

Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system

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Abstract With ecosystems increasingly supporting multiple invasive species, interactions among invaders could magnify or ameliorate the undesired consequences for native communities and ecosystems. We evaluated the individual and combined effects of rusty crayfish (*Orconectes rusticus*) and Chinese mystery snails [*Bellamya* (= *Cipangopaludina*) *chinensis*] on native snail communities (*Physa*, *Helisoma* and *Lymnaea* sp.) and ecosystem attributes (algal chlorophyll *a* and nutrient concentrations). Both invaders are widespread in the USA and commonly co-occur within northern temperate lakes, underscoring the importance of understanding their singular and joint effects. An outdoor mesocosm experiment revealed that while the two invaders had only weakly negative effects upon one another, both negatively affected the abundance and biomass of native snails, and their combined presence drove one native species to extinction and reduced a second by >95%. Owing to its larger size and thicker shell, adult *Bellamya* were protected from crayfish attack relative to native species (especially *Physa* and *Lymnaea*), suggesting the co-occurrence of these invaders in nature could have

elevated consequences for native communities. The per capita impacts of *Orconectes* (a snail predator) on native snails were substantially greater than those of *Bellamya* (a snail competitor). Crayfish predation also had a cascading effect by reducing native snail biomass, leading to increased periphyton growth. *Bellamya*, in contrast, reduced periphyton biomass, likely causing a reduction in growth by native lymnaeid snails. *Bellamya* also increased water column N:P ratio, possibly because of a low P excretion rate relative to native snail species. Together, these findings highlight the importance of understanding interactions among invasive species, which can have significant community- and ecosystem-level effects.

Keywords Biological invasion · Multiple invaders · *Bellamya* · *Orconectes* · Ecosystem change

Introduction

Biological invasions are widely recognized as a significant component of anthropogenic environmental change (Elton 1958; Vitousek et al. 1997; Byers et al. 2002). Worldwide increases in the number and geographic extent of invasions represent a major threat to native biodiversity in freshwater ecosystems, which collectively have higher rates of species endangerment and extinction than terrestrial or marine environments (Ricciardi and Rasmussen 1998; Dudgeon et al. 2006). In the US alone, more than 900 non-indigenous species have been introduced into inland waters and are collectively responsible for significant economic and ecological damage (Pimental et al. 2000; <http://nas.er.usgs.gov/>). Although research on biological invasions is often weighted toward terrestrial ecosystems (e.g., Puth and Post 2005), the importance of understanding and preventing

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non-indigenous species introductions in aquatic systems is underscored by the increasing number and rate of freshwater invasions, the high endemicity of freshwater ecosystems, and the importance of freshwater for human health and the economy.

Despite recent progress in invasion biology, a more complete understanding of invader impacts and the mechanisms through which they occur is essential to effective management and remediation of biological invasions. From well-studied cases, it is clear that invasions can lead to broad-scale changes in native populations, community structure, and ecosystem processes. High-profile examples in freshwaters include invasions by Nile perch in Lake Victoria, zebra mussels in North America (Johnson et al. 2006), spiny water fleas in Canada (Yan et al. 2002), American bullfrogs in the western USA (Hayes and Jennings 1986) and salt cedar (*Tamarix* spp.) in riparian areas of the southwest (Stromberg et al. 2007). For most invaders, however, our knowledge regarding specific ecological impacts remains limited (Pyšek et al. 2008), particularly at scales extending beyond the population level (see Parker et al. 1999). When community or ecosystem-level changes are associated with an invasion, the direct and indirect mechanisms responsible are often unknown or confounded by other forms of environmental change, precluding identification of the invader's role in observed shifts. This situation may severely undermine our ability to forecast how future increases or decreases (e.g., through eradication efforts) in invader abundances are likely to influence ecosystem conditions (Strayer et al. 2006).

As the number of biological invasions continues to increase, many ecosystems now support multiple non-indigenous species; for some environments and taxa, introduced species now outnumber native species (e.g., San Francisco Bay, California). Complex interactions among this “cocktail” of invaders can lead to a diversity of outcomes for native species and ecosystems, many of which are difficult to predict a priori (Grosholz et al. 2000; Crooks 2002; Bruno et al. 2005). Invaders may negatively affect one another through competition and/or predation, reducing or minimizing their joint impacts on native biota (Ross et al. 2004). In other cases, invaders may have no effect on each other (e.g., Cope and Winterbourn 2004) or exhibit facilitative interactions, increasing their ecological impacts and promoting establishment and spread (e.g., Ricciardi 2001; O'Dowd et al. 2003; Grosholz 2005). Because of the potentially complex interactions among invaders, experimental research to identify how invaders interact and their consequences for native communities and ecosystems is particularly valuable (e.g., Kiesecker and Blaustein 1998). Such efforts may be especially insightful in freshwater environments (see Lodge et al. 1994), where experimental approaches to understanding the multi-scale

effects of invasive species have historically been under-represented (Parker et al. 1999; Adams et al. 2003).

Here we experimentally investigated the individual and combined effects of two widespread freshwater invaders in the US, the omnivorous rusty crayfish (*Orconectes rusticus*) and the herbivorous Chinese mystery snail (*Bellamya chinensis*), on community and ecosystem properties of a benthic food web. We used mechanistic experiments to address the following questions:

1. What are the individual and combined effects of Chinese mystery snails and rusty crayfish on native snail growth, survival and reproduction?
2. How do these invasive species affect one another?
3. What are the direct and indirect consequences of these invaders for algal growth and nutrient availability?

