



# Nutrients influence the multi-trophic impacts of an invasive species unaffected by native competitors or predators

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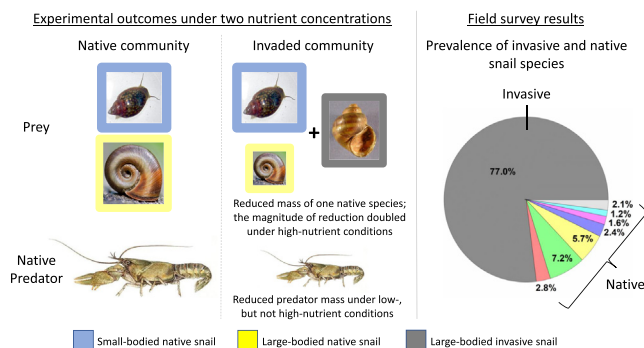
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## HIGHLIGHTS

- The impacts of invasive species are influenced by native competitors, predators, and water quality
- We assessed whether nutrient conditions affected competitive and predatory interactions with native species
- The invasive species out-competed a native species; the competitive effect doubled under high nutrient concentrations
- The native predator mass was less in ecological communities with invasive prey
- The abundance of the invasive snail species dominated the snail assemblage in a natural ecosystem

## GRAPHICAL ABSTRACT



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## ABSTRACT

Non-native species often lead to undesirable ecological and environmental impacts. Two hypotheses that predict establishment of non-native species are enemy release and biotic resistance. Support for these hypotheses in freshwater invasions is mixed. Experiments combined with field observations provide a complementary approach to understanding how interactions between native and non-native species lead to enemy release or biotic resistance. We tested experimentally whether these hypotheses provided insights into the invasion of the banded mystery snail (*Viviparus georgianus*), which has invaded the Great Lakes region and northeastern United States (US) from the southeastern US. Because freshwater systems vary widely in their nutrient concentrations due to natural and anthropogenic processes, we tested whether nutrient additions altered competitive and predatory interactions that regulate mechanisms of enemy release or biotic resistance. We evaluated the status of the mystery snail invasion in a 3-year field survey of Lake George (NY, US) to identify if field observations supported any experimental conclusions. The presence of the banded mystery snail led to a 14% and 27% reduction in biomass of a native competitor under low- and high-nutrient concentrations, respectively. The mystery snail also triggered a 29% biomass loss of a native snail predator, but only in low-nutrient concentrations. Field surveys indicated that the mystery snail dominated the snail community; of seven snail species, it comprised 77% of all snails. Results from the field surveys combined with experimental results indicate that neither competitors nor predators have likely suppressed the invasion of the banded mystery snail. This conclusion is consistent with competitive- and predatory-enemy release as we found no indication of biotic resistance via competition or

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predation from native species. Our results further highlight that the post-establishment impacts of invasive species are altered by the trophic state of freshwater ecosystems.

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## 1. Introduction

Successful establishment of invasive species can trigger changes in biodiversity (Molnar et al., 2008; Ricciardi et al., 1998; Sanders et al., 2003), ecosystem function (Vila et al., 2011), and species evolution (Mooney and Cleland, 2001). Two highly debated hypotheses that provide a framework to understand the successful or unsuccessful establishment of invasive species are the enemy release hypothesis (ERH) and the biotic resistance hypothesis (BRH). The ERH suggests successful invasion can be attributed to a lack of regulation of invasive species by native predators, competitors, or parasites in the invaded range (Blumenthal et al., 2009; Heger and Jeschke, 2014; Keane and Crawley, 2002; Pimm, 1987). In contrast to enemy release, the BRH posits that species diversity (filled niches) or interactions with native species (e.g., predation or competition) in the invaded range will limit the invasion success and, therefore, could reduce the post-establishment impacts of invasive species (Elton, 1958; Maron and Vila, 2001; Ricciardi et al., 2013). However, support for the ERH and BRH is mixed because species invasions are often complex due to direct and indirect species interactions and the context-dependent effects of abiotic conditions (Colautti et al., 2004; Elliott-Graves, 2016; Ricciardi et al., 2013). Experiments complemented with field observations are needed to further elucidate how context-dependent interactions between native and non-native species lead to enemy release or biotic resistance.

In freshwater ecosystems, tests of the ERH and BRH are rare (Jeschke et al., 2012b; Ricciardi and MacIsaac, 2011), despite a greater frequency of high-impact invaders than in marine (Alofs and Jackson, 2014; Ricciardi and MacIsaac, 2011) and terrestrial ecosystems (Moorhouse and Macdonald, 2015). Further, the extinction rate of freshwater animals is higher than terrestrial animals, and can even rival extinction rates observed in tropical forests (Burkhead, 2012; Ricciardi and Rasmussen, 1999). Thus, it is critically important to identify mechanisms that lead to invasion success and subsequent impacts in freshwater ecosystems, which could be exacerbating the loss or extirpation of native species (Gallardo et al., 2016).

Fresh waters vary widely in the availability and concentration of nutrients such as nitrogen and phosphorus. In oligotrophic systems, low-nutrient concentrations may restrict primary productivity and bottom-up effects (Elser et al., 2007). In natural or culturally eutrophic systems nutrient concentrations are much higher, which increases primary production available to consumers (Hecky and Kilham, 1988; McQueen et al., 1989). While nutrient availability can alter the dynamics and impacts of species invasions (Byers, 2002; Gallardo et al., 2016; Gonzalez et al., 2010), the role of nutrients in species invasions can either favor native or the invading species. For instance, higher nutrient concentrations have been shown to increase the propagule pressure and growth of invasive macrophytes (Xie et al., 2018) and enhance the production of invasive fish (Preston et al., 2018). On the other hand, higher nutrients can enhance biotic resistance from native communities and reduce the establishment of non-native species (Teixeira et al., 2017). Understanding the complex role nutrients have on the mechanisms of enemy release or biotic resistance is important for managing and predicting the impacts of freshwater invaders.

In this study, our primary objective was to test whether the theoretical framework of the BRH or ERH was useful in explaining a freshwater invasion in the Great Lakes Region of North America. We also wanted to determine whether any post-establishment impacts due to enemy

release or mechanisms of biotic resistance were altered by nutrient conditions. Specific sub-hypotheses of the ERH such as competitive-, predatory-, or mutualism-enemy release may have the greatest utility for theory building (Heger and Jeschke, 2014). Thus, we specifically addressed sub-hypotheses related to competitive- and predatory-enemy release (Colautti et al., 2004; Heger and Jeschke, 2014), which need empirical support in freshwater ecosystems (Heger and Jeschke, 2014; Jeschke et al., 2012a). We focused our investigation on the banded mystery snail *Viviparus georgianus*, which has invaded the Great Lakes Region of North America and the northeastern United States (US). Native to the southeastern US, unclear is if *V. georgianus* was intentionally or unintentionally released into the Hudson River and tributaries of Lake Michigan during the late 1800s or early 1900s (Clench, 1962; Mills et al., 1993). Quantitative data regarding the abundance of *V. georgianus* relative to native snail species is sparse (David et al., 2017), but it is well documented that *V. georgianus* has spread throughout the North American Great Lakes Region and the northeastern US (Bury et al., 2007). Yet, the impacts of *V. georgianus* are unknown except that it can consume native fish eggs (Eckblad and Shealy, 1972).

