

DENSITY EFFECTS OF NATIVE AND EXOTIC SNAILS ON GROWTH IN JUVENILE APPLE SNAILS *POMACEA PALUDOSA* (GASTROPODA: AMPULLARIIDAE): A LABORATORY EXPERIMENT

S. L. CONNER, C. M. POMORY AND P. C. DARBY

Department of Biology, University of West Florida, Pensacola, FL 32514, USA

(Received 6 December 2007; accepted 27 June 2008)

ABSTRACT

Pomacea paludosa (native Florida apple snail) is found in wetlands in the southeastern United States. *Pomacea insularum* is an exotic apple snail which has invaded wetlands in Florida and co-occurs with *P. paludosa*. The effect of changes in density on growth in native juvenile snails was studied by manipulating native juvenile density, native adult density and exotic adult density in a set of laboratory experiments. Growth decreased when native juvenile densities (without adults) increased from four to eight snails per 38-l aquarium. The presence of adults of either species decreased juvenile growth and also decreased juvenile survival. One exotic adult had the equivalent effect of three to four native adults. Based on the response of native juveniles to exotic adults, populations of the native apple snail *P. paludosa* could be negatively impacted by expansion of the exotic snail *P. insularum*.

INTRODUCTION

Density of individuals influences the biology of many organisms (e.g. Tanner, 1997; Dobson & Oli, 2001; Lorenzen & Enberg, 2001; Arenas, Viejo & Fernandez, 2002; Kammenga *et al.*, 2003). While density-based regulation takes place at the population level, the effects of density, or any other factor, must change biological function at the level of the organism (Murdoch, 1994; Abrams, 1995; Norris, 2004; Fordyce, 2006). One possible effect is a change in the rate at which an individual grows (Fowler, 1981). Decreased growth can delay sexual maturity and increase mortality, especially in younger members of a population (Smith & Smith, 2001).

Invasion of habitat by exotic species is one of the greatest threats to biological diversity (Bright, 1998; Van Driesche & Van Driesche, 2000; Brinson & Malvarez, 2002). Invasion by exotics can lead to declines in native populations, especially when natural predators, disease or other suppressants that might regulate the exotic population are absent (Byers, 1999; Lach *et al.*, 2000; Cowie, 2001). Negative interactions between native and exotic species include alteration of biological functions, displacement to suboptimal habitat and complete eradication of native species (Leppäkoski & Olenin, 2000; Myers *et al.*, 2000; Brinson & Malvarez, 2002; Simberloff, 2003).

Pomacea paludosa (Say) is the only native apple snail of wetlands in the southeastern United States (Thompson, 2004). *Pomacea paludosa* has a 1–1.5 year life span (Darby, Valentine-Darby & Percival, 2003). Females lay multiple clutches of eggs on emergent vegetation primarily in spring to early summer (Hanning, 1979; Turner, 1996). Adult snails die after the reproductive season (Hanning, 1979; Darby, Bennetts & Percival, 2008). Young hatch and mature during summer and fall, a time span when adult densities are normally decreasing due to their post-reproductive die off.

Interest in *P. paludosa* stems largely from the fact that it is the primary food source for *Rostrhamus sociabilis plumbeus* (Florida Snail Kite), an endangered hawk found in peninsular Florida (Sykes, Rodgers & Bennetts, 1995). It is also a food source for a variety of other animals including young alligators, various birds, turtles, fishes and aquatic insects (references

in Turner & Mikkelsen, 2004). Patterns of survival and abundance of adult *P. paludosa* have been documented in the field (Darby *et al.*, 2003, 2004; Karunaratne, Darby & Bennetts, 2006), but aspects of juvenile biology remain largely unknown.

Pomacea insularum (D'Orbigny) is an exotic snail, native to Argentina, and is part of the *Pomacea canaliculata* taxonomic group (Cazzaniga, 2002). It has recently invaded several lakes and wetlands in Florida (Rawlings *et al.*, 2007). *Pomacea insularum* is larger than *P. paludosa* (approximately three times larger as measured by shell length and four to five times larger by mass) (Darby, Mellow & Watford, 2007), lives longer than *P. paludosa*, and produces more eggs per egg cluster than *P. paludosa* (personal observations; Rawlings *et al.*, 2007) all making it an ideal candidate for invasive expansion. No information exists on how native juvenile snails respond to the presence of the exotic adults.

The effect of changes in density on growth in native juvenile snails was studied by manipulating native juvenile density, native adult density and exotic adult density in a set of laboratory experiments. The following questions were addressed: (1) At what density level does native juvenile snail density decrease native juvenile snail growth? (2) Does the presence or increase in density of native adult snails decrease native juvenile snail growth? (3) Does the presence or increase in density of exotic adult snails decrease native juvenile snail growth?

MATERIAL AND METHODS

Egg cluster collection and hatching

Egg clusters of *Pomacea paludosa* were collected from Wacissa Springs, Jefferson County, Florida, USA (30.3°N, 83.9°W). Vegetation with egg clusters was cut near the water line and transported to the laboratory where it was placed in a hatching tub (plastic tub 63.1 × 44.7 × 18.4 cm) containing 22.5 l of hard water (modified EPA recipe, see Corrao, Darby & Pomory, 2006). Florist's wire was tied around the tops of the vegetation to suspend it in an upright position in the tub, allowing hatchlings to fall into the water to mimic the natural hatching process. Hatchlings were taken out of the tub on a daily basis and placed in 38-l glass aquaria containing 32 l of

Correspondence: C.M. Pomory; e-mail: cpomory@uwf.edu

hard water, an air-driven corner filter and romaine lettuce leaves (food source) until enough hatchlings were available to start an experiment (about 2 weeks).

Adult snail collection

Native adult *P. paludosa* snails (shell length range 30–40 mm), defined as snails larger than approximately 30 mm shell length (Hanning, 1979; Darby *et al.*, 2003), were collected in the same area as egg clusters and placed in a separate holding tank (38-l aquarium containing 32 l of hard water and a corner filter) until juveniles hatched out. Adult snails were fed several leaves of romaine lettuce every 3 days. Exotic adult *P. insularum* snails (shell length range 70–80 mm) were collected from Lake Munson, Leon County, Florida, USA (30.0°N, 84.0°W) and maintained in a separate holding tank similar to that for native adult snails.