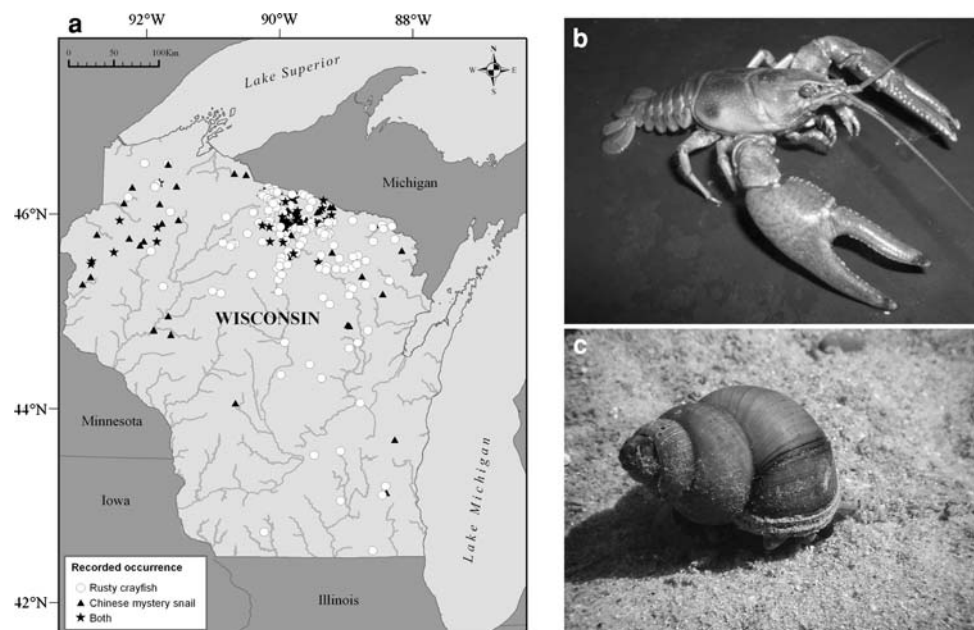
We focused on the response of native snails to invasion because of their importance in benthic food webs and because of their potential sensitivity to invasive crayfish (predators) and snails (competitors) (e.g., Dillon 2000; Carlsson et al. 2004). The rusty crayfish and the Chinese mystery snail are both widespread among inland lakes in the Laurentian Great Lakes region. While the impacts of rusty crayfish have been the subject of extensive research in northern temperate lakes (Lodge et al. 1994; Hein et al. 2006; McCarthy et al. 2006), no studies have addressed ecological effects of *Bellamya* or the interactions between these invaders. By examining the joint effects of an invasive predator and an invasive herbivore on the community and ecosystem properties of freshwater environments, this study aims to advance our understanding of multi-invader interactions. Given the larger size and thicker shell of *Bellamya* relative to native freshwater snails, we expected that it would be resistant to predatory crayfish, creating potential for multi-invader facilitation.

Materials and methods

Study system

The rusty crayfish (*Orconectes rusticus*, hereafter “*Orconectes*”; Fig. 1) has expanded its distribution over the last 50 years from its historical range in the Ohio River drainage to waters throughout at least 18 US states, Ontario, Quebec, Manitoba and the Laurentian Great Lakes (Olden et al. 2006). Vectors of rusty crayfish introduction include bait bucket discharge by recreational anglers, intentional releases by lake-users for nuisance weed control and commercial crayfish retailers, and natural inter-lake dispersal. In Wisconsin, *Orconectes* captures have increased from 7% of all crayfish records collected during the first 20 years of their invasion to 36% of all records

Fig. 1 **a** Distribution map of Wisconsin lakes found to support the Chinese mystery snail, *Bellamya chinensis* (*Bellamya*; dark triangles), rusty crayfish, *Orconectes rusticus* (*Orconectes*; white circles) or both invaders (dark stars) ($n = 242$ lakes sampled for both species). Invasions by **b** *Orconectes* and **c** *Bellamya* are widespread throughout Wisconsin



during the most recent 20 years (Olden et al. 2006). *Orconectes* can have substantial impacts on invaded freshwater ecosystems by feeding on organisms from multiple trophic levels, including benthic algae, macrophytes, invertebrates, snails, native congeners and fish (e.g., Lodge et al. 1994; McCarthy et al. 2006).

The Chinese mystery snail [*Bellamya* (= *Cipangopaludina*) *chinensis*, hereafter “*Bellamya*”] is a very large (up to 64 mm) viviparid snail native to Asia that was first observed in North America in the 1890s (Fig. 1; Clench and Fuller 1965; Jokinen 1982). The taxonomy of this group is incompletely resolved, and we follow Smith (2000) in using the genus name *Bellamya* (rather than the traditional *Cipangopaludina*). *Bellamya* was almost certainly introduced to the US multiple times through the aquarium trade or for culinary purposes (Mackie 1999), and is now widely distributed in lentic and slow-moving lotic water bodies across North America, including 27 US states and all of the Great Lakes (Clench and Fuller 1965; Dundee 1974; Jokinen 1982; Strayer 1987; Bury et al. 2007; <http://nas.er.usgs.gov/>). In Wisconsin, *Bellamya* was first recorded in the 1950s (Teskey 1954). These snails prefer muddy or sandy substrates, where they feed predominantly on epiphytic benthic algae, especially diatoms (Jokinen 1982). While little is known about its ecological significance or impacts on native fauna and flora, *Bellamya* can achieve very high densities in invaded lakes ($>40 \text{ m}^{-2}$, P. T. J. J., unpublished data; Branson 1977), and were reportedly removed from Lake Erie by the ton during the 1960s (Mills et al. 1993). Considering their often substantial biomass and the general importance of snails as benthic herbivores, we hypothesized that *Bellamya* would

have ecologically significant effects on invaded communities and ecosystems (Carlsson et al. 2004; Bury et al. 2007).

