J. Aquat. Plant Manage. 49: 32-36

Impact of invertebrates on three aquatic macrophytes: American pondweed, Illinois pondweed, and Mexican water lily

JULIE G. NACHTRIEB, M. J. GRODOWITZ, AND R. M. SMART*

ABSTRACT

The objective of this study was to investigate the impact of invertebrates on three native macrophytes: American pondweed (*Potamogeton nodosus* Poir.), Illinois pondweed (*P. illinoensis* Morong), and Mexican water lily (*Nymphaea mexicana* Zucc.). Biomass production of the three plant species was measured and compared under two conditions: one with an uncontrolled population of herbivorous invertebrates and one in which most herbivorous invertebrates were removed by an insecticide treatment. The insecticide effectively removed most plant-feeding insects, including those in orders Coleoptera, Diptera, Trichoptera, and Lepidoptera, but did not remove one invertebrate group likely to impact plants, Hemiptera (aphids). Differences in plant biomass due to feeding and nonconsumptive damage by remaining invertebrates were variable and dependent upon plant species. Nontreated samples of Mexican water lily exhibited high levels of insect damage (primarily herbivory), as well as case making and egg deposition, but biomass differences between treatments were not detected. The impacts of invertebrate herbivory and nonconsumptive damage were more pronounced in both pondweed species as nontreated biomass was significantly less than biomass of insecticide-treated pondweeds. Biomass of American and Illinois pondweed was reduced by 40 and 63%, respectively, due to invertebrate herbivory. Invertebrate herbivory, once thought to be insignificant to aquatic macrophytes, was shown to cause substantial biomass reductions in two of the three plant species studied.

Key words: herbivory, *Nymphaea mexicana*, *Potamogeton illinoensis*, *Potamogeton nodosus*.

INTRODUCTION

Native aquatic macrophytes are a valuable component of aquatic habitats. They provide important fish and wildlife habitat (Savino and Stein 1982, Heitmeyer and Vohs 1984, Dibble et al. 1996), improve water clarity and quality, and reduce rates of shoreline erosion and sediment resuspension (Smart 1995). Native plants, such as wild celery (*Vallisneria americana* Michx.), have also been shown to compete effectively against invasive macrophytes, thereby providing sustainable management of aquatic ecosystems (Smart et al. 1994, Smart 1995, Ott 2005, Owens et al. 2008).

Understanding the importance of native aquatic plants has prompted their use in an increasing number of revegetation projects. However, herbivory can negatively impact the establishment of plant founder colonies, consequently decreasing the success of revegetation projects (Lodge 1991, Dick et al. 1995, Doyle and Smart 1995, Doyle et al. 1997).

*First and third authors: US Army Engineer-Lewisville Aquatic Ecosystem Research Facility, 201 East Jones St., Lewisville, TX 75057; second author: US Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Corresponding author's E-mail: jnachtrieb@laerf.org.
Received for publication July 28, 2010 and in revised form October 13, 2010.

Cages can be constructed to protect plants from larger herbivores such as turtles, nutria, and crayfish, but excluding invertebrates is nearly impossible. Knowledge of the complex interactions between invertebrate herbivores and native macrophytes can aid in revegetation by improving plant species selection and timing and location decisions.

Although typically beneficial, plants can exhibit weedy growth, not only outside but also within their native range, prompting the need for control methods. Macrophytes such as American lotus (*Nelumbo lutea* [Willd.] Pers.), cattails (*Typha* spp.), and coontail (*Ceratophyllum demersum* L.) are commonly problematic within their native range of North America. Fanwort (*Cabomba caroliniana* A. Gray) can be weedy within its native range of North America as well as in Australia where it forms monospecific stands and is listed as one of Australia's 20 Weeds of National Significance (Schooler et al. 2006). Several species of spatterdock, waterlilies, and pondweeds native to North America are regarded as weeds in Holarctic countries (Sculthorpe 1967). Also, wetlands in the United Kingdom, the Netherlands, and Australia are being threatened by floating marshpennywort (*Hydrocotyle ranunculoides* L. f.), a species believed to be native to North America (EPPO 2006). A greater understanding of invertebrate impacts on native macrophytes could lead to the discovery of natural enemies with potential use as classical biological control agents in other areas of the world.