Experimental tank set-up and tank maintenance

Experiments were carried out using 38-l glass aquaria. All tanks contained 32 l of hard water and an air-driven filter. Opaque plastic sheeting was wrapped around each tank to reduce visibility across tanks, while still allowing light penetration. A recirculating heated water bath containing all tanks was used to stabilize water temperature, which ranged from 23 to 25°C. Several leaves (~40 g) of romaine lettuce were added every 4 days, providing continuous access to food. Light was controlled on a 12:12 h light:dark cycle.

Holding tanks containing juveniles from hatching and adults from field collection were maintained under the same conditions as the experimental tanks before and during experiments. Tanks were inspected daily and snails that died in experimental tanks were removed and replaced with similar-sized snails from the holding tanks to maintain constant densities. Replacement snails were marked with a silver pen and were not included in the final measurements.

Aquaria were cleaned once a week by wiping the inside of the glass with paper towels and siphoning particulate matter off the bottom. Siphoned water was passed through filter floss to remove debris and returned to each aquarium. Ammonia testing was done throughout the experiments using Hach test kits. An additional trial outside of the experiments was run for 30 days with five hatchlings and 10 native snails haphazardly selected from the holding tanks, but without air-driven filters or cleaning (worst-case maintenance scenario, which never happened during the actual experiments) to deliberately create relatively poor water-quality conditions in order to provide additional evidence that water quality was unlikely to have impacted the results of the experiments.

Snail measurements

Shell length was measured (nearest 0.01 mm, apex to end of aperture) with callipers every 2 weeks from the start of an experiment. Growth at each time point was calculated as change in shell length relative to initial measurement (Carter & Ashdown, 1984; Perry & Arthur, 1991). Growth rate was calculated as change in shell length for each time point relative to the previous time point and used to estimate the growth rate coefficient over the time of the experiment (Rao, 1958; Kshirsagar & Smith, 1995). Growth rate coefficients allow comparison of different treatments using ANOVA when all treatments are measured at the same time points and there are relatively few time points measured (full details with examples in statistical works by Rao, 1958; Kshirsagar & Smith, 1995). They are not designed to generate specific models of growth, only for relative comparisons to establish treatment effect.

Smaller coefficients reflect slower growth and, since all measurements take place at the same time points, indicate smaller size at the end of the experiment.

Experiment 1: native juvenile snail density

Experiment 1 was conducted as a completely randomized one-way ANOVA design having four density treatments with eight replicate tanks per density. Density treatments and snails were randomly allocated to 32 experimental tanks. Treatment densities were 4, 8, 16 and 32 juvenile snails per tank (=8, 16, 32 and 64 snails per m² of tank surface area and 0.125, 0.25, 0.5 and 1 snails per l of water volume). The experiment started on October 25 2003, and ended on December 6 2003.

Experiment 2: native adult and native juvenile snail densities

Experiment 2 was conducted as a completely randomized two-way factorial ANOVA design with two juvenile densities of 4 or 16 juveniles per tank as factor one, and four adult densities of zero, one, two or four adults per tank as factor two. Each treatment combination was replicated four times. Density treatments and snails were randomly allocated to 32 experimental tanks. The experiment started on May 25 2004, and ended on July 24 2004.

Several of the treatment replicates with the highest adult density of snails suffered complete juvenile mortality. For this reason, and given the completely randomized design focused on the question of growth, the treatments containing four adults were omitted from statistical analysis of growth.

Experiment 3: exotic adult and native juvenile snail densities

Experiment 3 was conducted and analysed similarly to Experiment 2, except that adults of the exotic snail *P. insularum* were used instead of native adults. Experiment 3 started on June 24 2005, and ended prematurely after 15 days due to a hurricane, so growth measurements were made for only one time point. Based on the results of Experiment 3, the procedure was modified to the design of Experiment 4.

Experiment 4: native adult, exotic adult and native juvenile snail densities

Experiment 4 was conducted as a completely randomized one-way ANOVA design having three treatments with eight replicate tanks per treatment. Density treatments and snails were randomly allocated to 24 experimental tanks. All tanks had four native juvenile snails per tank with treatments of zero adult, one native adult or one exotic adult snail per tank. Experiment 4 started on September 16 2005, and ended on November 11 2005.

Statistical analysis of growth

Comparison of mean growth rate coefficients among treatment groups was made using one-way ANOVA or two-way ANOVA with $\alpha = 0.01$. We set a more conservative α in order to account for multiple testing of a similar question across several experiments. Homogeneity of variance was tested with the Brown–Forsythe test (Brown & Forsythe, 1974). Data failing to meet assumptions were ln-transformed for statistical testing. Untransformed means are presented in the graphs and tables. A Fisher–Hayter multiple comparison test was used for pairwise comparisons (Hayter, 1986).

Experiments 2 and 4: native juvenile snail survival

Although not the original design goal of the study, survival at the end of Experiment 4, as related to the presence of exotic or native adult snails, was examined with a log-likelihood *G*-test with an $\alpha = 0.05$ as only one test was performed. A qualitative graphical comparison of percent survival by tank including Experiments 2 and 4 together was made for treatments containing four juvenile snails per tank.

RESULTS

Experiment 1: native juvenile snail density

Mean shell size of juveniles was not significantly different among treatment groups at the start of the experiment (Table 1; 1-way ANOVA $F_{3, 28} = 0.17, P = 0.92$). Mean growth coefficient of juveniles was larger for the lowest density treatment (four juveniles per tank) indicating a higher growth rate, with other density treatments (8, 16, 32 juveniles per tank) similar to one another (Fig. 1; 1-way ANOVA $F_{3, 27} = 9.41, P = 0.0002$).