To address our objectives, we conducted an experiment to test whether enemy release or biotic resistance could potentially explain pattern and process in the *V. georgianus* invasion of the Great Lakes and northeastern US regions. Specifically, the experiment tested for competitive interactions with native snails and predatory interactions with a native snail predator (i.e. crayfish). We also manipulated nutrient concentrations to determine whether bottom-up effects altered the outcomes of competitive and predatory interactions leading to enemy release or biotic resistance. We used data collected from a 3-year field study in a large lake ecosystem to determine the relative abundance of *V. georgianus* in relation to native snail species. These data were used to determine if the conclusions of our experiment were consistent with any field observations. We made the following five predictions, with the understanding that the ERH and BRH are potentially two mechanisms that are inversely correlated and where the sum of species interactions and abiotic influence would generate support for one hypothesis or the other (Fig. 1)

1. Biotic resistance would be supported if a native predator or a functionally similar native species reduced the abundance, survival, or growth of *V. georgianus*.
2. Enemy release would be supported if native predators and competitors did not reduce the abundance, survival, and growth of *V. georgianus*.
3. Elevated nutrient concentrations would reduce exploitative competition between native snails and *V. georgianus* because nutrients would increase periphytic food resources for the snail community. This could limit the impacts of *V. georgianus* on native competitors or the ability of the native community to resist invasion of *V. georgianus* through consumptive or competitive biotic resistance.
4. In a natural system, if the population size of *V. georgianus* is much lower than functionally similar native species, this might indicate that interactions with native species led to biotic resistance in the system.
5. In a natural system, if *V. georgianus* attains a much larger population size than functionally similar native species, it would suggest *V. georgianus* is either a better competitor than native snails or that native predators are not consuming *V. georgianus* to a degree that would suppress population size.

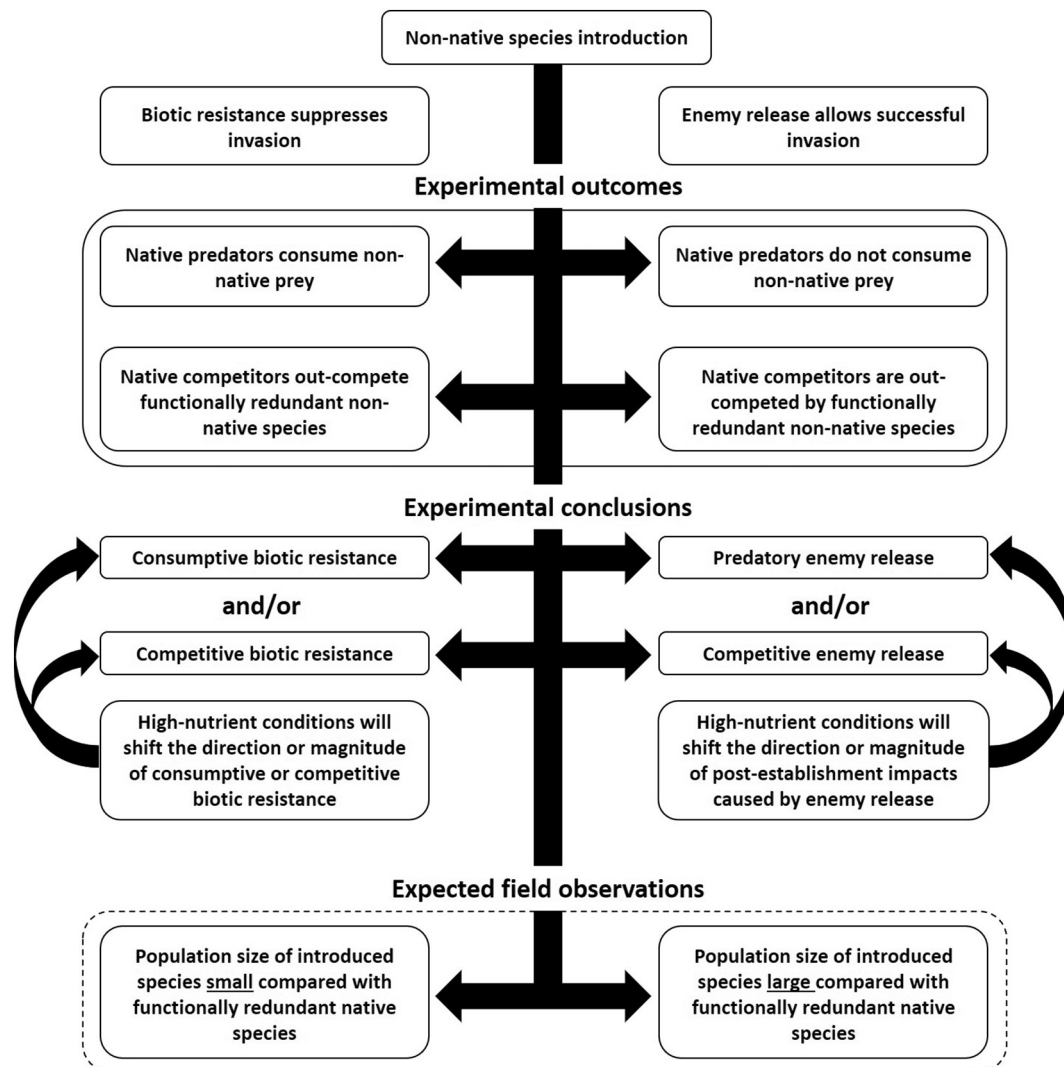


Fig. 1. Predictions of support for the biotic resistance hypothesis or enemy release hypothesis.

## 2. Methods

### 2.1. Experimental design and procedure

The experiment lasted for 68 d, from 21 August to 26 October 2016. We used a  $2 \times 2 \times 2$  randomized design in which we manipulated ecological communities. Manipulations included the presence or absence of a native snail predator (crayfish), the presence or absence of the non-native *V. georgianus*, and either ambient- or high-nutrient treatments. The additive design consisted of eight treatment combinations that we replicated four times for a total of 32 experimental mesocosms, which were 750-L cattle tanks located at Rensselaer Polytechnic Institute's Aquatic Ecology Laboratory (42°40'35.87"N; 73°42'2.05"W).