Available field data indicate that these two invaders frequently co-occur. A compilation of unpublished surveys conducted by the Wisconsin Department of Natural Resources (WDNR), the Great Lakes Indian Fish and Wildlife Commission, and the University of Wisconsin—Madison includes 550 and 266 water bodies sampled for *Orconectes* and *Bellamya*, respectively. Records were included only when detection and non-detection data were available and when invader occurrences were recorded by trained biologists or through vouchered specimens (see Johnson et al. 2008 for a complete description of the database). Among the 242 lakes sampled for both invaders, *Bellamya* occurred in 33.5%, *Orconectes* in 36.4%, and the invaders co-occurred in 18.2% of lakes (Fig. 1). While lakes were not sampled randomly (see Johnson et al. 2008), these data nevertheless suggest that both invaders are widespread in Wisconsin and that they frequently co-occur. The larger size and thicker shell of *Bellamya* relative to native snails indicate that it is likely more resistant to crayfish predation, suggesting the co-occurrence of these invaders may have important impacts on native communities.

Experimental manipulation

Design and establishment

We conducted a 2×2 experiment manipulating the presence of *Bellamya* and *Orconectes* to evaluate their

individual and combined effects on native snail communities, algal growth and water column nutrient concentrations (N and P). The experiment was conducted over an 8-week period in 24 outdoor mesocosms (1,200 l) randomly assigned to one of the following treatments: (1) native snails only, (2) native snails with *Bellamya*, (3) native snails with *Orconectes*, and (4) native snails with both invaders (six replicates per condition). Mesocosms were made of structural foam plastic and measured 1.6 × 1.7 m with a fill height of 0.5 m. While not equivalent to ecosystem-level manipulations, mesocosm studies can provide important insights about mechanistic interactions in aquatic food webs, including snail–predator interactions (e.g., Nyström et al. 1999; Turner and Chislock 2007). Prior to initiating the experiment, we seeded each mesocosm with ~1,000 l lake water, 18 kg commercial “play sand” and 27 kg coarse (1.9 cm diameter) gravel as substrate, two cinderblocks as refugia, and 28 g CaCO₃ to ensure sufficient Ca for shell growth. To provide inocula of algae and zooplankton, we added 150 ml lake mud and 100 ml concentrated zooplankton (dominated by *Daphnia pulicaria*) to each mesocosm. We supplemented nutrient levels by adding 1.40 g NH₄NO₃ and 0.097 ml of 85% H₃PO₄ (initial concentrations of ~450 µg l⁻¹ N and ~50 µg l⁻¹ P). Otherwise water chemistry mirrored Sparkling Lake, Vilas County, Wisconsin during June 2006 (see <http://www.limnology.wisc.edu/>). Mesocosms were covered with nylon mesh lids (1 mm mesh size) designed to permit sunlight while minimizing colonization by unintended fauna and flora.

Snail and crayfish additions

One week after establishing mesocosms, we added 45 individuals of each of the following native snail species: *Physa gyrina* (hereafter “*Physa*”, mean shell length ± 1 SE = 7.3 ± 1.5 mm), *Helisoma trivolvis* (hereafter “*Helisoma*”, 12.8 ± 1.0 mm) and *Lymnaea stagnalis* (hereafter “*Lymnaea*”, 25.3 ± 3.7 mm). These species occur commonly in lakes within the Northern Highlands Region of Wisconsin (Lewis and Magnuson 2000) and represent three pulmonate families with varying life history strategies: Physidae, Planorbidae and Lymnaeidae. For mesocosms assigned to invader treatments, we added 45 *Bellamya* (27.2 ± 3.5 mm) and/or two *Orconectes* (32.1 ± 0.4 mm carapace length). These densities—approximately 10 snails m⁻² and 0.2 crayfish m⁻²—are within the range normally observed in northern Wisconsin (Lewis and Magnuson 2000; Hein et al. 2006). Independent subsets of snails ($n = 30$ per species) and crayfish ($n = 10$) were individually measured, dried (60°C for 1 week), and weighed (shell and tissue). Because trematode parasite infections can be common in gastropods, with

significant effects on snail behavior and reproduction (e.g., Bernot 2003), we also dissected a subset ($n > 50$) of snails in each species to ensure the rarity of potentially confounding infections.

