Effects of Common Reed (*Phragmites australis)* Invasion and Glyphosate and Imazapyr Herbicide Application on Gastropod and Epiphyton Communities in Sheldon Marsh Nature Reserve.

A Thesis

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Abstract

Phragmites australis, the common reed, is an invasive macrophyte in many eastern North American wetlands. Reed often rapidly forms dense, near-monotypic stands by replacing native vegetation, which lowers plant diversity and alters wetland habitat structure. Accordingly, herbicides such as imazypr-based Habitat® and glyphosate-based AquaNeat® are often applied to reed stands in an attempt to control its establishment and spread. Although these herbicides are apparently not toxic to benthic organisms, they may indirectly affect them by altering available habitat structure via increased detrital litter, increased light penetration to surface waters and increased water temperature. Understanding the impacts of widespread herbiciding on benthic communities, as well as the impact of different herbicides on habitat conditions, should help wetland managers design control plans to reduce reed and conserve system biodiversity. I compared gastropod (i.e., snails) and epiphyton communities, and habitat conditions among large, replicated plots of unsprayed *Phragmites,* glyphosate-sprayed *Phragmites,* imazapyr-sprayed *Phragmites* and unsprayed *Typha angustifolia* (narrow-leaf cattail) in early the summer 2008 in a Lake Erie coastal marsh. I studied gastropods because they can greatly influence trophic structure in freshwater systems by consuming benthic algae and by serving as prey to sportfish. Moreover, I included *Typha angustifolia* in the study because it is another invasive plant common to many Lake Erie coastal wetlands. Relative abundances of gastropods were similar among treatments on

24 June and 30 June, but differed on 8 July. On 8 July, *Fossaria* spp. were particularly abundant in herbicide-treated plots, and *Promenetus umbilicatellus* was abundant in AquaNeat®- treated plots. Snail densities were greater in plots containing metaphyton (filamentous green algae) than in plots without metaphyton, and juvenile and small snails were abundant in metaphyton mats. Metaphyton presence was linked to increases in light penetration caused by herbiciding. Epiphytic algal densities, and chlorophyll *a* levels, an indicator of algal biomass, were low in all treatments, and diatom-dominated epiphyton communities were similar among treatments. Dry mass of benthic organic matter (BOM), which can provide available substrate for epiphyton growth and habitat for snails, was also similar among treatments.   Dissolved oxygen and water depths were similar among treatments, but water depths significantly declined with sampling date. A combination of low and highly variable water levels, low oxygen levels, and eutrophic conditions apparently shape snail and algal community structure in this marsh. My results suggest that both glyphosate- and imazapyr-containing herbicides have little effect on the herbivore-producer relationship and gastropod diversity in Sheldon marsh 1-year post-spraying.

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Fields of Study

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Introduction

Emergent vegetation plays a major role in determining the structural and functional organization of salt and freshwater marshes (Carter et al. 1991, Mitsch and Gosselink 2007). It provides physical habitat structure for fish, benthic invertebrates, and epiphyton (e.g., Fell et al. 2003, Laugaste and Reunanen 2005), and influences chemicophysical habitat conditions such as dissolved oxygen levels, nutrient concentrations, light availability, water movements, and temperature (Carter et al. 1991, Albay and Akcaaln 2003, Laugaste and Reunanen 2005). Eventual vegetation collapse and detrital build-up may also alter system hydrology by reducing erosion, trapping sediments, and interrupting patterns of water flow (Marks et al. 1994, Chambers et al. 1999). Thus, large-scale changes in emergent vegetation composition can potentially greatly influence many ecosystem processes, and hence benthic communities. Invasive macrophytes often rapidly spread to form near-monocultures by displacing native vegetation. This spread decreases habitat heterogeneity and alters habitat structure (Chambers et al 1999, Bickel and Closs 2009), which in turn impacts local benthic macroinvertebrate and algal communities (Strayer et al. 2003, Kelly and Hawes 2005, Bickel and Closs 2009) as well as soil microbial assemblages (Whitcraft and Levin 2008).

Phragmites australis (Cav.) Trin. ex Steud., (common reed) is a perennial grass that has been a minor component of North American wetlands until recently. An aggressive haplotype (M) from Europe was introduced to brackish marshes along the northeast coast ~80 years ago (Saltonstall 2002). Its rapid spread westward since has apparently been facilitated by anthropogenic disturbance (i.e., water diversion and draining) and by eutrophication from farm and urban runoff (Marks et al. 1994, Chambers et al. 1999). Reed recently became a nuisance in Great Lake coastal wetlands when lake water levels significantly dropped in 2000 (Wilcox et al. 2003, Back and Holomuzki 2008, Whyte et al. 2008). *Phragmites* has the ability to quickly spread by vegetative reproduction (i.e., rhizomes) into exposed mudflats created by low water levels (Marks et al. 1994, Chambers et al. 1999). Once established, reed can form dense, near-monocultures and displace native vegetation in as rapidly as one growing season (Marks et al. 1994, Chambers et al. 1999, Back and Holomuzki 2008).

Reed establishment and spread can greatly impact system physical processes. Vegetative growth of below-ground rhizomes can produce stems reaching ~3–4 m high at densities of ~200–300 stems m^{-2} and generate above-ground production >1 kg m^{-2} yr^{-1} (Marks et al. 1994, Warren et al. 2001). These heights and densities can reduce the amount of light reaching the marsh surface, which in turn can lower water temperature (Carter et al. 1991). Moreover, stems are high in cellulose and silica, and consequently, decompose slowly (> 2yrs) after their collapse and submergence, producing huge pools of detrital litter (Meyerson et al. 2000). Litter accumulation can lead to system-wide changes in geomorphology and hydroperiod (Chambers et al. 1999, Meyerson et al.

2000), and nutrient leaching from decomposing leaves and stems can increase nutrient levels such as nitrogen and phosphorus in surrounding waters (Asaeda et al. 2001, Karosienė and Kasperovičienė 2008). Litter accumulation can also increase the threat of fire, which can be particularly threatening to surrounding terrestrial ecosystems and nearby homes (Marks et al. 1994).

Reed invasion appears to have mixed effects on faunal abundance patterns. Dense, homogenous reed stands are generally unsuitable habitat for wading and migratory birds, as well as large mammals (Chambers et al. 1999). Moreover, reed litter accumulation can physically obstruct movements of fish (e.g., mummichogs) (Chambers et al. 1999), restricting them from breeding and foraging habitats. However, increased leaf litter from reed invasion may increase available habitat for some small mammals and reptiles (Marks et al. 1994). As for macroinvertebrates, work shows total density and species richness are lower in *Phragmites*-dominated wetlands than in *Spartina alterniflora* marshes (Angradi et al. 2001), but other work shows densities and diversity are similar between stands of *Phragmites* and native flora (Fell et al. 1998, Able and Hagen 2000, Warren et al. 2001, Kulesza et al 2008), or increase with *Phragmites* dominance (Holomuzki and Klarer 2009). Nutrient leaching from submerged reed litter into surrounding waters can promote epiphyton growth and alter algal community structure (Albay and Akcaalan 2002, Gosselain et al. 2005, Laugaste and Reunanen 2005). How these changes in epiphyton affect grazer communities is not known, but are important to understanding wetland trophic structure and herbivore spatial dynamics.