We filled each mesocosm with 650 L of aged (>48 h) tap water on 2 August 2016 and covered each mesocosm with a 60% shade cloth lid, which allowed enough light for primary production but prevented other organisms from entering the mesocosms (for information on municipal water source used, see <http://www.troyny.gov/departments/public-utilities/reports/>). On 4 August, we added two 40 × 20 × 20 cm mason blocks to each mesocosm. In tanks with a predator treatment, the blocks served as habitat refugia for snails from the crayfish predator in addition to the side walls of the tanks (crayfish are unable to climb the sides of the tanks). On the same day, we added 15 g of rabbit chow (Bunny 16; Blue Seal, Muscatine, IA, USA) and 100 g of leaf litter (*Quercus* spp.) to each mesocosm to serve as basal nutrient sources

and additional habitat structure. We also added two 15 × 15 cm tiles to serve as a standardized substrate to quantify periphyton biomass.

We added plankton to the mesocosms to mimic a more natural ecological community and because *V. georgianus* is a facultative filter feeder of algae and detritus, particularly as juveniles (Browne, 1978). On 5 August, we added a 0.5-L aliquot of phytoplankton and zooplankton to each mesocosm collected from three local New York lakes (Crystal Lake [42°38'33.80"N, 73°33'7.05"W], Burden Lake [42°36'52.00"N, 73°33'57.43"W], and Snyder Lake [42°39'35.81"N, 73°38'8.72"W]). We collected zooplankton from each lake using a 64- $\mu$ m mesh tow net. We concentrated and mixed collections from the three lakes before aliquoting 0.5 L into each mesocosm. We allowed primary producers and zooplankton to grow for 13 d before adding the experimental treatments.

On 18 August, we added the three functionally similar snail grazers. We added two native snails: the rams-horn snail *Helisoma trivolvis* and the pond snail *Physella acuta*. Both species are ubiquitous in lakes and wetlands throughout the Great Lakes region in North America and overlap with *V. georgianus* in this region. We collected all snail species from three locations: Crooked Lake (42°36'45.12"N, 73°31'29.64"W), Crystal Lake, and a private wetland (42°46'13.56"N, 73°38'42.83"W). We collected the snails from multiple locations because of access and we had to go to multiple systems achieve our desired stocking numbers for each species in the mesocosms. Snails were sampled with 500-micron D-frame net sweeps in areas of submerged vegetation. We randomly

sub-sampled 20 individual adults from the snails collected (preserved in ethanol prior to measuring), patted them dry, and weighed them to determine the aggregate wet biomass added to the mesocosms. We added 20 adult *P. acuta* (biomass 0.471 g; 0.0236 g/individual) and 20 adult *H. trivolvis* (biomass 13.113 g; 0.6557 g/individual) to all mesocosms. We added 20 adult *V. georgianus* (biomass 25.189 g; 1.2595 g/individual) to mesocosms assigned to the non-native snail treatment. Because the density of the three snail species varies widely in nature from a few  $\text{m}^{-2}$  to thousands  $\text{m}^{-2}$ , our intent was not to mimic natural densities. Rather, we added the same number of individuals to each tank and allowed competition, predation, and nutrients to shape the snail assemblages over the 68-d experiment. We did not standardize the initial populations by biomass because life history characteristics vary widely among the three snail species. *P. acuta*, for instance, attains a much smaller body size than the other snails, but grows and reproduces faster. Thus, rather than standardizing by biomass we chose to standardize by the number of individuals and assumed species interactions and life-history traits would dictate the experimental outcomes.

On 19 August, we added nutrients to the assigned mesocosms. The low-nutrient, aged tap water served as our ambient nutrient treatment. Our high nutrient treatment was meant to simulate eutrophic concentrations. We based the nutrient additions on concentrations reported for a typical eutrophic lake (Downing and McCauley, 1992). Our goal was to reach a concentration of 50  $\mu\text{g}$  of total phosphorus (TP)  $\text{L}^{-1}$  and a total nitrogen (TN) to TP ratio of 23:1. Thus, we added 0.05 mg P  $\text{L}^{-1}$  as sodium phosphate dibasic anhydrous ( $\text{Na}_2\text{HPO}_4$ ) and 1.15 mg N  $\text{L}^{-1}$  as ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ). We added these nutrients twice during the experiment; the second addition occurred on 2 October. Because nutrients are typically absorbed from the water column soon after introduction (e.g., Plath and Boersma, 2001) we did not measure TN:TP of the water column to verify that our dosage increased nutrients to the desired level.

We added the northern clearwater crayfish (*Faxonius propinquus*) as a snail predator to the assigned mesocosms on 21 August (defined as day 1 of the experiment). This species of crayfish is known have an opportunistic diet that includes native snails (Krist, 2002), many of which are from the same genera or species used in our experiment and found in our field study (Brown, 1998; Sura and Mahon, 2011). The carapace size of *F. propinquus* can exceed 35 mm in New York populations (Crocker, 1957), but attains a smaller size in cooler regions (Corey, 1988). Crayfish were an ideal model snail predator because they are known to have consumptive and non-consumptive effects on the native snail species used in our experiment (Auld and Relyea, 2008; Hoverman and Relyea, 2012; Hoverman and Relyea, 2016; Turner, 2004; Turner et al., 2006). The three snail species and crayfish also co-occur in the wild (e.g., Lake George, personal observation). We collected crayfish of similar size from Northwest Bay Brook, which is a tributary/wetland of Lake George (43°36'51.80"N, 73°36'44.88"W). Because crayfish are fiercely agonistic toward conspecifics, we only added a single crayfish to each predator-occupied mesocosm. We did not measure the size of the crayfish prior to introduction into the mesocosms to prevent further stress after transport from the field (length range of 3.5–4.0 cm from tip of rostrum to tip of telson). We randomly assigned crayfish to the mesocosms to prevent introducing size-bias among the experimental treatments.

We wanted to determine if environmental conditions varied among the experimental treatments. During the 68-d experiment, we sampled abiotic conditions, phytoplankton, periphyton, and zooplankton on days 26 (15 September) and 67 (25 October). We measured temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg O}_2 \text{L}^{-1}$ ), conductivity ( $\mu\text{S cm}^{-1}$ ), and pH with a calibrated YSI ProPlus Multiparameter Instrument (YSI, Yellow Springs, Ohio, USA).

We estimated phytoplankton abundance because *V. georgianus* can filter phytoplankton as a food source. To estimate phytoplankton abundance, we filtered two 350-mL samples of water collected from the

north side and center from each mesocosm through glass microfiber filters (1.2- $\mu\text{m}$  pore size; Whatman). We froze the glass microfiber filters until we conducted chlorophyll *a* analysis via fluorometry with acid correction (Arar and Collins, 1997).

To estimate periphyton biomass, we gently removed one 15 × 15 cm tile from each mesocosm, scrubbed and rinsed it three times, and filtered the slurry through pre-weighed and dried (at 60  $^{\circ}\text{C}$  for 24 h) glass microfiber filters. We dried periphyton-covered filters for 48 h at 60  $^{\circ}\text{C}$  and reweighed them to determine periphyton biomass on each tile.

