

NOTES

Investigating snails as potential biological control agents for invasive European frogbit (*Hydrocharis morsus-ranae*)

BIN ZHU*

INTRODUCTION

Many lakes, rivers, and ponds in the United States have been occupied by nonindigenous and invasive species that have altered ecosystem functions (Mills et al. 1994, Ricciardi 2001, Zhu et al. 2006, 2007). A floating aquatic plant—European frogbit (also called common frogbit, *Hydrocharis morsus-ranae* L.)—is one of the invasive species in North America that may profoundly affect the invaded ecosystems. European frogbit can form dense floating mats, has detrimental effects on native aquatic vegetation by blocking light (e.g., Catling et al. 1988), and affects animals by reducing plants and dissolved oxygen (e.g., Zhu et al. 2008). It can also block navigation channels, irrigation ditches, and water intake pipes, and it can reduce the aesthetic and recreational value of water bodies, thus decreasing tourism and real estate values (Catling et al. 2003). European frogbit has spread rapidly in the Great Lakes Basin and was recently found at several locations in New York State, including Sterling Creek in Cayuga County and a pond at the Audubon Center and Sanctuary in southern Chautauqua County (O'Neill 2007). It is, therefore, important to develop effective strategies to manage this invasive species.

Several different control strategies had been tried on European frogbit with varying levels of success. Mechanical harvesting and herbicides are two common methods reported to control European frogbit (Holz 1963, Renard 1963, Langdon 2007). For example, hand-pulling was used to eradicate this plant and showed some success from numerous environments (e.g., Langdon 2007). Chemicals, such as endothall [7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid] and diquat [6,7-dihydrodipyrido[1,2- α :2',1'- c]pyrazinediium ion] have been effective in controlling European frogbit in ditches (Holz 1963, Renard 1963). However, investigations on biological control of European frogbit are limited, despite abundant studies of biological control of other invasive aquatic plants, such as Eurasian watermilfoil (*Myriophyllum spicatum* L.), hydrilla [*Hydrilla verticillata* (L. f.) Royle], and water-chestnut (*Trapa natans* L.) (Sheldon and

Creed 1995, Ding et al. 2006, Cuda et al. 2008). For example, Sheldon and Creed (1995) studied aquatic weevil (the milfoil weevil) (*Euhrychiopsis lecontei* Dietz) as a biological control agent for Eurasian watermilfoil, and Ding et al. (2006) investigated the potential of the leaf beetle (the junsai mushi) (*Galerucella birmanica* Jac.) as a biological control agent for water-chestnut in the laboratory and in the field.

European frogbit is a food resource for many animals, including insects, rodents (e.g., beaver and mice), water birds, freshwater snails, and fish (Froemming 1954, Magomaev 1973, Sviridenko et al. 1988, Catling et al. 2003, Vaananen and Nummi 2003), suggesting the possible existence of biological control agents for this plant. Froemming (1954) observed that consumption of European frogbit stimulated egg production of the freshwater snails (the great pond snail) (*Lymnaea stagnalis* L.). Dabbling duck species (*Anas* L. spp.) have been documented to consume European frogbit in the eutrophic wetlands of central Finland (e.g., Vaananen and Nummi 2003). Magomaev (1973) also reported a 2-yr-old grass carp (*Ctenopharyngodon idella* Val.) can consume European frogbit at a rate of 740 g kg⁻¹ body wt d⁻¹ (translation: grams per kg of body weight per day). To date, there are no identified biological agents for this invasive plant. The objective of this study was to investigate the potential of snails as biological agents for European frogbit.