Data collection

During weeks 2 and 7 of the experiment, we measured water nutrient concentrations (N and P) and periphyton chlorophyll *a* levels on the mesocosm walls and on the sediment. In each mesocosm, water collected from three locations (using a 1.3-l tube sampler) was pooled, filtered (53 µm), and analyzed for total N and P using a persulfate digestion (see <http://lter.limnology.wisc.edu/protocols.html>). Periphyton was removed with a razor blade from 5-cm strips of flagging tape attached to the mesocosm walls (see Johnson et al. 2007). Sediment periphyton was collected and pooled from four locations within each mesocosm using a modified 60-ml syringe to quickly draw up the upper ~2 cm of sediment. Chl-*a* for mesocosm wall periphyton and sediment algae was analyzed after methanol extraction (see <http://lter.limnology.wisc.edu/protocols.html>). After 8 weeks, we drained the water from each mesocosm and enumerated snails and crayfish. Snails were weighed (aggregated wet mass) and individually measured. Crayfish were measured, dried (60°C for 1 week), and weighed.

Snail excretion

We measured rates of N and P excretion for each snail species in each treatment condition (except when insufficient numbers of snails remained alive due to crayfish predation). We placed a fixed number of snails into darkened, acid-washed containers with a known volume of water (*Bellamya*, four snails in 1.0 l; *Helisoma* and *Lymnaea*, ten snails in 0.2 l; and *Physa*, 40 snails in 0.1 l). After 1 h, we removed and weighed snails, collected excreted particles on pre-ashed and weighed glass fiber filters (1.0 µm pore size), and kept 100 ml filtrate. Filters were dried (60°C) to constant weight and, along with the filtrate, analyzed for total N and P using the persulfate digestion method.

Statistical analysis

We analyzed the effects of *Bellamya*, *Orconectes* and their interactions on the final abundance and wet mass of each native snail species using multivariate ANOVA (MANOVA) (log₁₀-transformed + 1 values; Quinn and Keough 2002). When evaluating the combined effects of two or more species, it is important to consider whether their effects should be strictly independent (i.e., the joint effects of both species on some response variable is simply the

sum of their individual effects when isolated) or may instead be non-independent (greater or less than the sum of their individual effects) (e.g., Wootton 1994; Sih et al. 1998). We evaluated whether effects of the two invasive species were independent using a two-way factorial ANOVA after \log_{10} -transforming snail densities (see Wootton 1994; Vonesh and Osenberg 2003; Fournier et al. 2006). We interpret a significant interaction term as indicating that the effect of the two invaders is non-independent. Although the addition of a constant (e.g., +1) to deal with zero values prior to log-transformation can alter this underlying biological relationship, this change is proportionately small (for our data) and unlikely to affect the results.

The effects of each invader on periphyton chlorophyll *a* levels (sediment and mesocosm walls), the N:P ratio in the water column, and on one another (size and/or abundance of the co-occurring invader) were evaluated using repeated-measures (RM) MANOVA. This analysis explicitly compared the value of each response variable between weeks 2 and 7 and among treatments. Finally, because the two invaders were stocked at different initial densities and biomasses, comparison of their effects on native species required calculation of an adjusted impact value (see Parker et al. 1999), in which the effects of each invader were converted to a per capita estimate as follows:

$$I_{ij} = \frac{(\Delta N_{ij} - \Delta N_i)}{\bar{B}_j}$$

where I_{ij} is the impact of invasive species j on the abundance of native species i , ΔN_{ij} is the average change in native species i abundance in treatments with invader j , ΔN_i is the average change in native species i abundance or biomass in the absence of either invader, and \bar{B}_j is the average abundance of invader j during the experiment. In this manner, we examined the impact of each invader relative to the change in native snail abundance in the absence of the invaders.

Results

Results of the mesocosm experiment indicated that both invaders negatively affected native snail abundance (Fig. 2a; MANOVA, *Bellamya*, Wilks' $\lambda = 0.56$, $F_{3,18} = 4.80$, $P = 0.013$; *Orconectes*, Wilks' $\lambda = 0.09$, $F_{3,18} = 64.72$, $P < 0.0001$). *Bellamya* negatively influenced the abundance of *Lymnaea* (ANOVA, $F_{1,20} = 14.32$, $P = 0.001$), whereas *Orconectes* strongly reduced the abundance of both *Lymnaea* and *Physa* (ANOVA, *Lymnaea*, $F_{1,20} = 173.4$, $P < 0.0001$; *Physa*, $F_{1,20} = 35.56$, $P < 0.0001$). *Physa* abundance increased by 110% in the absence of invaders, increased by 17% with *Bellamya* only,