To determine any direct or indirect effects on the zooplankton community, we collected five, 450-mL sub-samples, which were filtered through 64- $\mu\text{m}$  Nitex screening (total filtered water = 2.25 L). We collected 4 sub-samples in the four cardinal directions, halfway between the center of the mesocosm and the outside wall, and a fifth sample from the center of each mesocosm. We preserved zooplankton samples in 30% ethanol for later enumeration and identification. We identified zooplankton to the three major groups including cladocerans, copepods, and rotifers.

On day 68, we terminated the experiment and collected the snails and crayfish. We rinsed the remaining leaves and cinder blocks and removed them from each tank. We then filtered the entire contents of the tanks through a 500- $\mu\text{m}$  D-frame net. We immediately preserved the snails and crayfish in 70% ethanol for later enumeration and measurement. We measured aggregate biomass (g) for each of the snail species among the replicates. Combined with abundance estimates, this allowed us to infer changes in individual snail mass. If abundance did not differ among the treatments, we used aggregate biomass. We did this rather than dividing abundance by biomass to analyze average individual biomass because the size distributions are often highly skewed. For aggregate snail biomass of each species, we blotted the snails of a single species dry for each replicate and measured biomass to the nearest mg. We also measured crayfish length (mm; rostrum to telson) and biomass to nearest g.

## 2.2. Field surveys

The 3-yr field study was conducted in Lake George, a large oligotrophic and economically important lake in the Adirondack Park of New York State, USA. We conducted three field surveys during the summer months (June, July, and August) of non-native and native snails in each of 3 contiguous years of 2015–2017 to determine the relative abundance of *V. georgianus* in relation to native snail species. This allowed us to identify whether abundance patterns of snails in the field were consistent with potential mechanisms identified in our experiment. We sampled 30 sites for snails in Lake George (43°36'51.80" N, 73°36'44.88"W) using a Petite Ponar grab sampler because most sites had soft substrata. We took one grab at each site. Sampling of all 30 sites took 1–3 d each month, depending on weather. Sites were evenly distributed around the lake to ensure maximum coverage and occurred in <20 m of water. We filtered each sample from a site through a 1-mm sieve and preserved the sample in 70% ethanol for later enumeration and identification.

## 2.3. Statistical analyses

To analyze our experimental data, we used parametric analysis of variance (ANOVA). We used three-way, repeated-measures ANOVA (rm-ANOVA) to examine interactive and main effects of sampling date (day 26 or 67), predators (present or absent), the presence or absence of *V. georgianus*, or nutrients (ambient or high) on abiotic conditions, phytoplankton, zooplankton, and periphyton. For the rm-ANOVAs, we used the lmer function in lme4 package (tank as random effect, treatments as fixed effects) and car package to generate rm-ANOVA tables. If we detected any time-by-treatment interactions, we then analyzed the response variable within each sampling day using three-way

ANOVA. For abundance and biomass of the two native snail species, which were only measured at the end of the experiment, we used single-measure three-way ANOVAs. We examined the effects of nutrients, predators, and their interaction on the abundance and biomass of *V. georgianus* using two-way ANOVAs. We also used a two-way ANOVA to determine if there was an effect of nutrients, the presence of *V. georgianus*, and their interaction on crayfish predator size. If we detected significant interactive effects, we used Tukey's HSD post hoc tests to examine comparisons among the treatment levels.

We checked the assumption of equal variance for ANOVA with a Brown-Forsythe test. If the underlying assumption of constant variance was violated, we transformed the data. The only transformations needed were for periphyton on day 26 (rank transformation) and phytoplankton on day 26 ( $\log_{10}$ ). We conducted all rm-ANOVA analyses in R and two- and three-way single-measure ANOVAs were conducted in SigmaPlot 12.5. We used  $P \leq 0.050$  as a threshold to evaluate statistical support for our hypotheses.

In our field surveys, we examined the relative abundance (% composition) of each snail species collected throughout the 3 years of the survey. We calculated the frequency of occurrence of each snail species by dividing the number of samples that contained a given snail species by the total number of samples in our 3-years of snail surveys ( $n = 270$  samples). Finally, we calculated the mean number of individuals collected per sample when a species was detected. Because the number of samples that included a certain species was highly variable, we calculated 95% confidence intervals to compare the average abundance of each snail species when they were detected. If confidence intervals did not overlap, patterns were considered to be significantly different.

### 3. Results

#### 3.1. Experimental results

##### 3.1.1. Abiotic conditions

We found that temperature, dissolved oxygen, conductivity, and pH varied by sampling date ( $F_{1,24} \geq 58.6$ ,  $P < 0.001$ ), indicating abiotic conditions changed over time. However, we did not find any main effects, treatment interactions, or time-by-treatment interactions ( $F_{1,24} \leq 4.0$ ,  $P \geq 0.055$ ), suggesting experimental conditions were relatively similar among the experimental treatments.

##### 3.1.2. Plankton

We found no effects of nutrients, the invasive species, predator presence, or sampling date on the abundance of phytoplankton ( $F_{1,24} \leq 3.2$ ,  $P \geq 0.088$ ). We also found no effects of our experimental treatments on the three major groups of zooplankton. Cladoceran abundance did not differ among the experimental treatments or with sampling date ( $F_{1,24} \leq 2.6$ ,  $P \geq 0.110$ ). Copepod abundance varied by sampling date ( $F_{1,24} = 6.3$ ,  $P = 0.020$ ), but was unaffected by the experimental treatments ( $F_{1,24} \leq 3.3$ ,  $P \geq 0.081$ ). Similarly, we did not find any differences in rotifer abundance among the experimental treatments ( $F_{1,24} \leq 1.6$ ,  $P \geq 0.218$ ).

##### 3.1.3. Periphyton

We found an interaction between sampling date and the presence of *V. georgianus* ( $F_{1,24} = 4.8$ ,  $P = 0.038$ ) and a main effect of sampling date ( $F_{1,24} = 62.9$ ,  $P < 0.001$ ). Thus, we analyzed periphyton biomass within each sample date due to the treatment-by-date interaction. Midway through the experiment on the first sampling date (day 26), we did not find any main effects or interactions between nutrients, predator presence, or the presence of *V. georgianus* ( $F_{1,24} \leq 3.5$ ,  $P \geq 0.073$ ).

At the end of the experiment (day 67), we found an interaction between the predator and community treatment ( $F_{1,24} = 6.7$ ,  $P = 0.016$ ) and a main effect of nutrients ( $F_{1,24} = 12.3$ ,  $P = 0.002$ ). In the native community, periphyton biomass was 42% higher with a predator present compared with the no-predator treatment (Fig. 2;  $q = 4.5$ ,  $P$

$= 0.004$ ). In the presence of a predator, periphyton biomass was reduced by 30% in the invaded community compared to the native community ( $q = 4.6$ ,  $P = 0.003$ ). Periphyton biomass was 34% higher in the high-nutrient treatment compared to the ambient-nutrient treatment regardless of the presence of *V. georgianus* or predator treatment.

##### 3.1.4. Snail abundance and size

Average abundance, average aggregate biomass, and average individual biomass of snail prey among the experimental treatments can be found in Table 1.