Little information is available that quantifies the impact of invertebrate herbivores on native macrophyte biomass in North America. Early research indicated that while macrophytes were useful as a substrate for invertebrates and epiphytic growth, they provided little if any nutritional value (Shelford 1918). This same viewpoint was recently supported by Jolivet (1998); however, additional studies have shown importance of macrophytes as a food source for invertebrates. Among those, Soszka (1975) reported pondweeds can lose 50 to 90% of their leaf area from insect herbivory and non-consumptive destruction, mostly from lepidopterans, trichopterans, and dipterans. Leaf area damage as high as 56%, depending on plant species and locality, was documented by Sand-Jensen and Madsen (1989) and attributed to herbivory mostly by trichopterans and dipterans. Newman (1991) later identified five insect orders, Trichoptera, Diptera, Lepidoptera, Coleoptera, and Hemiptera, as containing most herbivores associated with aquatic macrophytes. Live macrophytes were also found to be engaged in aquatic food webs, sometimes to the extent that macrophyte biomass, productivity, and relative species abundance are dramatically changed by grazers (Lodge 1991). Finally, Cronin et al. (1998) determined that freshwater macrophyte herbivory is similar to that reported for terrestrial plants. This viewpoint differed widely from the early idea that macrophytes offered surface substrates only (Shelford 1918).

Although evidence has been collected to prove the existence of invertebrate herbivory of aquatic plants, the significance of this interaction is difficult to quantify. This study attempted to quantify invertebrate herbivory by comparing differences in biomass between grazed and ungrazed populations of macrophytes native to North America and commonly used in the southeastern United States for revegetation and invasive species exclusion efforts.

MATERIALS AND METHODS

This study was conducted in three 0.3 ha earthen ponds (40 m by 60 m) at the Lewisville Aquatic Ecosystem Research Facility located in Lewisville, Texas, (33E04'45"N, 96E57'30"W). Preparation of the study ponds included draining, mowing, rototilling, and installing a barrier to separate each pond lengthwise into two congruent sides. The barrier consisted of 5 cm by 10 cm mesh welded-wire fencing covered by pond liner (45 Mil EPDM Firestone pond liner, AZ Ponds and Supplies, Inc. Birdsboro, PA), creating two treatment areas per pond, an insecticide treatment, and an untreated herbivory area. The height of the fence was adjusted to fit the pond's contour, and the liner was measured to fit the height of the fence plus one meter. The extra meter of liner was buried in pond sediment to seal pond sides. Pond water was gravity-fed from Lake Lewisville, Lewisville, Texas, and supplied evenly to both sides of each pond.

On 27 May 2005 each treatment area was planted with five replicates each of three native macrophytes: American pondweed, IIllinois pondweed, and Mexican water lily. Each replicate was enclosed in a 91 cm dia by 120 cm tall cylinder (cage) constructed from 5 cm by 10 cm mesh welded-wire fencing anchored with 120 cm lengths of concrete reinforcing bar. Cages provided plant protection from disturbances such as turtles or ducks. Cages were spaced at equidistant intervals and positioned at equal depths by following the pond's contour. Amount of plants determined suitable for a cage varied by species due to plant size and growth rate. Each cage was planted with one of the following: three 1 L pots of American pondweed or Illinois pondweed, or one 1 L pot of Mexican water lily. Plants were removed from pots and planted directly into sediment. Each plant species was randomly placed within each treatment area. Ponds were maintained at a depth of approximately 1 m.

An insecticide, temephos (O,O'-(thiodi-4, 1-phenylene) O,O,O',O',-tetramethyl phosphorothioate) (Abate® 4-E, Clarke Mosquito Control Products, Inc. Roselle, IL), was applied once per week as an emulsifiable concentrate to one-half of each pond at a rate of 0.24 µL Abate formulation/L pond water. The temephos application system was constructed of 1.3 cm dia irrigation hose attached to the top of each cage within each temephos treatment area. One 3.78 Lph drip emitter was attached to the irrigation hose in the center of each cage so that temephos was directly applied to plants within the cage. One end of the hose was capped shut and the other end left open. Temephos was applied by attaching the open end of irrigation hose to a gas powered sprayer (FIMCO, No. Sioux City, SD), which forced the temephos solution into the hose and out through drip emitters.