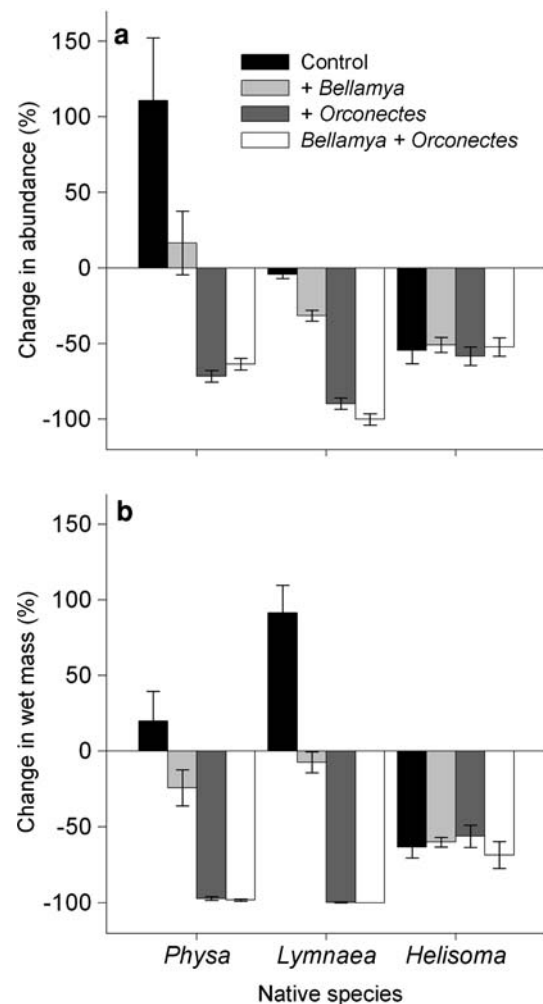


Fig. 2 Percentage change in **a** abundance and **b** wet mass of three native snail species, *Physa gyrina* (*Physa*), *Lymnaea stagnalis* (*Lymnaea*), and *Helisoma trivolvis* (*Helisoma*), during the 8-week mesocosm study with four invader treatments: no invaders (control), *Bellamya* only, *Orconectes* only and both invasive predators combined

and declined by >60% when *Orconectes* was present (alone or with *Bellamya*) (Fig. 2a). We also found a significant *Orconectes*-by-*Bellamya* interaction on *Lymnaea* abundance (ANOVA, $F_{1,20} = 5.28$, $P = 0.03$). *Lymnaea* abundance remained steady in the native treatment, but declined by 32% with *Bellamya*, and by 90% with *Orconectes*; *Lymnaea* went extinct in the combined invader treatment (Fig. 2a). Based on a model of independent enemy effects (e.g., Wootton 1994; Sih et al. 1998; Vonesh and Osenberg 2003), the expected decrease in the presence of both invaders was approximately 67% (i.e., less than the observed 100% reduction), suggesting that the presence of both invaders led to risk enhancement. Neither invader significantly affected the abundance of *Helisoma*, which declined sharply regardless of treatment condition. While both invaders affected the three native species similarly,

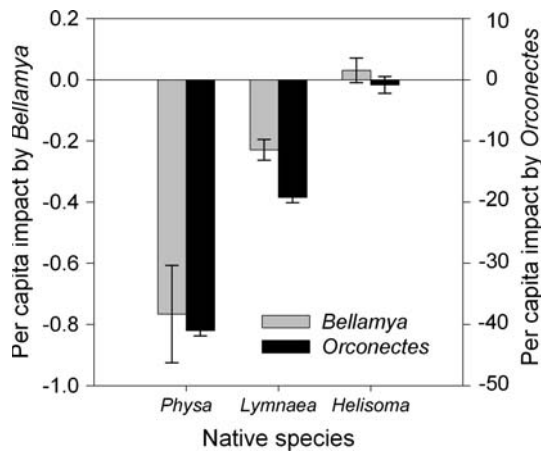


Fig. 3 Per capita impacts of the invaders *Bellamya* (gray bars, left axis) and *Orconectes* (black bars, right axis) on the abundance of each native snail species in the experiment. See text for details on how impact was calculated

the per capita effects of predatory crayfish ($n = 2$ per mesocosm) were consistently greater than those of *Bellamya* ($n = 45$ per mesocosm) (Fig. 3).

Bellamya and *Orconectes* had similarly negative effects on the final wet mass of native snail species (Fig. 2b; MANOVA, *Bellamya*, Wilks' $\lambda = 0.17$, $F_{3,18} = 28.69$, $P < 0.0001$; *Orconectes*, Wilks' $\lambda = 0.003$, $F_{3,18} = 1,845.02$, $P < 0.0001$). In the absence of either invader, *Lymnaea* mass increased by 91%; however, it decreased by 7% with *Bellamya* and by >85% with *Orconectes* (alone or with *Bellamya*) (*Bellamya* on *Lymnaea*, $F_{1,20} = 56.33$, $P < 0.0001$; *Orconectes* on *Lymnaea*, $F_{1,20} = 3867.2$, $P < 0.0001$). Similarly, *Physa* mass increased by 20% in the native snail treatment, decreased by 24% with *Bellamya*, and decreased by >95% with *Orconectes* (alone or with *Bellamya*) (*Bellamya* on *Physa*, $F_{1,20} = 6.66$, $P = 0.018$; *Orconectes* on *Physa*, $F_{1,20} = 395.28$, $P < 0.0001$). The observed declines in *Lymnaea* mass associated with *Bellamya* were due to changes in both survival and growth, as the log₁₀-transformed mean sizes of *Lymnaea* (but not of *Physa* or *Helisoma*) from *Bellamya* treatments were smaller than in the native treatments (MANOVA, Wilks' $\lambda = 0.102$, $F_{3,8} = 23.56$, $P < 0.0001$; ANOVA on *Lymnaea*, $F_{1,10} = 35.07$, $P < 0.0001$). Too few native snails remained in the *Orconectes* treatments to evaluate the effect of crayfish on snail growth. Neither invader significantly influenced changes in the wet mass of *Helisoma*, which declined in all treatments.