We found an effect of nutrients ( $F_{1,24} = 24.5$ ,  $P < 0.001$ ), but no effect of *V. georgianus*, predator presence, or any interactions on the abundance of the native *P. acuta* ( $F_{1,24} \leq 3.4$ ,  $P \geq 0.075$ ). Average *P. acuta* abundance  $\pm$  standard error (SE) among all treatments was  $503 \pm 51$  individuals, indicating that reproduction occurred. The abundance of *P. acuta* was  $2.2\times$  higher in the high-nutrient treatment compared to the ambient-nutrient treatment (Fig. 3A). We also found the aggregate biomass was  $2.1\times$  higher in the high nutrient treatment compared to the ambient treatment ( $F_{1,24} = 46.7$ ,  $P < 0.001$ ), but was not affected by the main or interactive effects of the other treatments ( $F_{1,24} \leq 2.9$ ,  $P \geq 0.100$ ; Fig. 3B).

We found no main effects or interactions of *V. georgianus*, predator, or nutrients on the abundance of native *H. trivolvis* ( $F_{1,24} \leq 3.3$ ,  $P \geq 0.083$ ). Average *H. trivolvis* abundance  $\pm$  SE among the treatments was  $35 \pm 6$  individuals, indicating that reproduction occurred. We found an interaction between nutrients and *V. georgianus* on the aggregate biomass of *H. trivolvis* (Fig. 4;  $F_{1,24} = 4.9$ ,  $P = 0.039$ ). Compared with the native community, aggregate biomass of *H. trivolvis* was reduced by 14% in the invaded community under the ambient-nutrient treatment ( $q = 3.9$ ,  $P = 0.011$ ) and by 27% in the high-nutrient treatment ( $q = 8.3$ ,  $P < 0.001$ ). Within the native community, nutrients increased the aggregate biomass of *H. trivolvis* by 24% ( $q = 6.2$ ,  $P < 0.001$ ).

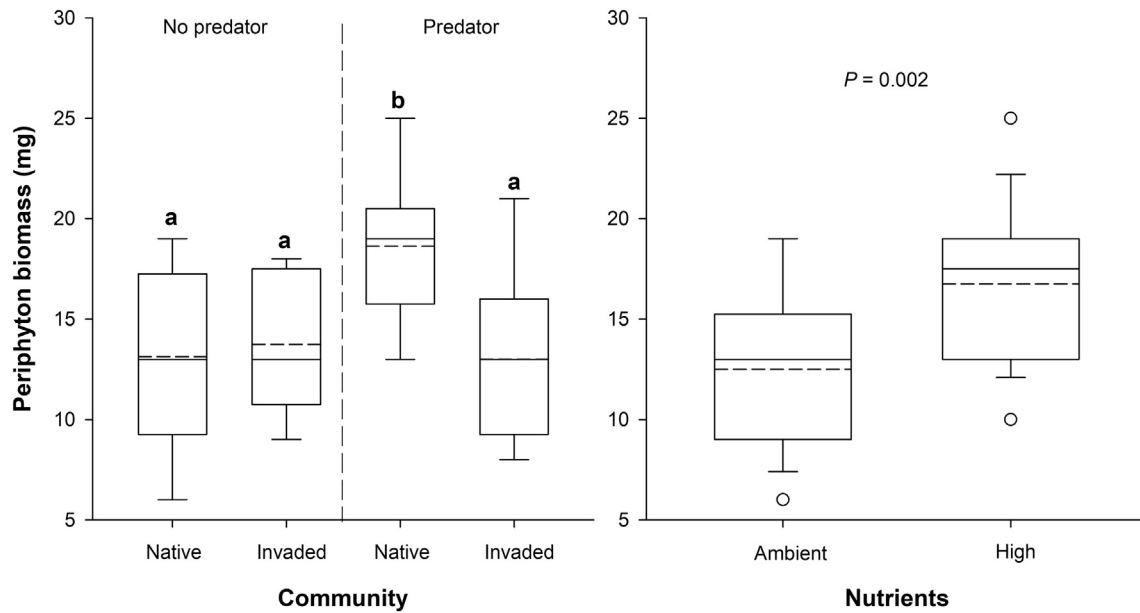
We recovered 20 *V. georgianus* in each tank (the original stocked density) except for 1 tank where we recovered 19 individuals, indicating high survival but no reproduction. We found no main effects or interactions of nutrients or predators on *V. georgianus* aggregate biomass ( $F_{1,12} \leq 1.6$ ,  $P \geq 0.226$ ). Average aggregate biomass  $\pm 1$  SE of *V. georgianus* among the 16 tanks was  $32.57 \pm 0.8$  g, which was 29.3% (7.38 g) higher than the stocked aggregate biomass.

##### 3.1.5. Crayfish size

We recovered all crayfish at the end of the experiment (100% survival). We found crayfish size was affected by the treatments. Average crayfish length (tip of rostrum to tip of telson) was smaller by 7.2% in the invaded community (435 mm) compared with native community (469 mm;  $F_{1,12} = 4.9$ ,  $P = 0.048$ ). We did not detect an effect of nutrients or a nutrient-by-*V. georgianus* interaction on crayfish length ( $F_{1,12} \leq 1.9$ ,  $P \geq 0.191$ ). We found an interaction between the nutrients and the presence of *V. georgianus* on crayfish biomass (Fig. 5;  $F_{1,12} = 5.1$ ,  $P = 0.044$ ). Under the ambient-nutrient treatment, crayfish biomass was reduced by 29% in the invaded community (2.5 g) compared with the biomass of individuals in the native community (3.5 g;  $q = 4.5$ ,  $P = 0.008$ ). No other pairwise comparisons were different.

#### 3.2. Field survey

Across sites, months, and years, our field surveys indicated that the non-native species *V. georgianus* was the most abundant snail species in Lake George. Over the 3-year survey, we collected six native species and the non-native *V. georgianus*. Among all snails collected, 77% were *V. georgianus* (Fig. 6A). The most abundant of the native snail species were *Gyraulus* spp. (7.2%), *H. trivolvis* (5.7%), and *P. acuta* (5.4%). We found that the non-native *V. georgianus* was detected in 96.0% of the samples. Among the native species, we detected *H. trivolvis*, *Gyraulus* spp., and *P. acuta* in 28.5%, 28.2%, and 22.2% of the samples, respectively (Fig. 6B). When detected, we found an average of 11.6 individuals of *V.*



**Fig. 2.** The interactive effects between the presence of *V. georgianus* and predators (left) and main effects of nutrients (right) on periphyton resources on the final day of the experiment (day 68). Each box shows the mean (—), median (—), the 25th and 75th percentiles (box), and the 10th and 90th percentiles (error bars). Outliers from these distributions are shown as single, unfilled data points. Letters above boxplots indicate statistical differences.

*georgianus* whereas we only found 1.6–3.2 individuals among the native species (Fig. 6C).