Habitat structure associated with macrophyte composition has a large influence on the algal abundance and community structure (Pillsbury and Lowe 1999, Whitcraft and Levin 2007, Karosienė and Kasperovičienė 2008). For example, living *Phragmites* stems tend to support higher epiphyton densities and more diatoms than *Typha angustifolia* L. (narrow-leaf cattail) stems, a common marsh macrophyte in Lake Erie coastal wetlands (Laugaste and Reunanen 2005, Kulesza and Holomuzki 2008, Holomuzki and Klarer 2009). Moreover, older live stems of reed tend to support higher densities of diatom-dominated epiphyton compared to younger stems, perhaps because of their spongy surface and increased availability of silica on older stems (Laugaste and Reunane 2005). However, toppled, submerged macrophytes also provide a large surface area for the colonization of microorganisms and have been largely overlooked as potential epiphyton substrate relative to standing live stems (Wetzel 1993, Warda 2002). Epiphyton development can increase with macrophyte collapse and detrital build-up as a result of increased light penetration and nutrient leaching (Karosienė and Kasperovičienė 2008). Reed-dominated systems are typically detritus-based systems because of this build-up (Komínková et al. 2000). Thus, studying the impact and suitability of reed detritus for epiphyton growth appears important to understanding these systems.

Light availability, water depth, and temperature also play important roles in algal growth and community structure (Albay and Akcaalan 2003, Gosselain et al 2005). Warm water and lower water levels facilitate epiphyton growth, whereas light availability plays an important role in determining algal assemblages. For example, green filamentous algae often increase with increased exposure to sunlight (Pillsbury and Lowe

1999, Whitcraft and Levin 2007), whereas diatoms typically thrive in relatively low light conditions (Pillsbury and Lowe 1999). As a result, changes in plant community structure that change these conditions may indirectly alter producer–herbivore interactions and system trophic economies (Bickel and Closs 2009).

Herbivorous and detritivorous gastropods serve as vital trophic links between primary producers and higher consumers in many wetlands (Kesler et al. 1986, Strong et al. 2008). Herbivorous gastropods can sometimes exert strong top-down effects on epiphyton and macrophyte communities (Elger et al. 2009, Liess et al. 2009). Snails are attracted to macrophytes because they provide a stable food source, access to the air-water interface, and a refuge from predators (Thomas and Daldorph 1994, Liess et al. 2009). Macrophytes also provide a suitable substrate for diatom-dominated epiphyton, which is a nutritious food source for many gastropods grazers (Kesler et al. 1986, Dillon 2000, Bayo et al. 2005). Moreover, bacterial films associated with epiphyton, filamentous-green algae, and macrophyte detritus may also serve as food sources for freshwater snails (Kesler et al. 1986, Gérard et al.2008, Dillon 2000, Bayo et al. 2005). Thus, macrophyte type and abundance can be a key determinant of snail abundance patterns.

Phragmites invasion is a major concern to wetland managers given its impact on plant diversity and habitat structure in these systems (Marks et al. 1994). Herbiciding is a common management technique used to control reed because of its low cost and ease of application (Marks et al. 1994, Back and Holomuzki 2008). Complete eradication of reed is often not feasible, but maintenance and control of reed spread is possible with yearly applications and multi-year spot-treatments (Ailstock et al. 2001, Warren et al. 2001, Back and Holomuzki 2008). Generally, the most commonly used herbicides to control reed have been glyphosate-based herbicides such as AquaNeat® (Riverdale Chemicals) and Glypro® (Dow AgroSciences) (Marks et. al 1994). In 2003, the imazapyr-containing herbicide Habitat® (BASF Corporation) was approved by the US EPA for invasive plants in aquatic systems (Patten 2003, Mozdzer et al. 2008). Both herbicide types work similarly by interrupting the synthesis of important amino acids (Entrix Inc 2003). However, the onset of necrosis (i.e., browning) after treatment can vary significantly between these herbicides. Plants treated with glyphostates will begin browning ~2 weeks post-application (Giesy et al 2000), whereas plants treated with imazapyr do not brown until several months after application (Entrix Inc. 2003, Back and Holomuzki 2008). Regardless of herbicide type, large-scale herbiciding of macrophyte beds eventually leads to dramatic changes in habitat structure through increased detrital amounts and alteration of abiotic factors such as sunlight penetration and water temperature (Ailstock et al. 2001, Whitcraft and Levin 2008), which will undoubtedly affect habitat conditions for natural communities.

Here, I evaluate the effects of glyphosate-treated, imazapyr-treated, and untreated patches of *Phragmites*, as well as untreated patches of *Typha angustifolia*, on snail and epiphyton community structure. *Typha angustifolia* is also invasive to many Great Lake coastal marshes, but is considered more ‘desirable’ than reed by some because it does not typically alter plant composition or wetland function (Findlay et al. 2008). Widespread senescence of emergent plants from herbicide application should increase detrital litter amounts and light penetration and alter physical habitat structure, which might impact epiphyton and gastropod trophic organization. I hypothesize that increased light penetration and warmer water temperatures in herbicide-treated plots will alter both epiphyton and gastropod community structure. Specifically, I predict that increased light penetration will increase the abundance of less nutritious green algae, and decrease the abundance of nutritious shade-tolerant diatoms. As a result, snail abundance, biomass, and diversity will be predictably lower in herbicide-treated plots with decreased nutritional availability. Examining the impacts of *Phragmites* and different herbicides on benthic diversity and habitat conditions will hopefully provide resource managers with information on best practices for reed control and the conservation of system biodiversity.

Methods

Study marsh

The study was done in Sheldon Marsh Nature Reserve (188 ha), located ~2 km west of Huron, Ohio (Fig. 1) on the south shore of Lake Erie. A 1.8-km long permanent sand bar separates much of the marsh from Lake Erie. However, a ~15-m wide channel at the western end of the sand bar directly connects the marsh to the lake (Fig. 1). So, water levels of the marsh are directly affected by Lake Erie water levels and seiches. The marsh is eutrophic (summer PO_4 levels ~112 μg/L), owing largely to runoff from its mainly urban watershed (Holomuzki and Klarer 2009). Water calcium levels exceed 50 mg/L (Holomuzki and Klarer 2009), and therefore is not a limiting determinant of gastropod shell formation and abundance (Lodge et al. 1987).