MATERIALS AND METHODS

A field survey and a laboratory experiment were conducted to evaluate the potential of snails to be biological control agents. In summer 2008, European frogbit was surveyed and collected using the standard rake-sampling technique (see details in Zhu and Georgian 2014) in 17 sites in the Great Lake Basin, including Oneida Lake ($n = 1$), Oswego River ($n = 1$), the southeastern shore of Lake Ontario ($n = 8$), St. Lawrence River ($n = 5$), and Lake Champlain ($n = 2$). Snails were collected from the samples and identified to family or species, following the identification characteristics provided in Thorp and Covich (2001). A subsample of 20 individual leaves at each site was examined to quantify the number and diversity of attached snails. Percentage of leaf damage for each leaf was estimated

*Author: Assistant Professor, Department of Biology, University of Hartford, 200 Bloomfield Avenue, West Hartford, CT 06117. Corresponding author's E-mail: zhu@hartford.edu. Received for publication February 14, 2014 and in revised form April 8, 2014.

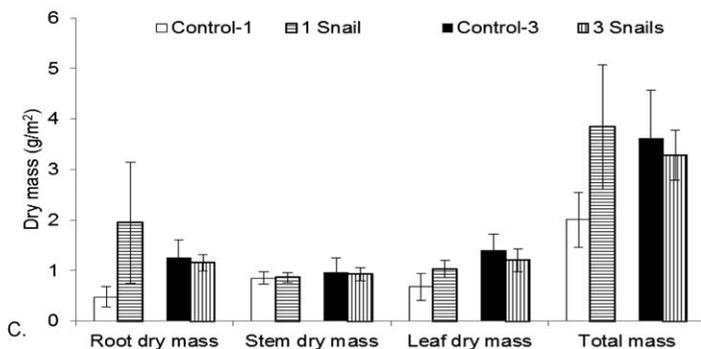
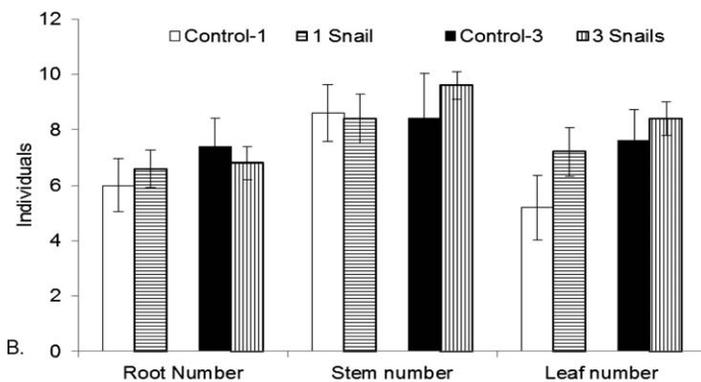
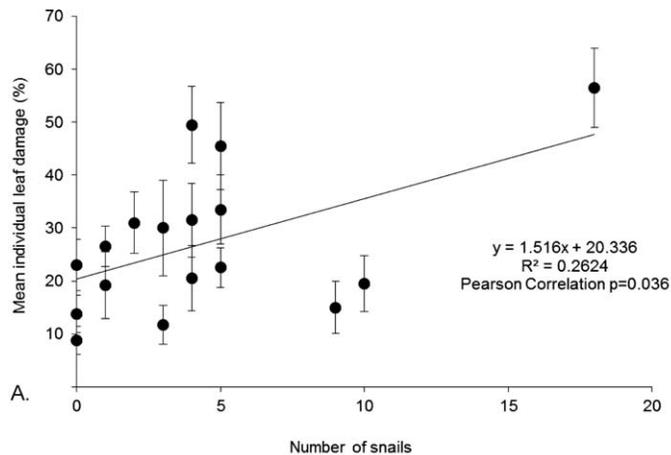


Figure 1. Effects of snail herbivory on growth of European frogbit: (A) Correlation between the number of snails and European frogbit leaf damage from the field survey. (B) Effects of different intensity of snail herbivory on the numbers of roots, stems, and leaves of European frogbit from the laboratory experiment. (C) Effects of different intensity of snail herbivory on root dry mass, stem dry mass, leaf dry mass, and total dry mass. An asterisk (*) indicates significant differences between the control groups (Control-1 or Control-3) and the treatment groups with two different snail densities (1 Snail or 3 Snails) at the level $\alpha = 0.05$.

visually. The mean percentage of leaf damage at each site was then correlated with the number of snails using the Pearson correlation.