#### 4. Discussion

Our experimental results appear to support the sub-hypotheses of competitive- and predatory-enemy release as potential mechanisms leading to successful invasion by *V. georgianus*. We found no indication that functionally similar native snails reduced the abundance, survival, or suppressed the growth of *V. georgianus*. In fact, *V. georgianus* biomass at the end of the experiment was greater than the biomass at the beginning of the experiment among all treatments. Thus, competition with the native snails in our experiment does not appear to generate a mechanism of biotic resistance. Although there can be a lag in the ability of native predators to control non-native prey (Carlsson et al., 2009) and consumption can drive biotic resistance in freshwater systems (Alofs and Jackson, 2014), *V. georgianus* was unaffected by the native crayfish predator, suggesting consumptive or non-consumptive effects did not yield support for biotic resistance. The dominance of *V. georgianus* from our 3-yr survey of the Lake George snail assemblage was clear. Such a dominant abundance and prevalence over native snails might

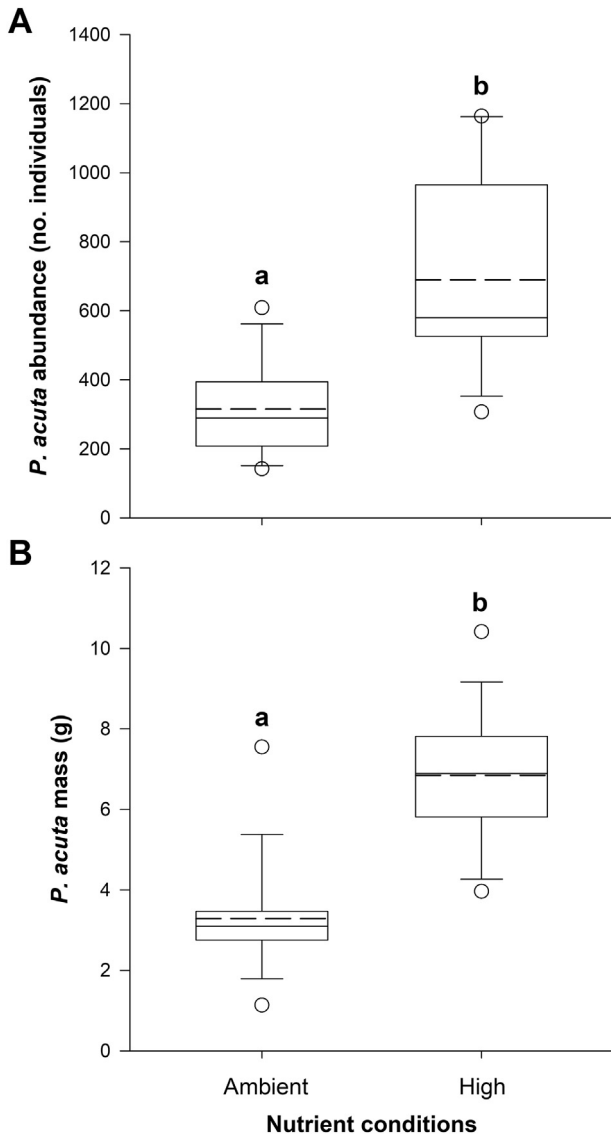
indicate—similar to our experimental conclusions—that neither competitors nor predators have controlled the *V. georgianus* invasion in Lake George. While limited to a single large oligotrophic lake, our field surveys seem to support our experimental conclusions that enemy release may account for the successful establishment, dominant abundance, and post-establishment impacts of *V. georgianus*.

Negative impacts of *V. georgianus* were manifested as reductions in the biomass of native species at multiple trophic levels. Competitive interactions, in favor of *V. georgianus*, led to the reduced biomass of *H. trivolvis*. There are multiple potential mechanisms that might favor *V. georgianus*, which are not necessarily mutually exclusive. One mechanism is that periphytic food resources were reduced or access was limited for *H. trivolvis*. Crayfish are well-known to elicit refuge-seeking behavior in freshwater snails. To avoid predation by crayfish, snails will move toward the water surface, which increases the biomass of benthic periphyton (Bernot and Turner, 2001; Turner et al., 1999; Turner et al., 2006). Indeed, we observed an increase in benthic periphyton biomass in the presence of crayfish indicating this anti-predatory migration behavior likely occurred in our experiment. However, we only observed this trend in the native community. When we added *V. georgianus* to the community, we observed reduced periphyton biomass

**Table 1**

Average abundance (number of individuals per tank), average aggregate biomass (g), and average individual biomass (g) of snail prey among the experimental treatments.

Snail species	Ambient nutrients				High nutrients			
	No predator		Predator		No predator		Predator	
	Native	Invaded	Native	Invaded	Native	Invaded	Native	Invaded
<i>Physella acuta</i>								
Abundance	295.00	252.50	329.00	385.75	727.50	849.50	701.75	479.75
Aggregate mass (g)	4.07	2.66	2.77	3.64	6.61	8.07	6.27	6.44
Mass (g)/individual	0.0138	0.0105	0.0084	0.0094	0.0091	0.0095	0.0089	0.0134
<i>Helisoma trivolvis</i>								
Abundance	54.75	36.75	19.25	23.25	62.00	31.75	30.50	22.75
Aggregate mass (g)	20.13	17.13	21.83	18.72	25.65	19.51	25.98	19.17
Mass (g)/individual	2.72	2.14	0.88	1.24	2.42	1.63	1.17	1.19
<i>Viviparus georgianus</i>								
Abundance	–	20.00	–	20.00	–	19.75	–	20.00
Aggregate mass (g)	–	34.20	–	30.80	–	32.75	–	32.28
Mass (g)/individual	–	1.71	–	1.54	–	1.66	–	1.61



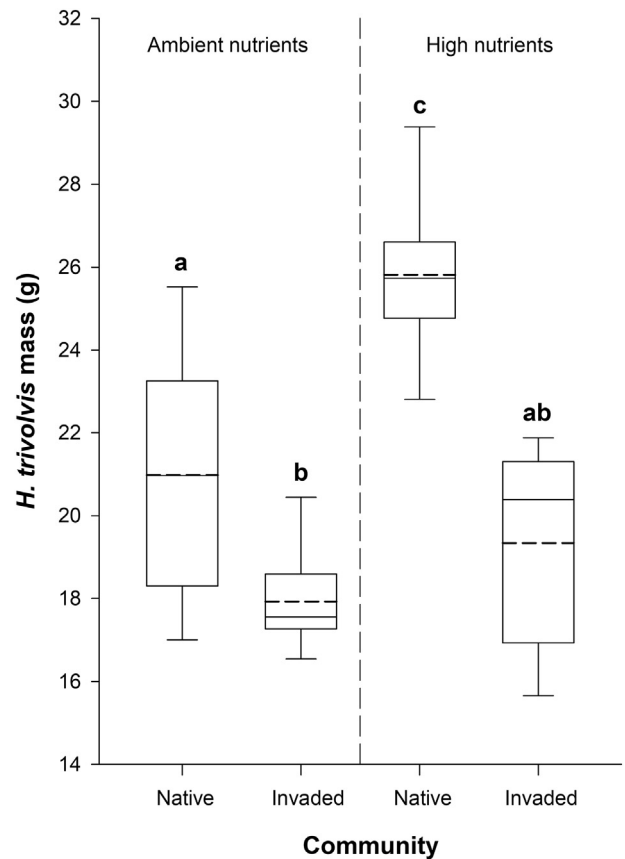
**Fig. 3.** The effects of nutrients on *Physella acuta* abundance (A) and biomass (B) at the end of the experiment. Letters above boxplots indicate statistical differences. Each box shows the mean (—), median (—), the 25th and 75th percentiles (box), and the 10th and 90th percentiles (error bars). Outliers from these distributions are shown as single, unfilled data points.