Experiment 2: native adult and native juvenile snail densities

Mean shell size of juveniles was not significantly different among treatment groups at the start of the experiment (Table 1; 2-way ANOVA Juvenile $F_{1, 18} = 2.32, P = 0.14$; Adult $F_{2, 18} = 1.05, P = 0.37$). Interaction terms on all two-way ANOVA tests were not significant (not shown). Main effects (Factor 1 juvenile density) mean growth coefficients of juveniles were not significantly different among the 4 and 16 juvenile density treatments (2-way ANOVA $F_{1, 18} = 3.03, P = 0.09$). Main effects (Factor 2 adult density) mean growth coefficient of juveniles was larger for the 0 adult density treatment indicating a higher growth rate, with the one and two adult density treatments similar to one another (2-way ANOVA $F_{2, 18} = 16.90, P = 0.00007$).

Simple effects (cell means within each adult density) mean growth coefficient of juveniles was larger for the four compared with the 16 juvenile density treatments within the zero adult density treatment, but not within the one or two adult density treatments (Fig. 2). This outcome indicates some biological

Table 1. Initial size of *Pomacea paludosa* from Experiments 1–4.

Experiment	Treatment main effects	Initial juvenile size mean (mm) ± SE	
1. Native juveniles	Juvenile 4	5.23 ± 0.07	
	8	5.22 ± 0.16	
	16	5.27 ± 0.09	
	32	5.19 ± 0.03	
2. Native juveniles	Juvenile 4	5.73 ± 0.14	
	16	5.97 ± 0.06	
	Native adults	Adult 0	5.83 ± 0.10
		1	5.73 ± 0.14
		2	6.00 ± 0.16
3. Native juveniles	Juvenile 4	7.91 ± 0.19	
	16	7.95 ± 0.14	
Exotic adults	Adult 0	8.31 ± 0.13	
	1	7.80 ± 0.17	
	2	7.68 ± 0.24	
4. Native juveniles Exotic or native adults	Adults 0	5.73 ± 0.06	
	1 native	5.78 ± 0.10	
	1 exotic	5.88 ± 0.07	

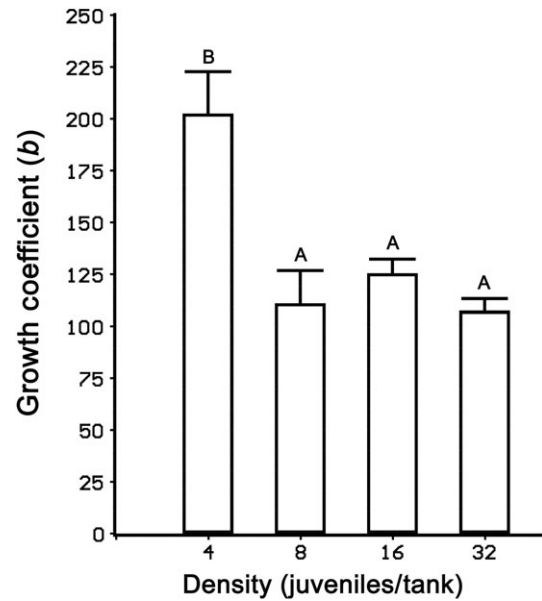


Figure 1. Experiment 1: native juvenile snail density. Growth coefficient (mean + SE) of *Pomacea paludosa* juveniles from treatments of 4, 8, 16 or 32 juveniles per tank. Treatments with different letters are significantly different based on the Fisher–Hayter multiple comparison test at $\alpha = 0.01$.

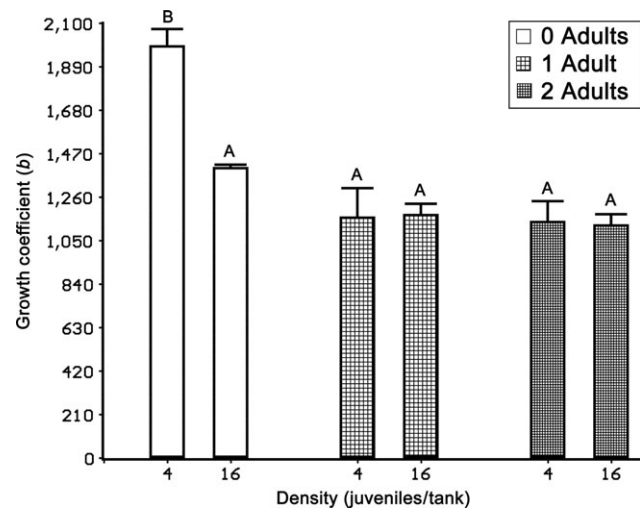


Figure 2. Experiment 2: native adult and native juvenile snail densities. Growth coefficient (mean + SE) of native *Pomacea paludosa* juveniles for simple effects treatments (individual cell means) containing 4 or 16 juveniles per tank in combination with 0, 1 or 2 native adults per tank. Treatments with different letters are significantly different based on the Fisher–Hayter multiple comparison test at $\alpha = 0.01$.

interaction effect, but interaction was not statistically significant ($P = 0.017$) using our predetermined alpha level of 0.01.

Experiment 3: exotic adult and native juvenile snail densities

Mean shell size of juveniles was not significantly different among treatment groups at the start of the experiment (Table 1; 2-way ANOVA Juvenile $F_{1, 18} = 0.03, P = 0.87$; Adult $F_{2, 18} = 2.79, P = 0.1$). Interaction terms on all two-way ANOVA tests were not significant (not shown). After 15 days

(only time point measured due to hurricane), mean growth of juveniles was significantly different across all three exotic adult density treatments with zero adult treatment > one adult treatment > two adult treatment; but not significantly different among juvenile density treatments (2-way ANOVA Juvenile $F_{1, 17} = 0.46$, $P = 0.51$; Adult $F_{2, 17} = 38.27$, $P < 0.00001$).

Experiment 4: native adult, exotic adult and native juvenile snail densities

Mean shell size of juveniles was not significantly different among treatment groups at the start of the experiment (Table 1; 1-way ANOVA $F_{2, 21} = 0.02$, $P = 0.82$). Mean growth coefficients of juveniles were different across all three adult density treatments with zero adult treatment > one native adult treatment > one exotic adult treatment (Fig. 3; 1-way ANOVA $F_{2, 15} = 19.51$, $P = 0.00007$).