We found a significant interactive effect of *Bellamya* and *Orconectes* presence on *Lymnaea* biomass (*Bellamya* \times *Orconectes*, $F_{1,20} = 34.29$, $P < 0.0001$), such that the effect of each invader was strongest in the absence of the other. However, this effect likely resulted from the fact that crayfish presence alone strongly (>95%) reduced

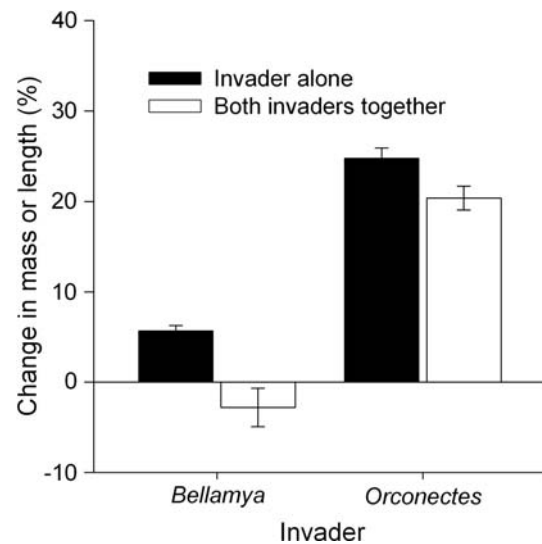


Fig. 4 Effect of the two invaders, *Bellamya* and *Orconectes*, upon one another. *Bellamya* had no effect on crayfish growth, whereas *Orconectes* reduced *Bellamya* abundance but not mass. Depicted are the percentage changes in snail wet mass (aggregated among all individuals) and crayfish lengths (averaged between individuals within the same mesocosm) over the 8-week experiment as a function of whether an invader occurred alone (black bars) or alongside the second invader (white bars)

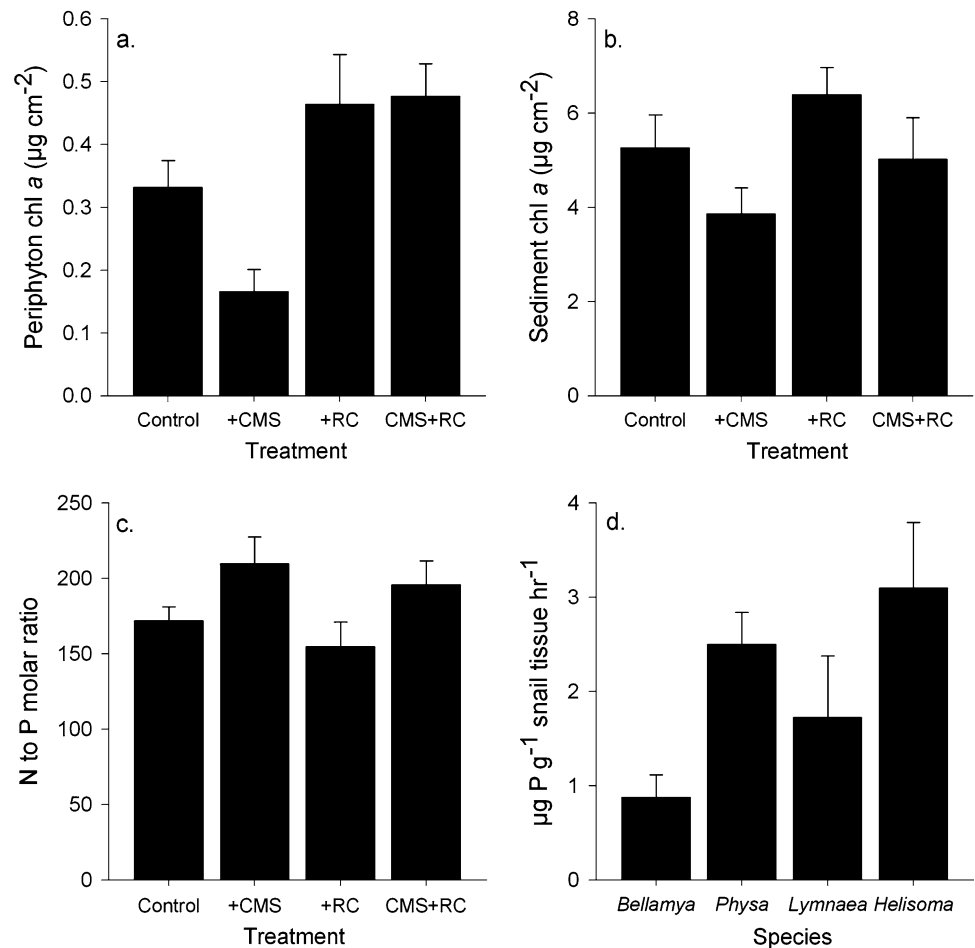
Lymnaea biomass, and the added effects of *Bellamya* were therefore minimal, even though *Lymnaea* went extinct when both invaders were present. The combination of both invaders could not drive biomass below zero.

Bellamya and *Orconectes* had negative or neutral effects upon each other. *Orconectes* caused a decline in *Bellamya* abundance but not in wet mass (Fig. 4; MANOVA, *Orconectes* on *Bellamya*, Wilks' $\lambda = 0.110$, $F_{2,9} = 36.36$, $P < 0.0001$; wet mass, $F_{1,10} = 0.97$, $P = 0.349$; abundance, $F_{1,10} = 69.29$, $P < 0.0001$). Overall, this result owed to the reduced abundance of small, juvenile *Bellamya* in the presence of *Orconectes*, likely as a result of either predation or lower reproductive output. For *Orconectes*, all crayfish survived for the duration of the experiment, and the presence of *Bellamya* had no significant effects on either the final size or mass of crayfish (averaged between individuals within the same mesocosm) (Fig. 4; ANOVA, $P > 0.05$).