To evaluate end of growing season differences in plant biomass due to invertebrate—plant interactions, all five replicates of each plant species per treatment area were selected and harvested for plant biomass at 4 months after planting (16 September 2005). One replicate was randomly selected, and invertebrates were harvested as well as biomass. Aboveground plant biomass within each cage was harvested and immediately placed into a plastic bag. Plant material was rinsed with water to remove sediments and algae, and dry weights were obtained by separating into species and drying to constant weights in an oven at 55 C for a minimum of 48 h. Replicates harvested for invertebrates were rinsed over a bucket to collect dislodged invertebrates. Internally feeding organisms were not expected to be recovered by these methods. Buckets were emptied into 710 μm sieves and all invertebrates collected were preserved in 70% ethanol. Invertebrates were later identified in the laboratory to the following taxonomic levels: Annelids to class, and Gastropoda and Insecta to genus (except for family Chironomidae, which was identified to subfamily).

Statistical Analyses

A one-way analysis of variance (ANOVA) was performed to differentiate treatment effects on total number of invertebrates collected. Nine invertebrate groups were analyzed separately including; Ephemeroptera, Oligochaeta, Coleoptera, Diptera, Trichoptera, Hemiptera, Lepidoptera, Odonata, and Gastropoda. Invertebrate effects on aquatic plants were quantified by comparison of plant dry biomass between treated and nontreated samples. Differences in plant biomass between treatments were analyzed with a one-way ANOVA for each plant species. Experimental data were analyzed at a significance level of $p < 0.05$ using STATISTICA version 8.0 (StatSoft, Inc., 2008, Tulsa, OK).

RESULTS AND DISCUSSION

Invertebrate Collections

Differences due to treatment (insecticide vs. no insecticide) in collected number of invertebrates varied based on invertebrate group (Figure 1). Two invertebrate groups, Ephemeroptera and Oligochaeta, were not analyzed because

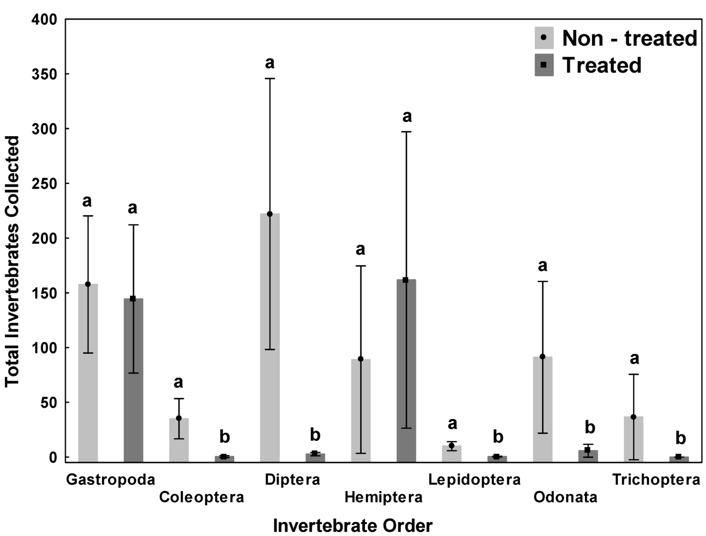

Figure 1. Total number of invertebrates collected per invertebrate order and treatment. Within each order, means with the same letter are not statistically different. One-way ANOVAs (DF = 1, 16): Gastropoda: p = 0.745, F = 0.109; Hemiptera: p = 0.310. F = 1.098; Coleoptera: p = 0.000, F = 18.861; Diptera: p = 0.001, F = 16.710; Trichoptera: p = 0.046, F = 4.673; Lepidoptera: p = 0.000, F = 27.831; and Odonata: p = 0.012, F = 7.990.

an average of fewer than three individuals was collected. Numbers of collected invertebrates from five orders (Coleoptera, Diptera, Trichoptera, Lepidoptera, and Odonata) were significantly reduced in treated areas (Figure 1) by 94 to 100% depending on invertebrate order. In two other groups, Gastropoda and Hemiptera, no significant differences were detected (Figure 1). The failure of temephos to eliminate these invertebrates precluded our ability to ascertain their effects on biomass of any of the plant species in this study. However, the 2 snail genera collected, *Physa* sp. and *Helisoma* sp. (Gastropoda: Physidae and Planorbidae, respectively), both feed primarily on epiphytic growth or detritus without damaging aquatic plants (Brown 2001, Smith 2001), and no obvious plant damage attributable to snail grazing was noted. Hemiptera samples consisted of 90% aphids in the genus *Rhopalosiphum* in nontreated and treated samples alike. *Rhopalosiphum* spp. can be damaging to plants and could therefore affect biomass results in treated areas (Blackman 1974).