Reed first appeared in a small isolated patch in the northeast corner of the marsh in 1998 (Back and Holomuzki 2008). However, it did not proliferate until 2000 (Back and Holomuzki 2008) when water levels in Lake Erie dropped (Wilcox et al. 2003). By 2001, reed covered approximately 11% of the emergent vegetation in the marsh (Back and Holomuzki 2008). Thereafter, annual applications of herbicides (glyphosates or Habitat®) from backpack sprayers have contained reed to ~6% of the emergent vegetation. The other dominant macrophytes in the marsh are *Typha angustifolia*, *Nelumbo lutea* (American Lotus), and *Sagittaria latifolia* (Broadleaf Arrowhead) (Holomuzki and Klarer 2009).The amphipod *Hyalella azteca* is numerically the dominant macroinvertebrate and is particularly abundant in *Nelumbo* beds with thick mats of duckweed (*Lemna minor*) (Holomuzki and Klarer 2009). The dominant snail is *Physella gyrina*. Macroinvertebrate diversity (Shannon-Weaver H' = 1.1), and % Ephemeroptera, Odonata, and Trichoptera (1.3%), an indicator of system ecological health (Stewart and Downing 2008), are low (Holomuzki and Klarer 2009).

The summer epiphytic algal community is dominated by diatoms. However, species dominance appears to differ among plant types. *Achnanthes lanceolata* and *Nitzschea palea* are dominant on submerged *Phragmites* stems, whereas *Eunotia bilunaris* and *Achnanthes hungarica* are dominant on *Typha* stems (Holomuzki and Klarer 2009).

Study design and herbicide treatments

A 300-m long, 20-m wide near-monotypic stand of reed in the northwest section of the marsh was divided into 15 20 x 20-m plots on 12 June 2007. Plots were randomly assigned an herbicide treatment of either 1) a 30% AquaNeat® solution, 2) a 5% Habitat® solution, or 3) no herbicide (control) (Fig. 1). These respective herbicide concentrations are strong, but often used to control reed (Back and Holomuzki 2008, Mozdzer et al. 2008). Herbicides were applied to assigned plots by hand using backpack sprayers on 14 June 2007. Five additional plots were demarcated on 4 June 2008 in a near-monotypic stand of unsprayed *Typha angustifolia* located ~60 m west of our westernmost

Phragmites plot (Fig. 1) to compare snail abundance patterns and determinants between plant types. However, cattail plots were smaller (ea. 4-m long by 20-m wide) than the reed plots because both the size of the *Typha* stand and the reserve boundary restricted their size.

Snail sampling and assemblage characterization

Snails were sampled in each plot on 24 and 30 June, and 8 July 2008 in a 10 x 10-cm quadrat outlined by PVC pipe. The quadrat was positioned 5-m into each plot at the halfway point along the open-water–emergent plant zone interface. Gastropods sampling took place near the center of plots to minimize potential edge effects posed by contiguous plots and treatments. One sample was taken in each plot on each date ($n = 60$ total samples). Submerged fallen stems and benthic organic matter (BOM; ≥ 2-mm particle size) were collected by hand and dip net (2-mm mesh) in each quadrat, placed in a Ziploc® bag, and stored in 95% ethanol. Snails were separated from plant material by hand in the laboratory, counted, and preserved in 95% ethanol. Snails were identified to genus or species using Pennak (1989) and Peckarsky et al. (1990) as primary references. After identification, snails were dried at 80°C for 24 h and weighed (± 0.001 g) to determine whether snail biomass differed among treatments.

Non-metric multidimensional scaling (NMDS) was attempted to graphically relate gastropod communities to herbicide/macrophyte treatments. The “slow-and-thorough” autopilot mode of NMDS in PC-ORD (McCune & Mefford 2006) generated a high stress value (27), suggesting the model was a poor fit for the data. Consequently, I did not use

NMDS to characterize snail communities. Instead, I characterized snail assemblages in treatments using the Shannon-Wiener diversity index (H') (Shannon and Wiener 1963), Kendall's coefficient of concordance (W), density estimates, and biomass. The Shannon-Wiener formula incorporates both species richness and evenness, and is expressed as:

$$H' = -\Sigma\, p_i \ln p_i$$

where p_i is the proportion of individuals found in the ith species. H' was computed for each study plot on each sampling date. Kendall's coefficient of concordance was used to compare rankings of relative abundances of dominant snail taxa across treatments (Sokal and Rohlf 1995). Densities were calculated from snail counts in quadrats for each plot, averaged for treatment on each date, and expressed as snails 100 cm^{-2}. Snail biomass estimates from each plot were also averaged for treatments for each sample date, and expressed as g 100 cm^{-2}.

Repeated measures (RM) ANOVAs were used to compare Shannon-Wiener diversity, and snail density and biomass among treatments across dates (main effect: herbicide/plant treatment; within subjects term: sample date) (SPSS 1999, Wilkinson 2000). Density and biomass were log10 (x+1)-transformed for analyses.

Environmental conditions in treatment plots

Plant detritus and epiphyton.— Amount of submerged macrophyte detritus and associated metaphyton (filamentous algal mats) collected in quadrats for snail samples was quantified to compare habitat, food supply, and epiphyton substrate availability among treatments. After snail removal, metaphyton was removed from samples with forceps, and benthic organic matter (BOM) was gently rinsed with tap water in a 2-mm sieve. BOM and metaphyton from each sample were separately dried at 100°C for 24 h and weighed (±0.01 g). Log-transformed dry masses of BOM were compared among treatments and sample dates using two-way RM ANOVA. Two BOM samples were combined and were therefore deleted from the analysis.

We also assessed potential epiphytic food availability for snails in treatments. A fallen, submerged *Phragmites* or *Typha* stem, depending on treatment plot, was collected from outside but within 0.5 m of the sample quadrat, and from a randomly chosen direction in each plot, on each sample day to determine epiphyton cell densities, chlorophyll *a* (chl *a*) concentrations, and assemblage. A 5–7 cm length of stem was cut, placed in a Whirl® pack bag, and immediately transported to the laboratory. Upon arrival, length and width of each cut stem were measured to calculate surface area for algal density estimates. Algae were removed from the entire stem using the blunt end of a scalpel, rinsed with distilled water into a beaker, and diluted to a known volume. A 10 mL subsample was taken from each sample prior to chl *a* extraction and preserved in Lugol's solution to determine algal assemblages. These samples were homogenized to produce a uniform suspension, and all units from a ~0.05 mL subsample were placed on a 22 x 22 cover slip and counted at 400X magnification. A unit was equivalent to a cell for colonial and unicellular algae or a 10-μm length of filamentous algae with relatively large cells. Either 250 cells were counted or 1000 fields (field area = 0.1257 mm^2) were viewed for each sample on 24 June and 8 July. Cells were identified to genus or species for assemblage determinations using Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) or Wehr and Sheath (2003).

For chl *a* determinations, each sample was vacuum filtered through a Gelman glass-fiber filter paper (pore size 1.0 µm) that was placed in 10 mL of buffered acetone at 4°C and stored in a dark freezer for 7–10 d. An extracted subsample (3 mL) was analyzed on a spectrophotometer at 664 and 740 nm. The sample was then acidified (0.1 mL 0.1 N HCL) and read at 665 and 750nm. Chl *a* concentrations were compared among treatments and all three sample dates using two-way RM ANOVA. Two samples contaminated with *Lemna minor* were deleted from the analysis.