Effects of invaders on chlorophyll a and nutrient concentrations

Both invaders significantly influenced algal growth within the mesocosms but in opposing directions (RM-MANOVA, *Bellamya*, Wilks' $\lambda = 0.325$, $F_{3,18} = 12.49$, $P < 0.0001$; *Orconectes*, Wilks' $\lambda = 0.269$, $F_{3,18} = 16.32$, $P < 0.0001$). *Bellamya* caused a decrease in sediment periphyton and a marginally significant decrease in wall periphyton

Fig. 5 Effects of invader treatment, control, Chinese mystery snails (+CMS), rusty crayfish (+RC), and of both invaders (CMS + RC), on **a** periphyton chlorophyll *a*, **b** sediment chlorophyll *a*, and **c** N:P molar ratio, and of snail species on **d** release of P by snails in experimental chambers, expressed as mass of P (in dissolved or particulate form) per unit mass of snail released per unit time



(ANOVA, sediment, $F_{1,20} = 9.531$, $P = 0.006$; wall periphyton, $F_{1,20} = 3.237$, $P = 0.087$), whereas *Orconectes* presence substantially increased periphyton chlorophyll *a* (Fig. 5a, b; ANOVA, $F_{1,20} = 19.47$, $P < 0.0001$). We also observed a time-by-*Bellamya*-by-*Orconectes* interaction on periphyton chlorophyll *a* (ANOVA, $F_{1,20} = 5.07$, $P = 0.036$), such that *Bellamya* reduced wall periphyton only in the absence of *Orconectes* (Fig. 5a). *Bellamya* also caused significant increases in the molar ratio of N to P in the water column, whereas *Orconectes* had no detectable effects on nutrient concentrations (Fig. 5c; MANOVA, time \times *Bellamya*, Wilks' $\lambda = 0.750$, $F_{3,18} = 6.672$, $P = 0.018$). Results of the snail excretion trials suggest that this effect may be due to a low rate of P excretion by *Bellamya* relative to native snails (Fig. 5d). Thus, the high biomass of *Bellamya* in combination with their low P excretion rates may reduce the amount of biologically available P and increase the N:P ratio.

Discussion

Many ecosystems now support multiple invasive species, and some have been invaded by hundreds of non-native

plants, animals, and micro-organisms (e.g., Ruiz et al. 2000; Ricciardi 2001; O'Dowd et al. 2003; Grosholz 2005). Past studies of species introductions largely emphasized invader impacts on particular taxa, focusing on conspicuous species or species that cause dramatic ecological impacts (Parker et al. 1999; Grosholz et al. 2000; Pyšek et al. 2008). As a result, we know comparatively little about how the effects of individual invaders may be attenuated or amplified as a result of interactions with other invaders, making the net consequences of biological invasions difficult to predict (Bruno et al. 2005). Field-based efforts need to be supplemented with experiments that examine the individual and combined effects of multiple invaders at several ecological scales, ranging from individuals to ecosystems (Ricciardi 2003; Strayer et al. 2006).

Results of our experiment demonstrate that, within experimental mesocosms, realistic densities of *Bellamya* and *Orconectes* each have significant effects on native snail communities and ecosystem properties. Relative to control treatments, rusty crayfish reduced the biomass of native *Lymnaea* and *Physa* snails by >90%, consistent with previous field studies illustrating the predatory effects of invasive crayfish (Lodge et al. 1994; McCarthy et al. 2006).

Competition by herbivorous Chinese mystery snails also caused substantial declines in the growth and abundance of native snails (Fig. 2). Despite this species' long invasion history, broad distribution within North America, and often high biomass within invaded environments, we are not aware of any previous study that has examined its ecological impacts.

We found evidence that the dual effects of both invaders on native snails were as severe as or worse than were their individual effects. Importantly, *Bellamya* and *Orconectes* had only weakly negative effects upon each other. With its large size and thick shell, *Bellamya* was substantially less vulnerable to crayfish predation than were *Physa* and *Lymnaea* (see also Osenberg and Mittelbach 1989; Brown 1998). As a result, the combination of both invaders had sharply negative effects on these native snails. The presence of both invaders led to risk enhancement for *Lymnaea*, such that *Lymnaea* was completely extirpated from all replicates in which *Orconectes* and *Bellamya* co-occurred but persisted (albeit at lower densities) when faced with either invader in isolation. Similarly, the biomass of *Physa* declined by >98% when confronted with both invaders. In contrast, *Bellamya* abundance declined by less than 15% in the presence of *Orconectes*, and biomass showed little change (<3%). With respect to *Lymnaea* and *Physa* biomasses, however, the combined presence of *Bellamya* and *Orconectes* led to risk reduction. This can likely be attributed to two factors. First, *Bellamya* negatively affected native snails indirectly through competition for resources; the presence of predatory crayfish, however, sharply reduced native snail density, thereby lessening the strength of competition between native and invasive snails and thus the effect of *Bellamya*. Second, because *Orconectes* often reduced native snail biomass by >90%, it was impossible for *Bellamya* to additionally drive this biomass below zero. We emphasize that a more complete understanding of the biological interactions between *Bellamya* and *Orconectes* could be achieved using a larger experimental scales (e.g., in situ experiments).