Experiments 2 and 4: native juvenile snail survival

By the end of Experiment 2 none of the tanks with zero, one or two native adults per tank experienced complete juvenile mortality; 50% of the tanks with four native adults per tank experienced complete juvenile mortality. By the end of Experiment 4 (with twice the number of tanks per treatment as Experiment 2) none of the tanks with zero adults, 25% of the tanks with one native adult, and 50% of the tanks with one exotic adult experienced complete juvenile mortality ($G^2 = 6.90$, $P = 0.031$). Survival of juveniles based on percent per tank for Experiments 2 and 4 together was greatest in treatments with zero adults, and lowest for the one exotic adult and four native adult treatments (Fig. 4). None of the adults died during the experiments.

Ammonia levels in experimental tanks varied between 0.01 and 0.3 ppm. As there is no reference value in the literature for our conditions, the additional ammonia test trial with juveniles

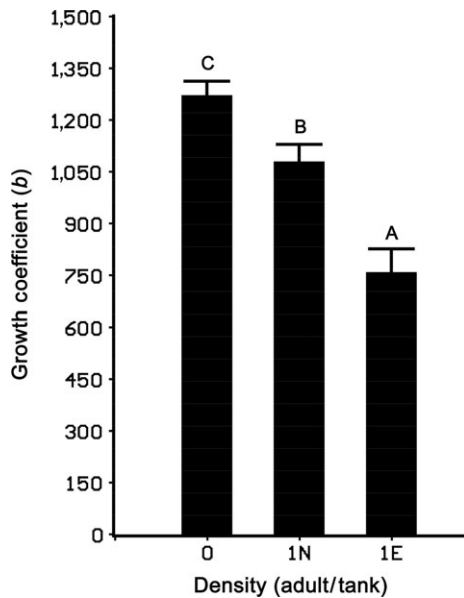


Figure 3. Experiment 4: native adult, exotic adult and native juvenile snail densities. Growth coefficient (mean \pm SE) of *Pomacea paludosa* juveniles with four juveniles per tank for treatments containing zero adults, one native *P. paludosa* adult (1N) or 1 exotic *Pomacea insularum* adult (1E) per tank. Treatments with different letters are significantly different based on the Fisher–Hayter multiple comparison test at $\alpha = 0.01$.

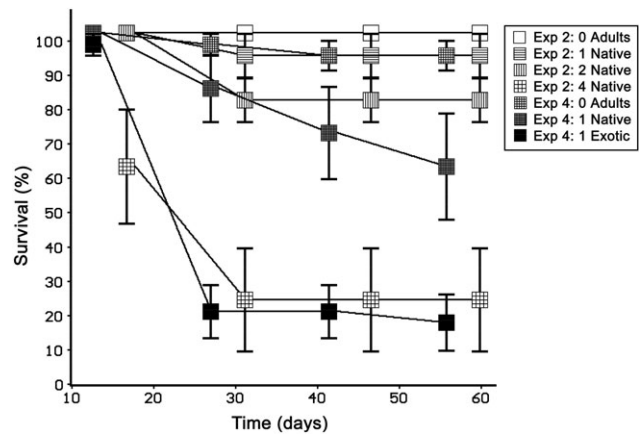


Figure 4. Experiments 2 and 4: native juvenile survival. Percent survival (mean \pm SE) of *Pomacea paludosa* juveniles for treatments containing no adults, native *P. paludosa* adults, or exotic *Pomacea insularum* adults from Experiments 2 and 4 with four juveniles per tank.

and adults, but without air-driven filters or cleaning, was used as a worst-case scenario for comparison and revealed levels varying between 0.3 and 1.5 ppm with 100% survival of juveniles over 30 days. Even without mechanical filtration the surface area of the tanks provided biological filtration. Field measures of ammonia in south Florida where apple snails are routinely collected vary between 0.001 and 1.1 ppm (Weaver & Payne, 2006).

DISCUSSION

In the field, the density of adult *Pomacea paludosa* is usually, but not always, less than 1 m^{-2} (Darby *et al.*, 2004; Karunaratne *et al.*, 2006). *Pomacea haustum* occurs at densities ranging between 0.2 and 40 m^{-2} (de Freitas & dos Santos, 1995). *Pomacea canaliculata* is found at densities of 2–4 m^{-2} when not under exotic expansion conditions (Ichinose *et al.*, 2000). Because of taxonomic confusion (Cazzaniga, 2002; Rawlings *et al.*, 2007), many studies of *P. canaliculata* are probably of other *Pomacea* species. Non-ampullarid gastropods, which are almost always much smaller in size, occur in much greater densities. Strzelec & Królczyk (2004) estimated densities between 12 and 193 m^{-2} for 14 species of gastropods found in rivers in Poland. Hill, Ryan & Schilling (1995) considered a density of 970 m^{-2} to be normal for the stream dwelling gastropod *Elimia claeaeformis*, and 50 m^{-2} to be a low-density treatment. Other freshwater density examples include *Hydrobia totteni* 50–19,000 m^{-2} (Wells, 1978), *Viviparus subpurpureus* and *Campeloma decisum* 100–1,700 m^{-2} (Brown, Varza & Richardson, 1989), and *Viviparus viviparus* 30–250 m^{-2} (Jakubik, 2007). Clearly *Pomacea* spp. are at the low end of the density spectrum. We found treatment effects with one adult in a tank (2 m^{-2} tank surface area). We have found 3–4 *P. insularum* adults m^{-2} in shallow water of Lake Tohopekaliga, Osceola County, Florida (P.C. Darby, unpubl.).

To our knowledge, density of juveniles in the field has not been measured. Based on estimates of typical egg cluster density and number of eggs per cluster during the April–June peak reproduction period, potential juvenile densities of *P. paludosa*, where adult snail densities are approximately 0.5 m^{-2} , would be 30–120 m^{-2} immediately after hatching (Darby *et al.*, 2008; P.C. Darby, unpubl.). For comparison, the levels used in our experiments ranged from 8 to 64 m^{-2} based on tank surface area. The water depth in the tanks used during the experiments (25 cm) is similar to the depth of water

levels often encountered where snails inhabit wetlands during peak reproduction (P.C. Darby, unpubl). Snails crawl and do not swim, so tank surface area should be relevant to interaction of individuals. Ramnarine (2004) used a stock density of juvenile *P. urceus* at 55 m^{-2} for an aquaculture tank experiment. Carlsson & Brönmark (2006) found juvenile *P. canaliculata* at densities between 22 and 58 m^{-2} in an enclosure experiment.