Non-metric multidimensional scaling (NMDS) was attempted to graphically relate epiphyton communities to herbicide/macrophyte treatments. Analysis was completed on dominant epiphyton taxa (species present in >75% of plots). The "slow-and-thorough" autopilot mode of NMDS was based on Curtis-Bray similarity matrices. Epiphyton communities in treatments were also characterized using the Shannon-Wiener diversity index (H'), Kendall's coefficient of concordance (W), and density estimates (10^3 cells/100 cm^2).

Physical factors.— Light levels in plots were measured on 8 July and 15 July 2008 to evaluate light effects on food (algae) levels. Light measurements were recorded by a Hobo® Light Intensity Logger (Onset Computer Corporation, Bourne, Maine, USA). Light intensity (log lum/m^2) was measured simultaneously at the water surface and above the emergent plant canopy at 15 sec intervals for 5 min in each plot between 0920 h and

1400 h on each date. Light meters at both heights were securely placed in water-tight clear plastic canisters. Canisters on the water were allowed to float on the surface in wave-free areas, whereas canisters over canopies were strung to poles at shade-free heights (~3 m high). Light readings over the 5 min period from both water surface and canopy localities were averaged and used to calculate the % difference in light between the canopy and water surface (% light penetration). Percent light penetration was arcsine-transformed and compared among plant/herbicide treatments and dates using two-way RM ANOVA. A significant treatment effect was followed by Bonferroni pairwise comparisons ($\alpha = 0.05$).

Water temperature and depth, and dissolved oxygen, other factors that might determine snail abundance patterns (Brown 2001), were recorded in each quadrat immediately prior to snail collections on each sample date and on 15 July at localities where snails had previously been collected. Temperature and dissolved oxygen were measured with an YSI model 95 probe, and depth was measured with a meter stick. These factors were compared among treatments and dates using two-way RM ANOVAs.

Results

Snail assemblages

A total of eight gastropod taxa were found on the three collection dates. Six of these species, *Physella* spp., *Fossaria* spp., *Promenetus umbilicatellus*, *Gyraulus parvus*, *Helisoma* sp., and *Stagnicola* sp. comprised >90% of all snails collected from plots on all dates (Fig. 2). The abundance rankings of these six species changed among treatments over time. Snail assemblages were similar among treatments on 24 June (Kendall's concordance, $W = 0.652$, $P < 0.025$) and 30 June ($W = 0.611$, $P < 0.05$), but not on 8 July ($W = 0.541$, $P > 0.05$). *Fossaria* spp. were most abundant in herbicide-treated plots, whereas *Promenetus umbilicatellus* was particularly abundant in AquaNeat® sprayed plots. *Physella* spp. were the most common snail taxa collected and were relatively abundant in all treatments (Fig. 2). *Pseudosuccinea columella* was uncommon, and only one specimen of *Ferrissia parallela* was found throughout the study. Low overall taxon richness contributed to low Shannon-Wiener diversity indices (Fig. 3A) that were similar across treatments and sample dates (RM ANOVA; treatment effect: $F_{3,11} = 1.779$, $P = 0.209$; date effect: $F_{2,22} = 0.342$, $P = 0.714$).

Snail densities were marginally different among treatments (RM ANOVA: $F_{3,16} = 2.675$, $P = 0.082$), but not among sample dates ($F_{2,36} = 0.258$, *P*= 0.774) (Fig. 3B). Densities were generally highest in herbicide-treated reed plots and lowest in *Typha* and control plots. Snail densities were significantly greater in plots that contained mats of metaphyton than those that did not (RM ANOVA: treatment effect: $F_{1,14} = 10.735$, $P = 0.006$; date effect: $F_{2,14} = 0.619$, $P = 0.546$), and mats mainly grew in herbicide-treated plots. Metaphyton was found in eight plots on 24 June (1 control, 2 AquaNeat®-treated, 2 Habitat®-treated, 3 *Typha*), five plots on 30 June (3 AquaNeat®-treated, 2 Habitat®-treated), and four plots on 8 July (2 AquaNeat®-treated, 2 Habitat®-treated). Combining dates, plots containing metaphyton averaged 40.71 ± 11.35 snails 100 cm^{-2}, whereas plots without metaphyton averaged 13.33 ± 2.16 snails 100 cm^{-2}. Despite this density difference, snail biomass did not significantly differ among treatments and sample dates (RM ANOVA; treatment effect: $F_{3,11} = 0.938$, $P = 0.455$; date effect: $F_{2,22} = 1.345$, $P = 0.281$) (Fig. 3C).

Detritus and epiphyton

BOM dry mass did not differ among treatments or among sample dates (RM ANOVA: treatment effect: $F_{3,14} = 1.347$, $P = 0.299$; date effect: $F_{2,28} = 1.051$, $P= 0.363$), even though detrital samples from AquaNeat®-treated plots were particularly large on 24 June and 8 July (Fig. 4). Likewise, chl *a* concentrations did not significantly differ among treatments (RM ANOVA: $F_{3,14} = 1.005$, $P = 0.419$), but did progressively decline with sample date ($F_{2,28} = 1.082$, $P = 0.008$) (Fig. 5A). In contrast, epiphyton densities were similar on sampling dates (RM ANOVA: $F_{3,15} = 1.498$, $P = 0.240$) but marginally different among treatments ($F_{3,15} = 3.277$, $P = 0.050$). Densities were slightly higher in

Typha plots than control plots (Bonferroni comparison, $P = 0.060$) mostly due to high densities on 24 June (Fig. 5B). Shannon-Wiener diversity indices were similar across treatments and dates (RM ANOVA; treatment effect: $F_{3,16} = 2.029$, $P = 0.153$, date effect: $F_{3,16} = 2.074$, $P = 0.170$) (Fig. 5C).

Diatoms dominated the algal communities in treatments (Fig. 6). On 24 June, the most abundant taxa consisted of three chlorophytes (*Oedogonium* sp., *Spirogyra* sp. *Ulothrix zonata*), and seven diatoms (*Aulacoseria sp., Fragilaria* sp., *Gomphonema* sp. *Navicula sp., Nitzschia sp.,* unidentified pennate diatoms, *Synedra sp.*), whereas on 8 July the dominant taxa consisted of two chlorophytes (*Oedogonium* sp., *Scenedesmus quadricauda*) and eight diatoms (*Aulacoseria sp., Cyclotella meneghiniana, Gomphonema parvulum, Fragilaria* sp., *Navicula confervacea, Navicula sp., Nitzschia sp*., unidentified pinnate diatoms) (Table 1). Rankings of most abundant algal assemblages were similar among treatments on both 24 June (Kendall's concordance, $W = 0.886$, $P < 0.001$) and 8 July ($W = 0.833$, $P < 0.001$). NMDS generated a high stress value (stress = 13.17, 1-dimensional solution) for algal assemblages on 24 June, thus I did not use the analysis for this date. NMDS for 8 July (stress = 6.46, 2-dimensional solution) included 11 species (2 chlorophytes, 9 diatoms) and showed no clear separation of algal assemblages among treatments (Fig. 7), suggesting algal communities were similar among treatments.