Macrophyte Biomass

Dry weights of Mexican water lily were not statistically different between treatments (Figure 2 A); however, herbivory and nonconsumptive damage from the adult coleopteran *Donacia cincticornis* Newman, larval lepidopteran *Synclita* sp., aphids of *Rhopalosiphum* sp., and odonate egg deposition were apparent on nontreated Mexican water lily. Changes in leaf density within cages were less obvious because new leaves were continuously emerging while highly damaged leaves were decaying. Undocumented observations from this study imply that leaf turnover rate increased in nontreated Mexican water lily plants, which were subjected to various types of invertebrate damage. This would make it difficult to determine biomass differences (i.e., measure herbivory) between treatments and could result in underestimates of the impact of invertebrates on Mexican water lily. Other researchers experienced similar difficulties in measuring invertebrate damage levels on other aquatic plant species. For instance, Wallace and O'Hop (1985) documented that leaf turnover rate of spatterdock (*Nuphar luteum* [L.] Sibth & Sm.) was higher at a site that experienced herbivory by the waterlily leaf beetle (*Pyrrhalta nymphaeae* [L.]) in contrast to where beetles were absent. At the herbivore site, leaves died faster but were replaced quickly as if plant growth was compensating for herbivory losses.

In contrast, treated Mexican water lily plants were mostly void of any signs of invertebrate damage other than that due to odonate egg deposition and *Rhopalosiphum* sp. Two anisopteran families, Aeschnidae and Petaluridae, as well as most zygopterans, are known to oviposit in aquatic plant tissue, which can leave holes in plants once the larva emerge. This endophytic trait can result in excessive damage to plant tissue by large numbers of females (Westfall and Tennessen 1996). Near harvest time, aphids were present in both treatment areas in large enough numbers to completely cover the floating leaves of Mexican water lily. While not problematic in small numbers, large aphid colonies are capable of removing enough of the plant's nutrients so that the plant prematurely breaks down plant tissue to replenish its nutrient