In Experiment 1 (juvenile density manipulation) an increase in juvenile density, in the absence of adults, from 4 to 8 or more juveniles per tank resulted in lower growth. *Pomacea canaliculata* experience density effects on growth in as little as a week at a density of two snails per m^2 (Tanaka *et al.*, 1999). Alves *et al.* (2006) experimented on growth in *P. lineata* and *P. bridgesi* at three densities and found no difference in growth rates in both species at densities equivalent to 16, 32 and 48 snails per tank in our experiment; this is a similar finding to our study at the higher density levels. Aufderheide *et al.* (2006) reported a density effect on growth of juvenile *Marisa cornuarietis*, another ampullariid snail, at a level equivalent to about six snails per tank in our experiment, but not at greater densities, again similar to our findings. Baur & Baur (1992) found that juveniles of the snail *Balea perversa* increased their growth rate when densities were lowered from 0.66 to 0.082 m^{-2} . Cameron & Carter (1979) found that the terrestrial snails *Cepaea nemoralis* and *C. hortensis* both experience lower growth at a density of 80 m^{-2} compared to 10 m^{-2} . *Helix aspersa*, the edible snail, can be raised commercially at densities of several hundred per m^2 (Dupont-Nivet *et al.*, 2000) and density effects on growth are seen at densities greater than 100 m^{-2} (Jess & Marks, 1995). *Mesodon normalis* is significantly smaller at age of reproduction when held at densities greater than 1041 m^{-2} (Foster & Stiven, 1996).

Based on the range of densities reported as having an effect on growth in different species, *Pomacea* appear to be more sensitive to initial density increases at relatively low densities compared to many other species. This suggests that their success is at least partly dependent on having large areas of suitable habitat to maintain populations at low density levels. Based on the effects of density in our experiments, we suggest rapid dispersal away from hatching areas with many egg clusters would be beneficial to the juvenile snails, perhaps contributing to the distribution pattern seen in adults (Karunaratne *et al.*, 2006). As the effect takes place at relatively low density levels and does not seem to increase with further increases in ecologically relevant density, competition is unlikely to be the cause. The response appears similar to a saturation effect (i.e. once a certain level is reached further increases do not change the outcome).

In Experiment 2 (native adult and native juvenile manipulation) juvenile *P. paludosa* grew significantly slower when exposed to as few as one adult *P. paludosa* per tank. In Experiment 3 (exotic adult and native juvenile manipulation), native juvenile growth decreased sooner than with native adults. The results of Experiment 4, with one exotic and one native adult, support that outcome, suggesting the presence of the exotic could negatively influence growth of native snails. Decreased growth rates in some species of snail result in the failure of juvenile snails to reach normal adult size (Williamson, Cameron & Carter, 1976; Cameron & Carter, 1979; Carter & Ashdown, 1984; Perry & Arthur, 1991; Baur & Baur, 1992; Foster & Stiven, 1996; Tanaka *et al.*, 1999; Moran & Emler, 2001). Smaller adult snails produce smaller eggs (Williamson *et al.*, 1976; Perry & Arthur, 1991; Tanaka *et al.*, 1999). Smaller eggs may have a reduced hatching rate (Winsor & Winsor, 1935; Eisenberg, 1965), which in turn could result in a decline in the overall population size (Berrie, 1970).

Survival of juveniles decreased with increasing density of adults, especially exotic adults. Eisenberg (1965) showed a

negative correlation between adult density and juvenile survival in the pond snail *Lymnaea elodes*, when density was over 719 m^{-2} . Higher density results in increased mortality and a delay in reproduction in the terrestrial *Limnicolaria flammea* (Egonmwan, 1992). Winsor & Winsor (1935) found that fitness in the pond snail *Lymnaea columella* is negatively correlated with initial hatchling density.

Based on ammonia testing, higher levels of ammonia did not occur with increased density of snails. Apple snails are resistant to nitrate and the hard-water formula is designed to provide calcium and buffering capacity (Lewis *et al.*, 2002; Corrao *et al.*, 2006). Food was provided *ad libitum* which would have been an additional source of minerals. The filter system was maintained during all experiments. In the first experiment, tanks with 8 and 32 juvenile snails had similar growth and all other experiments used only 4 or 16 juveniles per tank. Differences occurred between the 4 and 8 juvenile treatments and with the addition of one adult, which represents the lowest possible load on the filter system.

The mechanism of the density effect in the present set of experiments is not known. Potential reasons include interference with feeding and/or chemical suppressants. In all experiments food, in the form of romaine lettuce leaves, was always present and juveniles were seen on the leaves throughout each of the experiments; however, tanks were not monitored 24 h a day, so it is possible that the presence of adults could have reduced the amount of time juveniles fed. Growth in *P. paludosa* is affected by food composition (Shuford, McCormick & Magson, 2005); presumably changes in amount eaten would also have an effect. Kawata (1993) suggested behavioral interaction explained negative effects on growth in *Physa acuta*, although we saw no evidence of this during the course of our experiments.

Decreased growth in several species of snail held in captivity has been attributed to growth suppressive chemicals in slime trails (Williamson *et al.*, 1976; Hanning, 1979; Carter & Ashdown, 1984; Perry & Arthur, 1991), which would have been present during the experiments between times of cleaning. The slime trails of *Cepaea hortensis* decreased activity and growth in *C. nemoralis* when both species were held at densities of 40 m^{-2} (Cameron & Carter, 1979). Cameron & Carter (1979) also noted that the slime trail of a third more distantly related snail, *Helix aspersa*, had no effect on either *Cepaea* species. In contrast, Kawata & Ishigami (1992) found that growth of *Physa acuta* was faster in water conditioned by *Lymnaea* sp., so outcome based on interaction is not always in one direction in snails.