in the presence of the predator. This suggests that *V. georgianus* might not have sought refuge similar to the native snails and consumed periphyton in high risk areas (i.e. where the crayfish was present). If *V. georgianus* also foraged in refuge habitats, it might further reduce periphyton resources in refuge habitat. Therefore, the observed reduction in *H. trivolvis* biomass was perhaps due to the combination of restricted resource access to benthic resources following refuge-seeking behavior, as well as exploitative competition with *V. georgianus*.

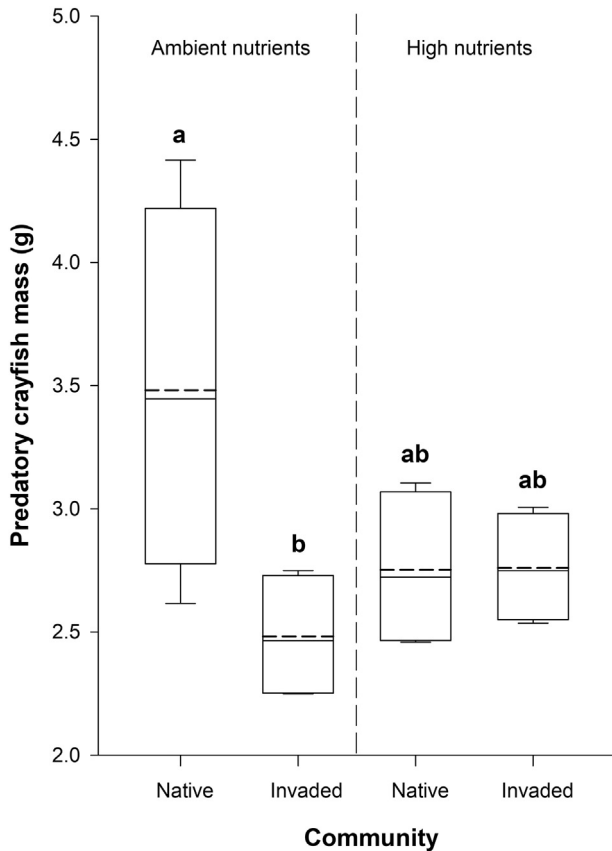
Although the crayfish is known to feed on the native snails used in our experiment (e.g., Brown, 1998; Sura and Mahon, 2011), we did not find any effects of the crayfish on the abundance or biomass of the native snails. It is possible that the crayfish did not have consumptive or non-consumptive effects on snails in our experiment. However, if this were the case, we would not have observed higher periphyton resources in the native community likely resulting from refuge-seeking behavior in the predator treatment (e.g., see Bernot and Turner, 2001; Turner et al., 2000; Turner et al., 2006). Another possibility for the lack of an effect on snail abundance or biomass could be due to compensatory reproduction or growth (e.g., Ali et al., 2003; Zipkin et al., 2008).

For instance, at low densities, *H. trivolvis* can exhibit compensatory reproduction that causes abundance to equalize over time (Johnson et al., 2012). If the consumption of native snails was countered by the compensatory effects of native snail reproduction due to a natural history of predatory-prey interactions, this would account for the lack of variability in abundance between the predator and no-predator treatments, although we currently lack data to fully explore this hypothesis.

While difficult to identify specific consumptive and non-consumptive effects in our study, our results suggest that *V. georgianus* was unaffected by the native crayfish predator. Other studies using larger crayfish and non-native snail prey show native crayfish (*Pacifastacus leniusculus*) readily consume the invasive Chinese mystery snail (*Bellamya chinensis*, Olden et al., 2009). This does not appear to be the case for *V. georgianus*, although it might be consumed by other crayfish not used in the current study. It is unlikely the size of *V. georgianus* mattered regarding its susceptibility to the native crayfish. Snail size does not necessarily predict vulnerability to a crayfish predators because of inducible defenses in shell morphology (e.g., thickness) can occur regardless of size (Hoverman et al., 2014). More plausible is that the operculum of *V. georgianus* combined with thick shells of larger, older individuals prevent access to soft tissues by crayfish predators (e.g., Hoverman and Relyea, 2009). Applied to natural systems, if other native predators do not adapt to consume dominant invaders—such as *V. georgianus* in Lake George—the lack of biotic resistance could disrupt freshwater food webs (Carlsson et al., 2009). We know from 3 years of field surveys that the proportion of *V. georgianus* consumed by the fishes of Lake George is higher compared with native snails (Hintz,



**Fig. 4.** The interactive effects between the presence of *V. georgianus* and nutrients on the biomass of *H. trivolvis* after the 68-day experiment. Each box shows the mean (—), median (—), the 25th and 75th percentiles (box), and the 10th and 90th percentiles (error bars). Outliers from these distributions are shown as unfilled data points. Letters above boxplots indicate statistical differences.



**Fig. 5.** The interactive effects between the presence of *V. georgianus* and nutrients on the biomass of crayfish predators after the 67-day experiment. Each box shows the mean (—), median (—), the 25th and 75th percentiles (box), and the 10th and 90th percentiles (error bars). Outliers from these distributions are shown as single, unfilled data points. Letters above boxplots indicate statistical differences.