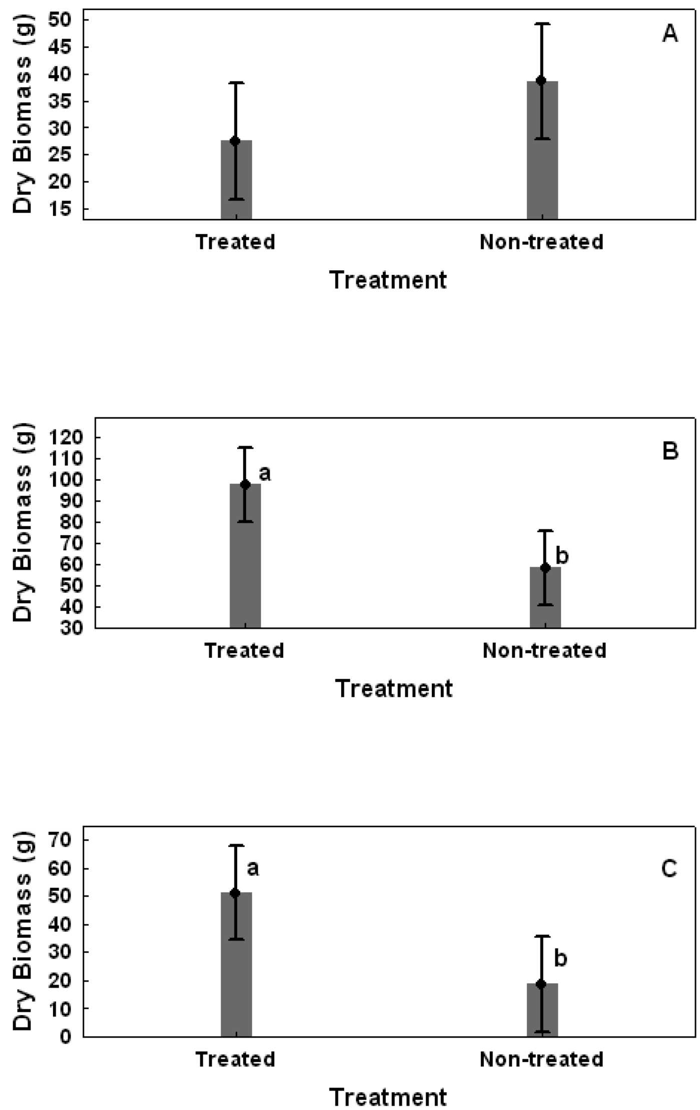

Figure 2. Mean (±0.95 confidence interval) dry biomass (g) of Mexican water lily (A), American pondweed (B), and Illinois pondweed (C) collected per treatment area. Means with the same letter are not statistically different. One-way ANOVA. Mexican water lily (A): $p = 0.144$, $F = 2.258$, $DF = 1, 28$; American pondweed (B): $p = 0.003$, $F = 10.568$, $DF = 1, 28$; and Illinois pondweed (C): $p = 0.010$, $F = 7.668$, $DF = 1, 28$.

supply (Blackman 1974), which can halt plant growth and ultimately cause death. Without aphids in treated samples, biomass may have increased at a rate greater than nontreated plants. Therefore, even though plant conditions from the two treatments were clearly different, combined effects of possible increased leaf turnover rate in nontreated plants and aphid herbivory in treated plants made it difficult to identify biomass differences due to the impact of invertebrates on Mexican water lily. To document leaf turnover rate in future studies, leaves should be marked at emergence and days to leaf death should be noted to compare leaf turnover rate between treatments.

Both pondweeds followed similar trends throughout the study (Figures 2B and 2C). Treated plants were rarely damaged by herbivores other than *Rhopalosiphum* sp. and odonate egg deposition, while nontreated plants sustained damage mostly from a combination of *Rhopalosiphum* sp., lepidopteran larvae of *Synclita* sp. and *Paraponyx* sp., and dipteran larvae of *Hydrellia discursa* Deonier and *H. bilobifera* Cresson. Biomass differences between treatments for both pondweeds were significant (Figures 2B and 2C). Nontreated dry weights of American and Illinois were reduced by 40 and 63%, respectively, when compared to treated dry weights. Because aphids were present in large quantities in both treatments, differences in plant biomass were most likely attributable to the lepidopterans and dipterans identified above. Unlike Mexican water lily, an increase in leaf turnover rate was not observed for the pondweeds. Invertebrate herbivory and nonconsumptive damage were shown to significantly impact both pondweeds.

Future research should focus on invertebrate herbivory on other native species of aquatic plants in controlled research settings as well as natural conditions in water bodies. The impacts invertebrate herbivory may have on revegetation efforts (e.g., reduced competitive potential against nuisance species, reduction of tolerance to species-selective herbicide applications) merit investigation. Studies should be designed to include comparisons of leaf turnover rates in the presence and absence of herbivorous insects. More important, individual plant and insect combinations should be studied to further our knowledge of possible host-specific biological control agents for native plants for use in areas where they become problematic.

ACKNOWLEDGEMENTS

This research was conducted under the US Army Corps of Engineers Aquatic Plant Control Research Program, US Army Engineers Research and Development Center. Permission to publish was granted by the Chief of Engineers. We would like to thank Dr. Judy Shearer and Dr. Gary Dick for review of the paper and students and employees at the Lewisville Aquatic Ecosystem Research Facility for technical assistance.

LITERATURE CITED

Blackman R. 1974. Aphids. London and Aylesbury (UK): Ginn & Company Limited.

Brown KM. 2001. Mollusca: Gastropoda. In: Thorp JH, Covich AP, editors. Ecology and classification of North American freshwater invertebrates. 2nd edition. San Diego (CA): Academic Press. p. 297-329.

Cronin G, Wissing KD, Lodge DM. 1998. Comparative feeding selectivity of herbivorous insects on water lilies: aquatic vs. semi-terrestrial insects and submersed vs. floating leaves. Freshwater Biol. 39:243-257.