Regardless of the mechanism, it is clear that the presence of any adults reduces growth in *P. paludosa* juveniles, and that exotic adults had a more dramatic effect on both growth and survival of the native juveniles. One exotic adult had the equivalent effect of three to four native adults, which is probably at least partly due to the larger size of the exotic. It would be advantageous for similar species, such as *P. paludosa* and *P. insularum*, to produce inhibitory substances that act on closely related species that may be using similar resources such as food, egg laying substrate, mating space, predator cover and aestivating substrate. As the exotic adults are much larger than the native adults, interference or chemical release could have been greater, accounting for the greater effect of the exotic. Exotic adults in the field can be even larger than individuals used in the experiments (Rawlings *et al.*, 2007), so any size related effect would be greater. Asymptotic size is an inherent property of a species and exotic invasion takes place at the level of individuals, not masses, so the replacement of natives by exotics should have a larger impact even if only based on size differences. The native species has evolved a life history in which adult snails

die off annually leaving fewer adults to interact with juveniles; in contrast, the exotic adult snails, with a multi-year life span, may be present throughout this time.

The effect of the presence, or absence, of native adults is supporting evidence of a benefit that might be driving the evolution of adult post-reproductive die off as part of the life history of *P. paludosa*. If each generation does better with decreases in adult density while juveniles are in the growth phase, the characteristic of post-reproductive die off could be selected (Stearns, 2000; Roff, 2002).

Physiological tolerance of the exotic *P. insularum* to environmental conditions of temperature, pH, salinity and oxygen (Ramakrishnan, 2007) indicate it should do well in wetland habitats of the southeastern United States where *P. paludosa* occurs (see discussion by Rawlings *et al.*, 2007). Both species have similar responses to food type (Sharfstein & Steinman, 2001; Shuford *et al.*, 2005; Boland *et al.*, 2008). Juveniles consume more vegetation per mass than adults (Boland *et al.*, 2008), and potential juvenile density right after hatching is distinctly different from adult density making studies on juveniles crucial to understanding interactions between species. Brown (1982) found negative competitive effects when habitat and food type greatly overlapped in *Lymnaea elodes* and *Physa gyrina*. Exotic invasion by the freshwater *Potamopyrgus antipodarum* has caused problems with native gastropods in rivers in the western USA (Richards, Cazier & Lester, 2001; Hall, Dybdahl & VanderLoop, 2006; Lysne & Koetsier, 2008). Snail grazing can greatly affect vegetation (Brönmark, 1989), and exotic *Pomacea* spp. have become problems in many areas of the world (Cowie, 2002). The endangered snail kite has trouble feeding on the exotic *P. insularum*, so it is not equivalent to the native snail in meeting the birds foraging requirements (Darby *et al.*, 2007). Assuming the general pattern of response found in the laboratory translates to the field, we suggest an increase in the exotic apple snail *P. insularum* has the potential to reduce growth in the native apple snail *P. paludosa* where both species are present.

ACKNOWLEDGEMENTS

We thank Nancy Glass and Sarah Kell for assistance during the experiments. This work was partially supported by the Florida Department of Environmental Protection and the University of West Florida.

REFERENCES

- ABRAMS, P.A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *American Naturalist*, **146**: 112–134.
- ALVES, T., LIMA, P., LIMA, S.F.B., FERRI, A.G., BARROS, J.C. & MACHADO, J. 2006. Growth of *Pomacea lineata* and *Pomacea bridgesi* in different stock densities. *Thalassas*, **22**: 55–64.
- ARENAS, F., VIEJO, R.M. & FERNANDEZ, C. 2002. Density-dependent regulation in an invasive seaweed: responses at plant and modular levels. *Journal of Ecology*, **90**: 820–829.
- AUFDERHEIDE, J., WARBRITTON, R., POUNDS, N., FILEMPEADOR, S., STAPLES, C., CASPERS, N. & FORBES, V. 2006. Effects of husbandry parameters on the life-history traits of the apple snail, *Marisa cornuarietis*: effects of temperature, photoperiod, and population density. *Invertebrate Biology*, **125**: 9–20.
- BAUR, A. & BAUR, B. 1992. Responses in growth, reproduction and life span to reduced competition pressure in the land snail *Balea perversa*. *Oikos*, **63**: 298–304.
- BERRIE, A.D. 1970. Prolonged inhibition of growth in a natural population of the freshwater snail *Biomphalaria sudanica tanganyicensis* (Smith) in Uganda. *Annual Tropical Medical Parasitology*, **62**: 45–51.
- BOLAND, B.B., MEERHOFF, M., FOSALBA, C., MAZZEO, N., BARNES, M.A. & BURKS, R.L. 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of applesnails (*Pomacea*). *Journal of Molluscan Studies*, **74**: 47–54.
- BRIGHT, C. 1998. *Life out of bounds: Bioinvasion in a borderless world*. W.W. Norton, New York.
- BRINSON, M.M. & MALVAREZ, A.I. 2002. Temperate freshwater wetlands: types, status, and threats. *Environmental Conservation*, **29**: 115–133.
- BRÖNMARK, C. 1989. Interactions between epiphytes, macrophytes and freshwater snails: a review. *Journal of Molluscan Studies*, **55**: 299–311.
- BROWN, K.M. 1982. Resource overlap and competition in pond snails: an experimental analysis. *Ecology*, **63**: 412–422.
- BROWN, M.B. & FORSYTHE, A.B. 1974. Robust tests for the equality of variances. *Journal of the American Statistical Association*, **69**: 364–367.
- BROWN, K.M., VARZA, D. & RICHARDSON, T.D. 1989. Life histories and population dynamics of two subtropical snails (Prosobranchia: Viviparidae). *Journal of the North American Benthological Society*, **8**: 222–228.
- BYERS, J.E. 1999. The distribution of an introduced mollusk and its role in the long-term demise of a native congeneric species. *Biological Invasions*, **1**: 339–352.
- CAMERON, R.A.D. & CARTER, M.A. 1979. Intra- and interspecific effects of population density on growth and activity in some helioid land snails (Gastropoda: Pulmonata). *Journal of Animal Ecology*, **48**: 237–246.
- CARLSSON, N.O.L. & BRÖNMARK, C. 2006. Size-dependent effects of an invasive herbivorous snail (*Pomacea canaliculata*) on macrophytes and periphyton in Asian wetlands. *Freshwater Biology*, **51**: 695–704.
- CARTER, M.A. & ASHDOWN, M. 1984. Experimental studies on the effects of density, size, and shell colour and banding phenotypes on the fecundity of *Cepaea nemoralis*. *Malacologia*, **25**: 291–302.
- CAZZANIGA, N.J. 2002. Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda: Ampullariidae). *Biotell*, **26**: 71–81.
- CORRAO, N.M., DARBY, P.C. & POMORY, C.M. 2006. Nitrate Impacts on the Florida Apple Snail, *Pomacea paludosa*. *Hydrobiologia*, **568**: 135–143.
- COWIE, R.H. 2001. Invertebrate invasions on Pacific Islands and the replacement of unique native faunas: A synthesis of the land and freshwater snails. *Biological Invasions*, **3**: 119–136.
- COWIE, R.H. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts, and management. In: *Molluscs as crop pests* (G.M. Barker ed.), 145–192. Cabi Publ, Wallingford, UK.
- DARBY, P.C., BENNETTS, R.E. & PERCIVAL, H.F. 2008. Dry down impacts on apple snail (*Pomacea paludosa*) demography: implications for wetland water management. *Wetlands*, **28**: 561–575.
- DARBY, P.C., MELLOW, D.J. & WATFORD, M.L. 2007. Food handling difficulties for snail kites capturing non-native apple snails. *Florida Field Naturalist*, **35**: 79–85.
- DARBY, P.C., VALENTINE-DARBY, P.L. & PERCIVAL, H.F. 2003. Dry season survival in a Florida apple snail (*Pomacea paludosa* Say) population. *Malacologia*, **45**: 179–184.
- DARBY, P.C., VALENTINE-DARBY, P.L., PERCIVAL, H.F. & KITCHENS, W.M. 2004. Florida apple snail (*Pomacea paludosa*) responses to lake habitat restoration activity. *Archiv für Hydrobiologie*, **161**: 561–575.
- De FREITAS, J.R. & DOS SANTOS, M.B.L. 1995. Current advances on the study of snail-snail interactions, with special emphasis on competition process. *Memórias do Instituto Oswaldo Cruz*, **90**: 261–269.
- DOBSON, F.S. & OLI, M.K. 2001. The demographic basis of population regulation in Columbian ground squirrels. *American Naturalist*, **158**: 236–247.
- DUPONT-NIVET, M., COSTE, V., COINON, P., BONNET, J.-C. & BLANC, J.-M. 2000. Rearing density effect on the production