Overall, these results may help to explain why *Bellamya* and *Orconectes* frequently co-occur among Wisconsin lakes. This pattern likely reflects that both species are invading similar types of environments and/or the conditions created by one species may be conducive to establishment of the second invader. Lakes in our study region are subjected to increased human activity and disturbance, which likely lead to greater propagule pressure and enhanced probability of establishment success for both species (Havel et al. 2005; Johnson et al. 2008). However, because rusty crayfish reduce native snail abundance (Lodge et al. 1994; McCarthy et al. 2006; this study) with only limited effects on *Bellamya*, crayfish invasions could also indirectly facilitate the subsequent establishment and

spread of *Bellamya* by weakening competition for algal resources. In a similar example, the non-native amethyst gem clam (*Gemma gemma*) expanded its range in San Francisco Bay only after the invasion of European green crab (*Carcinus maenas*), which preferentially consumes native clams (Grosholz 2005). However, because rusty crayfish also had weakly negative effects on *Bellamya*, particularly with respect to production of new snails, the net benefit conferred by such predator-mediated competition is not resolved.

Although predatory rusty crayfish had substantially greater per capita effects on native species than did *Bellamya* (a competitor), both invaders affected the three native snail species in a similar fashion, with the strongest effects on *Physa*, followed by *Lymnaea*, then *Helisoma*. Neither invader, however, significantly affected the density or biomass of *Helisoma*, which declined sharply in all treatments (including the control). These patterns may result from ecological trade-offs between predation resistance and resource competition. *Physa* and *Lymnaea* have thin shells that offer little protection against crayfish (Brown 1998; Nyström and Perez 1998; Nyström et al. 1999; Rundle and Brönmark 2001), but are more adapted to rapidly convert available resources into growth and reproduction. Both species increased significantly in either abundance (*Physa*) or growth (*Lymnaea*) in the control treatment, but declined in the presence of either invader. In contrast, the thick shell of *Helisoma* affords greater protection from crayfish and fish predators (Stein et al. 1984; Osenberg and Mittelbach 1989; Brown 1998; Rundle and Brönmark 2001); competition with the other native snails for algal resources may have prevented this species from increasing significantly, even in the absence of either invader. However, this does not explain why *Helisoma* failed to increase in abundance or biomass in the presence of crayfish, which reduced the abundance of other native snails.

Our experimental results underscore the importance of individual and interactive effects of invasive species on community and ecosystem properties. *Orconectes* substantially increased the amount of periphyton, most likely as an indirect result of a trophic cascade: crayfish predation reduced the abundance of grazing native snails, thereby increasing the amount of periphyton in the system. Similar results from crayfish predation have been reported by Nyström et al. (1999, 2001). Grazing by *Bellamya*, in contrast, negatively affected periphyton levels on the mesocosm walls and especially the sediment, where *Bellamya* were most commonly observed during the experiment. *Bellamya* also increased the ratio of total N to P in the water column, which can have important effects on algal community structure in natural systems. We suggest this intriguing finding may be explained, in part, by results of the snail

excretion trials. Per unit dry mass, *Bellamya* excreted less P in dissolved or particulate form than any of the three native snail species, suggesting that the tissues of this invader might represent a “sink” for P, reducing its availability in the environment and thereby increasing the N:P ratio. Similarly, Evans-White and Lamberti (2006) reported that consumer identity could influence nutrient recycling, likely via differences in excretion patterns. Given the importance of N:P in ecological stoichiometry and in controlling algal growth, additional experimental and field efforts are needed to clarify and substantiate this finding and evaluate its significance. Carlsson et al. (2004) found that introduced apple snails in Southeast Asia can drive wetlands from a macrophyte-dominated, clear water state to a turbid, phytoplankton-dominated state with concomitant shifts in the biological community, highlighting the potential significance of invasive snails on ecosystem condition.

Our findings highlight the potential importance of interactions between *Bellamya* and *Orconectes* in affecting native snail communities. As found in several previous studies, predation by invasive crayfish sharply reduced native snail biomass (Lodge et al. 1994; Nyström et al. 1999, 2001); by virtue of its large size and thick shell, however, *Bellamya* was relatively unaffected by *Orconectes*, but did have significant competitive consequences for two of the three native snail groups. How results from these experimental systems scale up to entire lake ecosystems is an important but as of yet unanswered question. Given that these experimental communities included only a subset of the possible native benthic species and single initial density of each invader, we fully expect that the patterns observed here will vary both within and among larger lake ecosystems. Broad-scale field surveys, preferably in combination with in situ experiments, represent an important next step in definitively assessing the effects of each invader and their interactions in nature.

Quantifying the impact of biological invasions is a complex and challenging endeavor (Strayer et al. 2006). Our findings highlight the importance of quantifying the direct and indirect interactions among invasive species. The need for ecologists to address such questions is underscored by the now commonplace occurrence of multiple invaders within many ecosystems. Identifying the multi-trophic impacts of biological invasions is a particularly pressing issue in freshwater ecosystems, which have endured widespread invasions by non-indigenous species and exhibit high levels of species imperilment and extinction risk.

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