A laboratory experiment was also initiated to evaluate snail herbivory on European frogbit growth. Twenty individual plants of European frogbit with similar sizes, collected from Oneida Lake, NY, were randomly placed in 20, 18.9 L (5-gallon), white plastic containers (0.3 m by 0.35 m) with 15 L of lake water collected from Oneida Lake in

each container (one plant per container). The containers were randomly and equally divided into two growth chambers¹ because of space limitations, and the plants grew 7 d to adjust to the new environment: temperature at 25 C and the maximum light intensity ($\sim 830 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 14 h d⁻¹ (Zhu et al. 2008). The containers were then randomly assigned into four treatments, with five replicates each: (1) treated with no snails (Control-1), (2) treated with one snail (1-Snail, $\sim 15 \text{ snails m}^{-2}$), (3) treated with no snails (Control-3), and (4) treated with three snails (3-Snails, $\sim 45 \text{ snails m}^{-2}$). The first two treatments were arranged randomly in one growth chamber, and the latter two in the other. Snails used in the experiment were the tadpole physa (*Physa gyrina* Say), which was the species most frequently found on European frogbit in the field samples. The experiment lasted 4 wk from July 8, 2008, to August 5, 2008. The position of the containers was randomly switched each week to decrease potential differences in light intensity at different locations in the growth chambers. Parameters, including root number, stem number, leaf number, and wet mass, were quantified at the beginning of the experiment to compare whether the initial plant conditions were similar. At the end of experiment, plant growth was measured as root number, stem (including stolon) number, leaf number, root dry mass, stem dry mass, leaf dry mass, and total dry mass. Dry mass was determined after drying at 65 C for 72 h.

Statistical analysis

Pearson correlation analysis was conducted to study the relationship between the number of snails and leaf damage in the field survey. The initial plant parameters, including root number, stem number, leaf number, and wet mass, were compared between the control groups and the treatment groups (i.e., Control-1 vs. 1-Snail and Control-3 vs. 3-Snails) using ANOVA (Kuehl 2000). European frogbit growth at the end of the experiment was compared between the control groups and the treatment groups using analysis of covariance (ANCOVA) with initial plant condition as the covariant (Kuehl 2000). No transformations were needed following tests for heteroskedasticity. All analyses were conducted using IBM SPSS Statistics 21.²

RESULTS AND DISCUSSION

In the field survey, 74 snails were found present on 108 individual European frogbit plants, which is a small fraction of all the European frogbit plants collected from the 17 sites. The snail density was estimated to be 9.6 snails m⁻². The snails were classified into three different families—*Physa* Drap. spp. ($n = 42$; 56.8%), *Lymnaea* Lam. spp. ($n = 16$; 21.6%), and *Helisoma* Swain. spp. ($n = 16$; 21.6%). Pearson correlation revealed a weak, but significant, positive correlation between the number of snails and the leaf damage in the field survey ($n = 17$, Pearson correlation = 0.512, $P = 0.036$; Figure 1A). The significant correlation indicates negative impacts of snail herbivory on European frogbit growth: higher leaf damage was associated with plants containing higher number of snails. However, the

correlation was highly affected by one data point (snail number = 18; leaf damage mean \pm SD = $56.5 \pm 7.4\%$, Figure 1A). If that data point was excluded, the correlation would be nonsignificant ($n = 16$, Pearson correlation = 0.140, $P = 0.604$). Because of low abundance of snails in nature and the weak correlation between the number of snails and the leaf damage, it, therefore, seems unlikely that snail herbivory would be an effective control for European frogbit in natural aquatic ecosystems.