- performance of the edible snail *Helix aspersa* Müller in indoor rearing. *Annals of Zootechnology*, **49**: 447–456.
- EGONMWAN, R.I. 1992. The effects of population density on growth rate in *Limicolaria flammea* Müller (Pulmonata: Achatinidae). *Journal of Molluscan Studies*, **58**: 57–64.
- EISENBERG, R.M. 1965. The regulation of the density in a natural population of the pond snail, *Lymnaea elodes*. *Ecology*, **47**: 889–906.
- FORDYCE, J.A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, **209**: 2377–2383.
- FOSTER, B.A. & STIVEN, A.E. 1996. Experimental effects of density and food on growth and mortality of the southern Appalachian land gastropod, *Mesodon normalis* (Pilsbry). *American Midland Naturalist*, **136**: 300–314.
- FOWLER, W.F. 1981. Density dependence as related to life history strategy. *Ecology*, **61**: 602–610.
- HALL, R.O., DYBDAHL, M.F. & VANDERLOOP, M.C. 2006. Extremely high secondary production of introduced snails in rivers. *Ecological Applications*, **16**: 1121–1131.
- HANNING, G.W. 1979. *Aspects of Reproduction in Pomacea paludosa (Mesogastropoda: Piliidae)*. Thesis, Florida State University.
- HAYTER, A.J. 1986. The maximum familywise error rate of Fisher's least significant difference test. *Journal of the American Statistical Association*, **81**: 1000–1004.
- HILL, W.R., RYON, M.G. & SCHILLING, E.M. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology*, **76**: 1297–1309.
- ICHINOSE, K., WADA, T., YUSA, Y. & KUBOTA, T. 2000. Influence of habitat differences brought about by environmental changes on the densities of adults and eggs of *Pomacea canaliculata*. *Proceedings of the Association for Plant Protection of Kyushu*, **46**: 78–84.
- JAKUBIK, B. 2007. Egg number-female body weight relationship in freshwater snail (*Viviparus viviparus* L.) population in a reservoir. *Polish Journal of Ecology*, **55**: 325–336.
- JESS, S. & MARKS, R.J. 1995. Population density effects on growth in culture of the edible snail *Helix aspersa* var. *maxima*. *Journal of Molluscan Studies*, **61**: 313–323.
- KAMMENGA, J.E., SPURGEON, D.J., SVENDSEN, C. & WEEKS, M. 2003. Explaining density-dependent regulation in earthworm populations using life-history analysis. *Oikos*, **100**: 89–95.
- KARUNARATNE, L.B., DARBY, P.C. & BENNETT'S, R.E. 2006. The effects of wetland habitat structure on Florida apple snail density. *Wetlands*, **26**: 1143–1150.
- KAWATA, M. 1993. Relative importance of direct and indirect interaction among individual snails. *Researches on Population Ecology*, **35**: 69–77.
- KAWATA, M. & ISHIGAMI, H. 1992. The growth of juvenile snails in water conditioned by snails of a different species. *Oecologia*, **91**: 245–248.
- KSHIRSAGAR, A.M. & SMITH, W.B. 1995. *Growth curves*. Marcel Dekker Inc., New York.
- LACH, L., BRITTON, D.K., RUNDELL, R.J. & COWIE, R.H. 2000. Food preference and reproductive plasticity in an invasive freshwater snail. *Biological Invasions*, **2**: 279–288.
- LEWIS, P.A., KLENIM, D.J., LAZORCHAK, J.M., NORBERG-KING, T.J., PELTIER, W.H. & HEBER, M.A. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater invertebrates. *EPA-821-R-02-013*. U.S. Environmental Protection Agency, Cincinnati, OH.
- LEPPÄKOSKI, E. & OLENIN, S. 2000. Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biological Invasions*, **2**: 151–163.
- LORENZEN, K. & ENBERG, K. 2001. Density-dependent growth as a key mechanism in the regulation of fish populations: Evidence from among-population comparisons. *Proceeding of the Royal Society of London*, **269**: 40–54.
- LYSNE, S. & KOETSIER, P. 2008. Comparison of desert *Valvata* snail growth at three densities of the invasive New Zealand mudsnail. *Western North American Naturalist*, **68**: 103–106.
- MORAN, A.L. & EMLET, R.B. 2001. Offspring size and performance in variable environments: Field studies on a marine snail. *Ecology*, **82**: 1597–1612.
- MURDOCH, W.W. 1994. Population regulation in theory and practice. *Ecology*, **75**: 271–287.
- MYERS, J.H., SIMBERLOFF, D., KURIS, A.M. & CAREY, J.R. 2000. Eradication revisited: Dealing with exotic species. *Trends in Ecology and Evolution*, **15**: 316–320.