Physical conditions

Typha and control plots had significantly less light penetration than herbicide-treated plots (RM ANOVA: $F_{3,16} = 34.431$, $P < 0.001$; all Bonferroni comparisons, $P < 0.001$) (Fig.8). *Typha* and control plots extinguished similar amounts of light (Bonferroni comparison, $P = 0.190$), and both plot types extinguished significantly more light on the second sample date than on the first (RM ANOVA: $F_{1,16} = 5.265$, $P = 0.036$). Light penetration was marginally negatively related to chl *a* levels, but not diatom densities (chl *a*: $r = –0.439$, $P = 0.060$; diatom density: $r = –0.278$, $P = 0.235$). Light penetration also apparently affected water temperature. Water temperatures differed among treatments ($F_{3,16} = 11.915$, $P < 0.001$; Table 2) and were higher in AquaNeat®-treated plots than in control and *Typha* plots (all Bonferroni comparisons, $P < 0.05$). Temperatures were also higher in Habitat®-sprayed plots than *Typha* plots (Bonferroni comparison, $P = 0.001$). Dissolved oxygen did not vary among treatment types (RM ANOVA: $F_{3,16} = 0.285$, $P= 0.836$), but varied widely across sampling dates ($F_{3,48} = 58.310$, $P < 0.021$). Water depths in plots declined with sample date ($F_{3,48} = 19.856$, $P < 0.001$) but did not vary among treatments (RM ANOVA: $F_{3,16} = 62.501$, $P = 0.170$).

Discussion

Vegetation type and herbiciding had a time-varying impact on snail communities. Relative frequencies of snail species were similar among treatments on 24 and 30 June, but varied among treatments on 8 July. Specifically, *Fossaria* spp. and *G. parvus* were more abundant in herbicide-treated plots than in untreated reed and cattail plots. Moreover, snail densities were generally higher in herbicide-treated plots than in control and *Typha* plots. However, snail *H'*-diversity and overall biomass remained similar among treatments across sample dates. Bickel and Closs (2009) similarly found that mollusk abundance, but not taxon richness and biomass, significantly increased when physical habitat structure in a lake littoral zone was altered by cutting the invasive macrophyte *Lagarosiphon major* (oxygen weed). Kulesza et al. (2008) found that snail densities were greater in stands of *Phragmites* treated with glyphosate ~3 months earlier than in untreated *Typha* stands, and attributed this density difference to greater diatom food supplies on standing reed stems. They suggested that phytotoxic leachates from live *Typha* stems produced an allelopathic effect that reduced epiphyton abundance relative to reed stems. In contrast, I found that densities of diatom-dominated epiphyton were marginally greater on *Typha* than on reed stems, suggesting once submerged, decaying *Typha* stems lose their allelopathic effect.

So, why were snail densities greater in herbicide-treated than in herbicide-free plots? As decaying submerged macrophyte leaves enter the detrital pool, density of snails are likely to increase (Menédez 2005), presumably from greater detrital food supplies and substrate availability for epiphyton colonization (Warada 2002). However, I found detrital biomass to be similar among treatments. Rather, metaphyton abundance was generally greater in herbicide-treated plots than in control and *Typha* plots. Coincidently, many juvenile and small snails were found in metaphyton mats, which might explain similarities in snail biomass in treatments. Structurally complex metaphyton can harbor rich diatom-dominated epiphyton (Burkholder 1996), trap fine BOM (Holomuzki and Short 1990), and provide macroinvertebrate prey a refuge from predators (Dewitt et al. 1999). Indeed, snails <10 mm are particularly vulnerable to crayfish predation (Dewitt et al. 1999), whereas snails <4 mm are vulnerable to fish predation (Lodge et al 1994). Moreover, metaphyton presence was linked to herbiciding-caused increases in light penetration; an expected result given green algal abundance is positively linked to light availability (Pillsbury and Lowe 1999, Whitcraft and Levin 2007). Another consequence of increased light penetration was significantly warmer water temperatures in herbicide-treated than in untreated plots. Warmer temperatures, to a point, can speed snail growth (Aufderheide et al. 2006), expedite reproduction (Jigyasu and Singh 2010), and enable an individual to sooner reach a potential refuge from some predators (Alexander and Covich 1991, Auld 2008). Thus, herbiciding leads to dramatic changes in habitat conditions caused by an increase in toppled stems and greater light penetration, which increases habitat suitability for gastropods (Bickel and Closs 2009, Liess et al. 2009).

All snails found in treatments were pulmonates belonging to the families Lymnaeidae (*Fossaria* spp., *Stagnicola* sp.), Physidae, and Planorbidae (*Promenetus umbilicatellus*, *Gyraulus parvus*, *Helisoma* sp.). Multiple species of physids were found but were identified only to genus due to phylogenetic disagreements in the literature (e.g. Pip and Franck 2008) and difficulty with identification from only morphological characteristics (U.S. EPA 1982). It is likely that two *Fossaria* species, *F. obrussa* and *F. modicella*, were present in plots. All species collected feed on both detritus and algae, specifically diatoms, but diet and food preferences may vary slightly between families, species body sizes, and ontogenetic stages within species (Kesler et al. 1986, Dillon 2000). For example, juvenile planorbids feed heavily on small diatoms and detritus, whereas adults feed more on large diatoms and blue-green algae (Dillon 2000). Physids generally feed on detritus, attached bacteria and algae (Kesler et al. 1986) and have been observed consuming *Phragmites* detritus (Bayo et al. 2005). Juvenile lymnaeids seem to prefer small diatoms, whereas adults typically consume larger diatoms, other microalgae, and have a strong preference for carrion (Kesler et al. 1986, Dillon 2000). Overall, most snails generally consume benthic biofilms rather than shred actual plant matter.

Detrital and epiphytic food not associated with metaphyton may still have played some role in promoting higher snail densities in herbicide-treated plots. *Phragmites* is relatively unpalatable to many detritivores due to its high fiber content and sclerenchymatous outer tissues (Armstrong 1996). Furthermore, *Phragmites* stems also contain high levels of Acid Detergent Lignin (ADL), which may limit microbial decomposer activity, and hence slow conditioning, making stems less palatable to detritivores (Ágoston-Szabó and Dinka 2008). However, early necrosis from herbiciding may have expedited microbial colonization and litter degradation, and thus increased palatability. Moreover, when allowed to decompose >1 year, similar to the timeframe in my study, submerged leaves of *Phragmites* decompose faster than leaves of *Typha* (Mason and Bryant 1975, Warren et al. 2001), which may facilitate detritivory. Thus, overall detrital quality may have affected snail abundance patterns in treatments. In addition, nutrient-rich diatoms were the most abundant epiphyte, and algal densities were highest in *Typha* plots. It is possible that increased grazing pressure by snails may in part account for lower algal densities in herbicide-treated plots. Still, algal densities and chl *a* levels were relatively low in all treatments; a surprising result given Sheldon is a eutrophic marsh. I suspect low and widely fluctuating water levels are responsible for low algal abundance across treatments. Lake Erie seiches coupled with already low water levels in the marsh and lake likely temporarily exposed some benthic habitats in plots to air (M. Grote, OH Department of Natural Resources, personal communication), and temporary drying can significantly stunt algal growth (Albay and Akcaalan 2003).