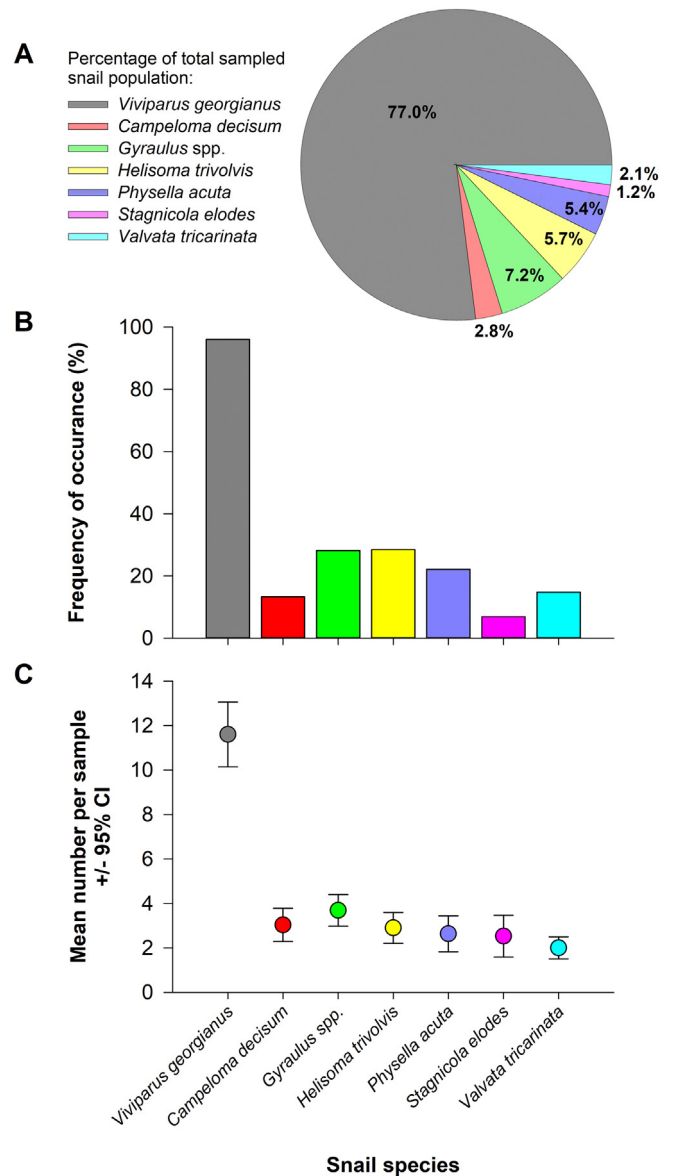
unpublished data), but the present study indicates *V. georgianus* is still the dominant snail species despite any fish consumption.

Unexpectedly, under low-nutrient conditions, crayfish biomass was lower in the invaded community compared with the native community. One possibility for this pattern is that competition occurred between *V. georgianus* and the crayfish predator, perhaps due to fewer periphytic resources we observed in low-nutrient conditions. The species of crayfish used in our experiment is well known to consume the native snail prey (Brown, 1998; Sura and Mahon, 2011), but this species is also opportunistic readily consuming periphyton (Corey, 1988). If access to higher quality native snail prey was reduced due to native prey seeking refuge, this may have encouraged the crayfish predator to consume lower quality periphytic resources. Further, *V. georgianus* is a facultative filter feeder of algae and detritus (Browne, 1978), which may have supplemented its diet when competing for limited periphytic resources with the crayfish even though we found no differences in phytoplankton among the experimental treatments. Overall, our results indicate that a native predator can compete with an invader that is functionally similar to the predator's prey and the predator itself—such an interaction was also dependent on nutrient concentration. We acknowledge we do not have diet or behavioral data to pin down the mechanism of competition between *V. georgianus* and the crayfish predator, but this could be the focus of further research.

We have shown that the negative multi-trophic impacts of *V. georgianus* varied with nutrient concentration. Although the directionality of species interactions in our experiment did not change, we found support for our prediction that nutrients would alter the magnitude of the impacts of *V. georgianus*. Exploitative competition with *V. georgianus* reduced *H. trivolvis* biomass, but this reduction was doubled

under high-nutrient concentrations. These interactions suggest that increasing nutrient concentrations can magnify competitive effects between native and non-native species in fresh waters. Further, the negative effects of *V. georgianus* on biomass of the crayfish predator were limited to low-nutrient concentrations. Overall, our results suggest that the trophic state of a freshwater ecosystem matters when identifying the post-establishment impacts of invasive species.

We are mindful our study has limitations. First, it is worth considering whether we would have observed similar outcomes in the experiment if we had added more of either of the native snail species to compensate for the additional biomass of *V. georgianus*. However, invasion does not follow a substitutive pattern. When a species is introduced, it enters regardless of the population size of the native community. Here, our goal was to manipulate a community to mimic an introduction. Snaydon (1991) illustrates that criticisms of additive designs for confounding overall density with competitor proportions



**Fig. 6.** Results from three years (2015–2017) of field surveys of the Lake George snail community: (A) percent composition of each species among all samples ( $N = 270$ ), (B) frequency of occurrence of each snail species (i.e., % of samples that detected each species), and (C) mean number of snails per sample  $\pm$  95% confidence intervals in when a snail species was detected.



are unconvincing “because overall density has no biological or statistical meaning.” Further, unlike replacement designs, additive designs allow for interpretable measures of competitive ability and severity (see Snaydon, 1991), which were the objectives of the competition investigation in our experiment. Ultimately, a hybrid design should be considered in future studies of similar nature (Byrnes and Stachowicz, 2009). Second, our field study did not cover the pre-introduction time period for *V. georgianus*. This is an unrealistic expectation in most species invasions because invaders are often discovered accidentally. The *V. georgianus* invasion began over 100 years ago making pre-invasion study impossible. Third, our field data were collected from a single oligotrophic lake. While the field study indicates that native competitors and predators have done little to suppress the invasion of *V. georgianus*—consistent with our experimental results—we are mindful that our conclusions are limited to a single lake and does not span lakes of varying trophic state. Such efforts might yield further insight into the invasion of *V. georgianus* throughout the Great Lakes region. Fourth, the effects of *V. georgianus* on the mass of the crayfish predator under ambient nutrient conditions could have resulted from a size bias at the onset of the experiment. We did not measure the initial size of the crayfish to minimize stress. Since we randomly assigned similarly sized crayfish to the mesocosms, the probability of introducing a size bias into any one treatment is low. We would also expect the experimental treatments to shape growth patterns and drown out any potential small size bias at the onset given the duration of the experiment and crayfish growth rates (e.g., Hill et al., 1993). Lastly, we did not study how *V. georgianus* was affected by crayfish predators and functionally similar competitors in its native range. From our experiment and field study, it appears that neither the native predator or functionally similar and ubiquitous snail competitors are influencing the abundance of *V. georgianus* in the invaded range. Understanding the interactions between *V. georgianus* and predators and competitors in its native range would provide additional insight into the role of enemy release as a potential driver of the *V. georgianus* invasion.

#### 4.1. Conclusion

As the globalization of earth's species pools continues, predicting the success and impacts of invasions is critical (Ricciardi and Rasmussen, 1998). Although understanding what drives successful invasions and post-establishment impacts is complex (Hintz et al., 2017; Strayer et al., 2006), it is essential for advancing theory and the creation of ecologically-sound management plans. Some have questioned the utility of the ERH in invasion ecology, others have suggested sub-hypotheses of the ERH are more useful for theory building. Our experimental and field investigations yield support for the ERH as a plausible explanation for an under-studied freshwater invasion leading to negative impacts on native species. Importantly, our experimental results also illustrate that theoreticians and ecological managers should consider the trophic state of an ecosystem in the study of freshwater invasions.

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#### Declaration of competing interest

The authors declare no conflict of interest.

#### Author contributions

All authors contributed to conceptualization of the study, data collection, and review of the manuscript.

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