Dibble ED, Killgore KJ, Harrel SL. 1996. Assessment of fish-plant interactions. In: Miranda LE, DeVries DR, editors. American Fisheries Society Symposium 16: Multidimensional approaches to reservoir fisheries management. p. 347-356.

Dick GO, Smart RM, Keiser ED. 1995. Populations of turtles and their potential impacts on aquatic plants in Guntersville Reservoir, Alabama. Joint Agency Guntersville Project Aquatic Plant Management, Tennessee Valley Authority Report.

Doyle RD, Smart RM. 1995. Competitive interactions of native plants with nuisance species in Guntersville Reservoir, Alabama. In: Proceedings of the Aquatic Plant Control Research Program. 29th Annual Meeting. Vicksburg (MS): US Army Engineer Waterways Experiment Station. Miscellaneous Paper A-95-3. p. 237-242.

Doyle RD, Smart RM, Guest C, Bickell K. 1997. Establishment of native aquatic plants for fish habitat: test plantings in two north Texas reservoirs. Lake Reserv Manage. 13:259-269.

[EPPO] European and Mediterranean Plant Protection Organization. 2006. *Hydrocotyle ranunculoides*. EPPO Bulletin 36 (1): 3-6. doi:10.1111/j.1365-338.2006.00945.x.

Heitmeyer ME, Vohs PA Jr. 1984. Distribution and habitat use of waterfowl wintering in Oklahoma. J Wildl Manage. 48:51-62.

Jolivet P. 1998. Interrelationship between insects and plants. Boca Raton (FL): CRC Press. 309 p.

Lodge DM. 1991. Herbivory on freshwater macrophytes. Aquat Bot. 41:195-224.

Newman RM. 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. J North Am Benthol Soc. 10:89-114.

Ott RA. 2005. Influence of native macrophytes and herbivory on establishment and growth of *Hydrilla verticillata* in pond-scale mesocosms. [dissertation]. [Nagadoches (TX)]: Stephen F. Austin University. 138 p.

Owens CS, Smart RM, Dick GO. 2008. Resistance of Vallisneria to invasion from hydrilla fragments. J Aquat.Plant Manage. 46:113-116.

Sand-Jensen K, Madsen TV. 1989. Invertebrates graze submerged rooted macrophytes in lowland streams. Oikos. 55:420-423.

Savino JF, Stein RA. 1982. Predator-prey interactions between largemouth bass and bluegills as influenced by simulated, submerged vegetation. Trans Am Fish Soc. 111:225-266.

Schooler S, Julien M, Walsh GC. 2006. Predicting the response of *Cabomba caroliniana* populations to biological control agent damage. Aust J Entomol. 45:327-330.

Sculthorpe SP. 1967. The biology of aquatic vascular plants. London (UK): Edward Arnold.

Shelford VE. 1918. Conditions of existence. In: Ward HB, Whipple GC, editors. Freshwater biology. New York (NY): John Wiley. p. 21-60.

Smart RM. 1995. Preemption: an important determinant of competitive success. In: Proceedings of the aquatic plant control research program, 29th annual meeting. Vicksburg (MS): US Army Engineer Waterways Experiment Station. Miscellaneous Paper A-95-3. p. 231-236.

Smart RM., Barko JW, McFarland DG. 1994. Competition between *Hydrilla verticillata* and *Vallisneria americana* under different environmental conditions. Vicksburg (MS): US Army Engineer Research and Development Center. Technical Report A-94-1.

Smith DG. 2001. Pennak's freshwater invertebrates of the United States. 4th edition. Porifera to Crustacea. New York (NY): John Wiley and Sons. 638 p.

Soszka GJ. 1975. Ecological relations between invertebrates and submerged macrophytes in the lake littoral. Ekol Polska 23:393-415.

Wallace JB, O'Hop J. 1985. Life on a fast pad: waterlily leaf beetle impact on water lilies. Ecology. 66(5):1534-1544.

Westfall M. Jr, Tennessen KJ. 1996. Odonata. In: Merritt RW, Cummins KW, editors. An introduction to the aquatic insects of North America. Dubuque (IA): Kendall/Hunt Publishing Company. p. 164-216.