Of the top 10 most epiphyton abundant taxa, *Cyclotella, Fragilaria, Melosira varians, Navicula, Nitzchia, Synedra* and several species within the genus *Gomphonema* have a high tolerance to eutrophication in lakes and streams (Kelly and Whitton 1995, Wehr and Sheath 2003). Likewise, all snails found in sample plots were pulmonates, which can survive temporary dry-outs, low oxygen levels, and eutrophication (Strong et al. 2007). The high abundance of eutrophication-resistant diatoms and desiccation-resistant snails indicates that nutrient and water levels are primary determinants of community assemblages in Sheldon Marsh and in other Lake Erie coastal wetlands like it. Moreover, oxygen levels can drop to ~1 mg/L at night in reed beds in these systems (J. Holomuzki, unpublished data), which could be a key factor affecting snail diversity assemblage.

Wetland managers often seek to control reed spread because of negative impacts to wetland plant diversity and migratory bird habitats, as well as increased fire risks to marshes located near residential areas (Marks et al. 1994, Meyerson et al. 200). With no control program, reed often quickly spreads (Back and Holomuzki 2008), and may eventually dominate entire emergent zones. Herbiciding as a management technique is both inexpensive (Back and Holomuzki 2008) and has relatively little direct impact on benthic communities (Kulesza et al. 2008). However it is important to determine unintended indirect ecological consequences to large-scale herbicide treatments to provide resource managers guidance for the restoration of reed-invaded marshes. In this study, I investigated the indirect impacts of herbicide application to epiphyton and gastropod communities. My results suggest that both glyphosate- and imazapyr-containing herbicides have little short-term effect on the herbivore-producer relationship and gastropod diversity in Sheldon marsh. Studies are needed that compare habitat conditions and benthic community structure in reed stands treated with different herbicides and concentrations at different application times (e.g., early summer vs. late summer), as well as investigations on more long-term impacts of application. I studied benthic communities 1- year after herbicide application, however, complete stem collapse may not occur for 2-years post-herbiciding. As stems continue to collapse, both BOM and metaphyton biomass may increase, further altering habitat structure. My study did not account for BOM time of submergence, which could have masked temporal variation in epiphyton attachment and successional patterns in epiphyton community structure. The impact of detritus age, stem type, time of submergence, and subsequent impacts on epiphyton communities should be studied to better understand the implications of detrital amount and quality on benthic community organization in these freshwater coastal wetlands.

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Appendix A

Tables and Figures

		% Relative Abundance			
Date	**Taxa**	**Control**	**AquaNeat®**	**Habitat®**	***Typha***
24 June	*Aulacoseira* sp. (d)	16.40	19.33	17.92	27.44
	Unidentified Pennate Diatoms	10.81	14.31	15.07	23.04
	Oedogonium sp. (g)	7.68	2.63	29.29	8.25
	Nitzschia sp. (d)	6.94	7.59	9.16	6.92
	Navicula sp. (d)	4.50	12.99	6.17	6.14
	Synedra sp. (d)	9.12	3.78	4.20	3.10
	Spirogyra sp. (g)	0.00	8.06	1.89	0.00
	Ulothrix zonata (g)	0.00	2.41	0.55	3.44
	Fragilaria sp. (d)	1.02	3.45	0.00	2.28
	Gomphonema sp. (d)	2.85	3.75	1.75	0.30
8 July	*Aulacoseira* sp. (d)	27.56	17.88	26.15	31.56
	Unidentified Pennate Diatoms	21.29	19.75	17.52	11.86
	Navicula sp. (d)	14.03	8.63	10.57	6.93
	Nitzschia sp. (d)	5.58	10.37	3.70	6.00
	Oedogonium sp. (g)	6.90	0.85	10.30	0.70
	Gomphonema parvulum (d)	2.14	5.86	5.36	3.85
	Cyclotella meneghiniana (d)	2.45	3.62	3.78	5.68
	Scenedesmus quadricauda (g)	0.61	2.52	1.01	8.54
	Fragilaria sp. (d)	1.17	7.69	0.00	0.00
	Navicula confervacea (d)	4.21	0.00	1.44	2.61

Table 1. Relative abundance (%) of the ten most abundant algae (d= diatom; g = green alga) in each treatment on 24 June and 30 July.

Date	**Treatment**	**Depth (cm)**	**DO (mg/L)**	**Temp (˚C)**
24 June	Control	18.24 ± 2.40	29.80 ± 11.25	26.54 ± 0.53
	AquaNeat ®	13.80 ± 1.68	51.68 ± 18.90	27.88 ± 0.55
	Habitat ®	17.20 ± 0.90	40.94 ± 14.88	28.34 ± 0.51
	Typha	18.90 ± 1.94	29.66 ± 13.58	25.46 ± 0.33
30 June	Control	19.48 ± 2.62	39.92 ± 9.52	21.96 ± 0.24
	AquaNeat ®	18.56 ± 0.85	67.6 ± 6.22	22.06 ± 0.19
	Habitat ®	20.56 ± 3.35	50.94 ± 5.48	21.98 ± 0.17
	Typha	19.06 ± 1.10	14.86 ± 2.05	21.68 ± 0.07
8 July	Control	11.80 ± 2.27	10.3 ± 0.62	27.26 ± 0.88
	AquaNeat ®	10.70 ± 1.56	27.36 ± 11.89	30.52 ± 0.86
	Habitat ®	9.36 ± 1.44	13.26 ± 2.37	29.52 ± 1.09
	Typha	16.10 ± 1.13	11.28 ± 0.99	24.44 ± 0.10
15 July	Control	11.32 ± 1.69	21.26 ± 3.07	24.24 ± 0.63
	AquaNeat ®	13.50 ± 1.87	17.48 ± 4.80	26.76 ± 1.00
	Habitat ®	13.58 ± 1.26	27.5 ± 6.69	25.82 ± 0.53
	Typha	18.88 ± 0.41	9.9 ± 1.04	22.88 ± 0.23

Table 2. Average (± 1 SE) water depth (cm), dissolved oxygen (DO; mg/L), and temperature (˚C) for each experimental treatment across 4 sampling dates in 2008.