- NORRIS, K. 2004. Managing threatened species: the ecological toolbox, evolutionary theory and declining-population paradigm. *Journal of Applied Ecology*, **41**: 413–426.
- PERRY, R. & ARTHUR, W. 1991. Shell size and population density in large helicid land snails. *Journal of Animal Ecology*, **60**: 409–421.
- RAMAKRISHNAN, V. 2007. Salinity, pH, temperature, desiccation and hypoxia tolerance in the invasive freshwater apple snail *Pomacea insularum*. PhD Dissertation, University of Texas, Arlington.
- RAMNARINE, I.W. 2004. Quantitative protein requirements of the edible snail *Pomacea urceus* (Muller). *Journal of the World Aquaculture Society*, **35**: 253–256.
- RAO, C.R. 1958. Some statistical methods for comparison of growth curves. *Biometrics*, **14**: 1–17.
- RAWLINGS, T.A., HAYES, K.A., COWIE, R.H. & COLLINS, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology*, **7**: 97.
- RICHARDS, D.C., CAZIER, L.D. & LESTER, G.T. 2001. Spatial distribution of three snail species including the invader *Potamopyrgus antipodarum*, in a freshwater spring. *Western North America Naturalist*, **61**: 375–380.
- ROFF, D.A. 2002. *Life history evolution*. Sinauer Associates, Sunderland.
- SHARFSTEIN, B. & STEINMAN, A.D. 2001. Growth and survival of the Florida apple snail (*Pomacea paludosa*) fed 3 naturally occurring macrophyte assemblages. *Journal of the North American Benthological Society*, **20**: 84–95.
- SHUFORD, R.B.E., MCCORMICK, P.V. & MAGSON, J. 2005. Habitat related growth of juvenile Florida applesnails (*Pomacea paludosa*). *Florida Scientist*, **68**: 11–19.
- SIMBERLOFF, D. 2003. Confronting introduced species: A form of xenophobia? *Biological Invasions*, **5**: 179–192.
- SMITH, R.L. & SMITH, T.M. 2001. *Ecology and field biology: sixth edition*. Addison Wesley Longman, San Francisco.
- STEARNS, S.C. 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften*, **87**: 476–486.
- STRZELEC, M. & KRÓLCZYK, A. 2004. Factors affecting snail (Gastropoda) community structure in the upper course of the Warta river (Poland). *Biologia, Bratislava*, **59**: 159–163.
- SYKES, P.W., RODGERS, J.A. & BENNETT'S, R.E. 1995. Snail kite (*Rostrhamus sociabilis*). In: *The birds of North America, No. 171* (A. Poole & F. Gill eds), 1–32. Academy of Natural Sciences, Philadelphia and American Ornithologists' Union, Washington, DC.
- TANAKA, K., WATANABE, T., HIGUCHI, H., MIYAMOTO, K., YUSA, Y., KIYONAGA, T., KIYOTA, H., SUZUKI, Y. & WADA, T. 1999. Density-dependent growth and reproduction of the apple snail, *Pomacea canaliculata*: a density manipulation experiment in a paddy field. *Research Population Ecology*, **41**: 253–262.
- TANNER, J.E. 1997. The effects of density on the zoanthid *Palythoa caesia*. *Journal of Animal Ecology*, **66**: 793–810.
- THOMPSON, F.G. 2004. *An identification manual for the freshwater snails of Florida*. University of Florida Press, Gainesville.
- TURNER, R.L. 1996. Use of stems of emergent plants for oviposition by the Florida applesnail, *Pomacea paludosa*, and implications for marsh management. *Florida Scientist*, **59**: 34–49.

- TURNER, R.L. & MIKKELSEN, P.M. 2004. Annotated bibliography of the Florida applesnail, *Pomacea paludosa* (Say) (Gastropoda: Ampullariidae), from 1825 to 1999. *Nemouria*, **48**:1–188.
- VAN DRIESCHE, J. & VAN DRIESCHE, R. 2000. *Nature out of place*. Island Press, Washington, DC.
- WEAVER, K.C. & PAYNE, G.G. 2006. Chapter 2A: status of water quality in the Everglades protection area. In: *2006 South Florida Environmental Report, vol. 1*, South Florida Water Management District (<http://my.sfwmd.gov>).
- WELLS, F.E. 1978. The relationship between environmental variables and the density of the mud snail, *Hydrobia totteni* in a Nova Scotia salt marsh. *Journal of Molluscan Studies*, **44**:120–129.
- WILLIAMSON, P., CAMERON, R.A.D. & CARTER, M.A. 1976. Population density affecting adult shell size of *Cepaea nemoralis* L. *Nature*, **263**: 496–497.
- WINSOR, C.P. & WINSOR, A.A. 1935. Longevity and fertility in the pond snail, *Lymnaea columella*. *Journal of the Washington Academy of Science*, **25**: 302–307.