Under laboratory conditions, no significant differences between the control groups and the treatment groups were detected at the beginning or end of the experiment. At the beginning, root number (ranging from 4 ± 0 to 5 ± 0.8), stem number (ranging from 3.2 ± 0.2 to 3.8 ± 0.2), leaf number (ranging from 3.2 ± 0.2 to 3.8 ± 0.2), or wet mass (ranging from 18.7 ± 2.0 to 25.5 ± 3.5 g/m²) did not differ among treatments (all $P > 0.05$, ANOVA). That demonstrated that all the plants were similar at the start of the experiment. At the end of experiment, root number, stem number, and leaf number did not differ between the control group and the treatment group, regardless the density of snails (Figure 1B). Similarly, there were no significant differences between the control and treatment groups at either low or high snail densities (Figure 1C). These results suggest that snail predation did not have any effect on the growth of European frogbit under laboratory conditions. The density of snails used in the laboratory (~ 15 or 45 individuals m⁻²) was high, compared with the density that was found in the field survey (~ 9.6 snails m⁻²). Even at such high densities, no significant negative effects were observed. Combining findings from both the field survey and laboratory experiments, it is concluded that it is unlikely for snails, specifically the tadpole physa, to be biological control agents. Additionally, this species is often found abundant in macrophyte stands in almost any permanent or intermittent freshwater habitat (Dillon 2000) and was reported to consume considerable amount of macrophytes as well as detritus, diatoms, filamentous algae, and fungi (Sheldon 1987, Newman et al. 1996). Those observations indicate that this species is a generalist instead of a specialist, further suggesting a low potential for this species to become biological control agents.

Therefore, this study highlights the need for further investigations to discover potential biological agents for invasive European frogbit. There was damage on leaves from the field observations, and perhaps, that damage was caused by organisms that do not stay on the plants but can move around easily. Other snail species could possibly be candidates of biological agents because some freshwater snails, such as the great pond snail and the decollate snail (*Rumina decollata* L.), were reported to consume European frogbit as well (Froemming 1954). Organisms in the native range of European frogbit should, in particular, be investigated to search as biological control agents.

This study also provides useful information for introducing the potential biological agents to North America when they are successfully identified in the native range of European frogbit. Typically, in the classical biological control program, before or during oversea explorations for host-specific organisms, surveys are usually conducted in

the invaded places to determine whether there are native organisms, such as snails, insects, or pathogens that damage the plant (Strong and Pemberton 2000, Ding et al. 2006). This allows for an understanding of potential interactions between the extant species and those potentially to be released from overseas. Identification of those extant species is, therefore, an important procedure in the biological control program.

SOURCES OF MATERIALS

¹Conviron E15, Controlled Environments Ltd, 590 Berry Street, Winnipeg, MB R3H 0R9, Canada.

²IBM Corporation, 1 New Orchard Road, Armonk, NY 10504.

ACKNOWLEDGEMENTS

This work was supported by the New York State Great Lakes Protection Fund (Small Program). I sincerely thank Meredith Eppers, Bryan Harris, and Joseph Sullivan from the Hobart and William Smith Colleges for assisting sample collection and Dr. Lars G. Rudstam from the Cornell University for snail identification.