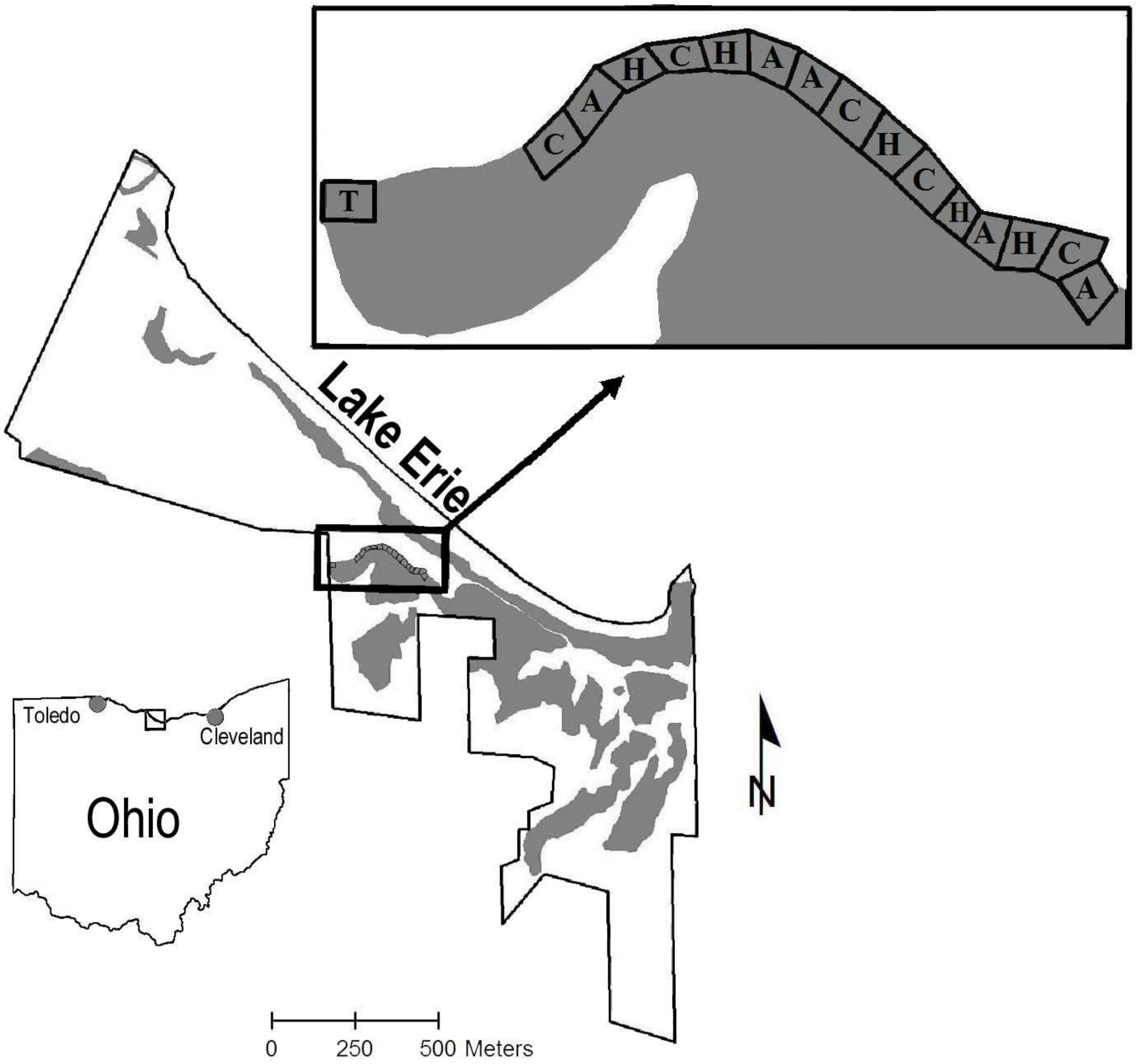

Figure 1. Sheldon Marsh Nature Reserve in Huron, Ohio. Gray patches represent the emergent plant zone. Sampling plots are labeled as C= Control (Unsprayed *Phragmites*), A=AquaNeat®, H=Habitat®, T=Unsprayed *Typha*. The rectangle demarcating *Typha* contains the five sampling plots.

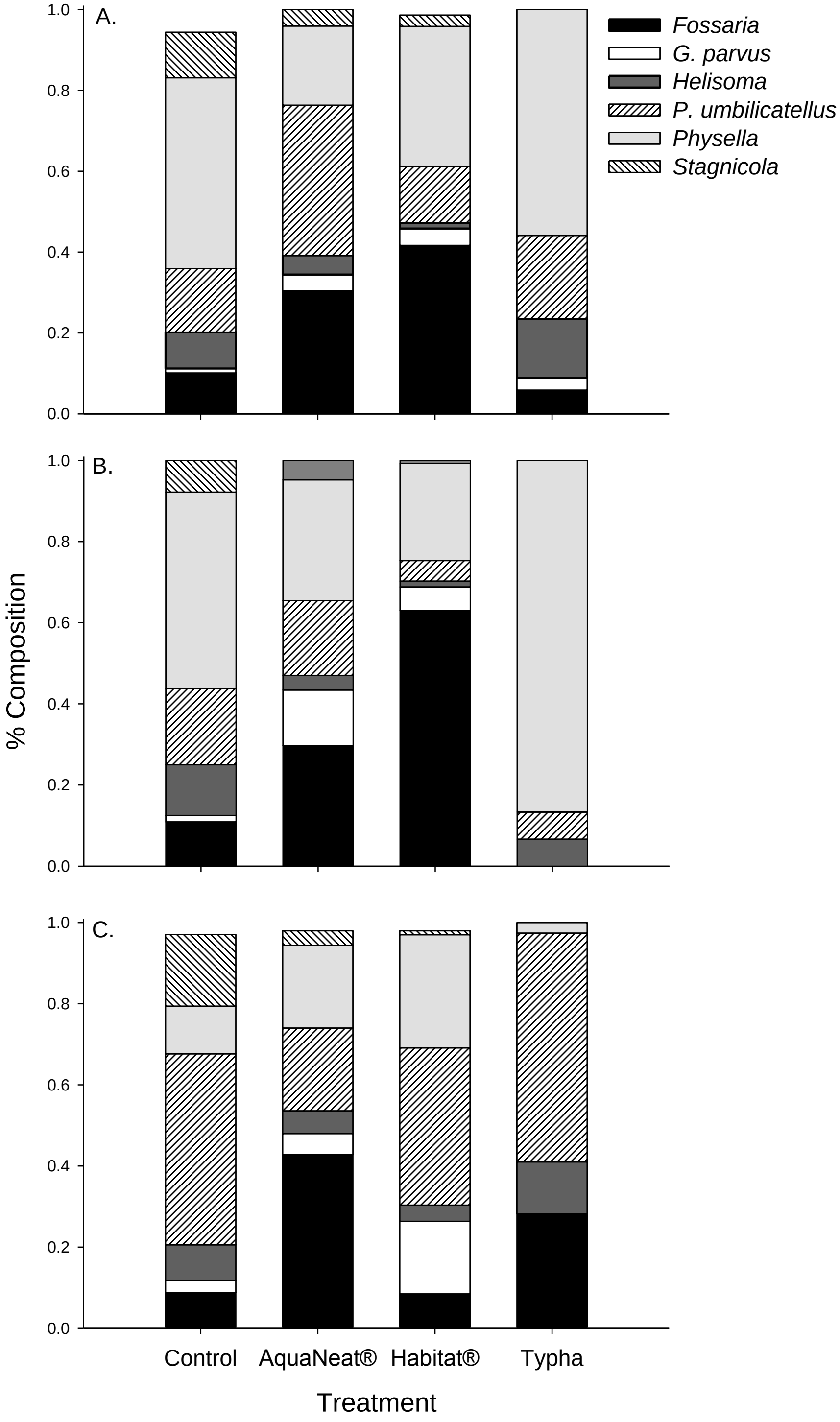

Figure 2. Mean composition (%) of common snail species found in each treatment for A) 24 June B) 30 June and C) 8 July 2008.

