trends in variation for body whorl size emerged despite the potential for errors in landmark placement along the curvature of the body whorl, although random error in landmark placement might have reduced the significance of the observed patterns. However, the possible inconsistences of landmark placement contributed rather little to the overall results, as only 7.4 % of the variation was explained by the Principle Component axis that carried body whorl variation (PC2) (Fig. 5b). Future studies should use semi-landmarks on snail body whorls to eliminate inconsistencies in placement, thereby improving confidence in quantitative comparisons of curved surfaces (Mitteroecker and Gunz 2009; Sheets 2011).

The observed parallel responses fit a priori expectations based on biomechanical analysis of snail shell shape in water flow (Dussart 1987; Vermeij 1995; Statzner and Holm 1989; Haase 2003; Minton et al. 2007, 2011). Our past work suggests that water flow variation is an important determinant of shell variation in invasive *Po. antipodarum* populations (Kistner and Dybdahl 2013). The analysis of *Py. robusta* shell shape variation is also consistent with these expectations. This pattern is most clearly seen by comparing snails of both species from Reservoir and the River sites 2 and 3. The Reservoir site provides a stable lentic environment while snails located from the River sites experience higher flow rates (Table 1). Longer spires and smaller apertures exhibited by the Reservoir snails are consistent with lentic conditions (Vermeij 1995) while the short and stout shells exhibited by River 2 and 3 snails provide greater protection against the free-flowing currents along the Snake River (Dussart 1987). Therefore, shell shape variation across three of the four sites is consistent with environmental responses to water flow, although other unmeasured factors are likely contributing to this variation (Minton et al. 2007, 2011).

Although the patterns of variation were consistent among sites, *Po. antipodarum* at the River 1 site was an exception. Here, the two species differed greatly in the shape of their body whorl, spire length, and aperture size. The shell form of *Py. robusta* snails at River 1 is consistent with the high flow velocity of this site while the *Po. antipodarum* shell form at this site is not. This discrepancy may be due to the fact that water velocity at River 1 was nearly twice as fast as that at River 2 and 3. It is possible that, compared to *Py. robusta*, *Po. antipodarum* avoid strong current by moving into more sheltered habitats (Haase 2003), reducing the influence of current on shell shape. It is also possible that the two species are responding to different abiotic and biotic variables at this site. Minton et al. (2011) found that spring snail shape was correlated with water pH and temperature. Direct and indirect effects of predation can also alter shell shape (Bourdeau 2009, 2010). It is plausible that *Po. antipodarum* exhibited smaller body whorls and longer spires at River 1 site than their native counterparts as a result of increased predation in invasive populations. Snails may respond to predators by exhibiting elongated spires that allow greater contraction of soft tissue (Bourdeau 2009). Multiple snail fitness measurements in conjunction with environmental data would provide stronger evidence for adaptive shell responses to water flow. An examination of the fitness of different shell forms across multiple environments is required to verify that *Po. antipodarum* shell shape variation is adaptive.

While our results are consistent with the adaptive explanation, it remains unclear whether phenotypic plasticity, transgenerational non-genetic inheritance of plastic responses, or genetically based evolutionary change is the driving force behind *Po. antipodarum*'s shell variation. In their native range, there is evidence that *Po. antipodarum* exhibits plasticity in shell shape along a water flow gradient (Haase 2003). It is possible that plasticity has allowed shell shape in *Po. antipodarum* to quickly respond to local conditions and express variation similar to that seen in *Py. robusta*. The parallel shell forms of the invader and native might result from the plasticity in *Po. antipodarum* which could account for the rapid matching of the native snail *Py. robusta* (Schweitzer and Larson 1999; Legar and Rice 2003). Invasive species invading novel environments often display increased plasticity compared to populations in the native range and populations of related native species (McDowell and Lee 2002; Yeh and Price 2004; Lee et al. 2003; Dybdahl and Kane 2005; Lombaert et al. 2008). While both native and invasive snails exhibit similar patterns of variation in shell morphology along the Snake River, populations of *Po. antipodarum* do not exhibit as much shell variation as *Py. robusta* along the same sites (Fig. 4). This may be due to incomplete phenotypic plasticity, defined as an incomplete adaptive response to the new fitness optimum (Ghalambor et al. 2007). It may take several generations for an invasive species to achieve a perfectly adaptive plastic response via natural selection on plasticity (Richards et al. 2006).

Adaptive evolution is another possible explanation for the variation in shell shape seen in *Po. antipodarum* (Drown et al. 2010; Kistner and Dybdahl 2013). If native *Py. robusta* represents the optimal phenotype at each site, then the similarity in shell shape among sites suggests that invasive *Po. antipodarum* has come to mimic the native shape within a relatively short time scale. Clonal populations of *Po. antipodarum* have reached such high density in the invaded range (Kerans et al. 2005; Hall et al. 2006; Riley et al. 2008) that genetic variation is possible via rapid accumulation of mutations (Butin et al. 2005) and there is evidence for genetic variation in invasive *Po. antipodarum* populations in the Snake River (Dybdahl and Kane 2005; Hershler et al. 2010). Alternatively, epigenetic changes might be responsible for rapid change in invasive species, especially when plastic responses can be inherited across generations (Bonduriansky et al. 2011). A reciprocal transplant experiment between low and high flow sites could directly test whether observed shell variation is due to evolved specialization or phenotypic plasticity.

Our results support an adaptive explanation for shell variation in a widespread invasive snail. We found that *Po. antipodarum* has responded to environmental conditions in a similar manner as its native counterpart, *Py. robusta*. Shell shape variation was consistent with differences in water velocity. Short and stout shell shells were found in high flow environments while long narrow shell shells were found in lentic habitats. Our findings are consistent with other studies on snail morphology across flow gradients (Statzner and Holm 1989; Haase 2003; Minton et al. 2007, 2011). Although these results are compatible with an adaptive response in the invasive snail, an association between measures of fitness and shell shape across a wider sample of well-characterized sites would help to confirm our conclusion. If shell morphology responds adaptively, then that capacity might help explain this invader's success across broad environmental gradients.

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