LITERATURE CITED

- Catling PM, Miltrow G, Haber E, Posluszny U, Charlton WA. 2003. The biology of Canadian weeds, 124: *Hydrocharis morsus-ranae* L. Can. J. Plant Sci. 83:1001–1016.
- Catling PM, Spicer KW, Lefkovitch LP. 1988. Effects of the floating *Hydrocharis morsus-ranae* (Hydrocharitaceae), on some North American aquatic macrophytes. Nat. Can. 115:31–137.
- Cuda JP, Charudattan R, Grodowitz MJ, Newman RM, Shearea JF, Tamayo ML, Villegas B. 2008. Recent advances in biological control of submerged aquatic weeds. J. Aquat. Plant Manage. 46:15–32.
- Dillon RT, Jr. 2000. The ecology of freshwater molluscs. Cambridge University Press, UK. 509 pp.
- Ding J, Blossy B, Du Y, Zheng F. 2006. *Galerucella birmanica* (Coleoptera: Chrysomelidae), a promising potential biological control agent of water chestnut, *Trapa natans*. Biol. Control 36:80–90.
- Froemming E. 1954. Problematic constituents in several marsh plants. Pharmazie 9:766–769.
- Holz W. 1963. Chemical weed control in ditches; trials on the control of submerged plants In: Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung, Stuttgart–Hohenheim, Germany.
- Kuehl RO. 2000. Design of experiments: statistical principles of research design and analysis. 2nd ed. Pacific Grove, CA: Duxbury. 666 pp.
- Langdon S. 2007. Eradication of European Frogbit (*Hydrocharis morsus-ranae*) from the Grasse River in the Town of Clare, St. Lawrence County, New York. Ray Brook, NY: Adirondack Park Invasive Plant Program 2007 Season Report. 23 pp.
- Magomaev FM. 1973. The daily diet of two- and three-year-old grass carp. Sb. Nauchn. Tr. VNIIPRKh. 10:192–196.
- Mills EL, Leach JH, Carlton JT, Secor CI. 1994. Exotic species and the integrity of the Great Lakes. Bioscience 44:666–676.
- Newman RM, Kerfoot WC, Hanscom Z III. 1996. Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. Ecology 77:2312–2323.
- O'Neill CR, Jr. 2007. European frog-bit (*Hydrocharis morsus-ranae*)—Floating invader of Great Lakes Basin waters. Brockport: New York Sea Grant Invasive Species Factsheet Series 07-1.
- Renard C. 1963. The use of diquat and paraquat to control aquatic plants In: Compte rendu de la 5^e Conférence du COLUMA (Comite Francais de Lutte contre les Mauvaises Herbes. Versailles, France.
- Ricciardi A. 2001. Facilitative interactions among aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? Can. J. Fish. Aquat. Sci. 58:2513–2525.
- Sheldon SP. 1987. The effect of herbivorous snails on submerged macrophyte communities in Minnesota USA lakes. Ecology 68:1920–1931.

- Sheldon SP, Creed RP, Jr. 1995. Use of a native insect as a biological control for an introduced weed. *Ecol. Appl.* 5:1122–1132.
- Strong DR, Pemberton RW. 2000. Ecology-biological control of invading species—risk and reform. *Science* 288:1969–1970.
- Sviridenko VG, Lapittskaya SK, Avseenko SV, Tolkachev VI. 1988. Chemical characterization of aquatic plants and helophytes eaten by the European beaver. *Rastit. Resur.* 23:621–625.
- Thorp JH, Covich AP. 2001. Ecology and classification of North American freshwater invertebrates. 2nd ed. 1056 pp.
- Vaananen VM, Nummi P. 2003. Diet of sympatric dabbling ducks in eutrophic wetlands. *Suom. Riista.* 49:7–16.
- Zhu B, Eppers ME, Rudstam LG. 2008. Predicting invasion of European frogbit in the Finger Lakes of New York. *J. Aquat. Plant Manage.* 46:186–189.
- Zhu B, Fitzgerald DG, Mayer CM, Rudstam LG, Mills EL. 2006. Alteration of ecosystem function by zebra mussels in Oneida Lake, NY: impacts on submerged macrophytes. *Ecosystems* 9:1017–1028.
- Zhu B, Georgian SE. 2014. Interactions between invasive Eurasian water-milfoil and native water stargrass in Cayuga Lake, NY, USA. *J. Plant Ecol.* In press. doi:10.1093/jpe/rtt063.
- Zhu B, Mayer CM, Heckathorn SA, Rudstam LG. 2007. Can dreissenid attachment and biodeposition affect submerged macrophyte growth? *J. Aquat. Plant Manage.* 45:71–76.