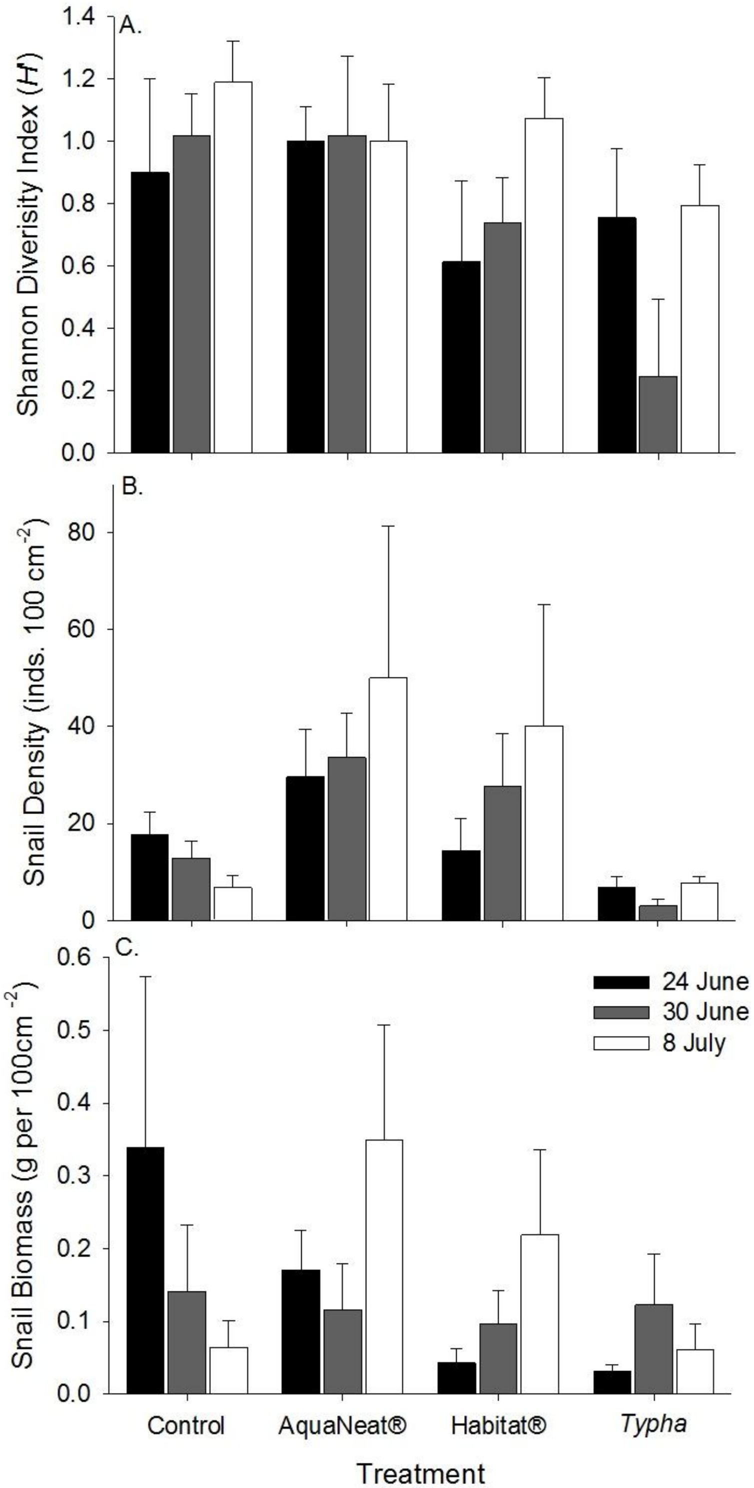

Figure 3. Mean (± 1 SE) A) Shannon-Weiner Diversity Indices (*H'*) B) densities (individuals 100 cm^{-2}) and C) dry masses (g 100 cm^{-2}) of snails in the four treatments on each sampling date.

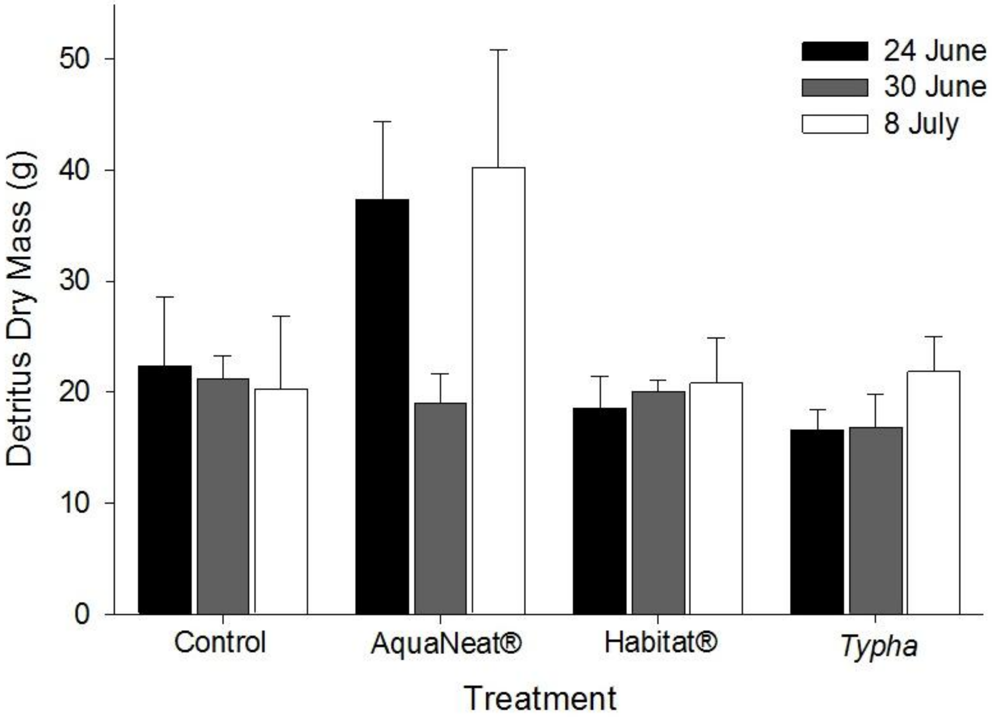

Figure 4. Mean (± 1 SE) dry mass (g) of BOM in each treatment on each sample date.

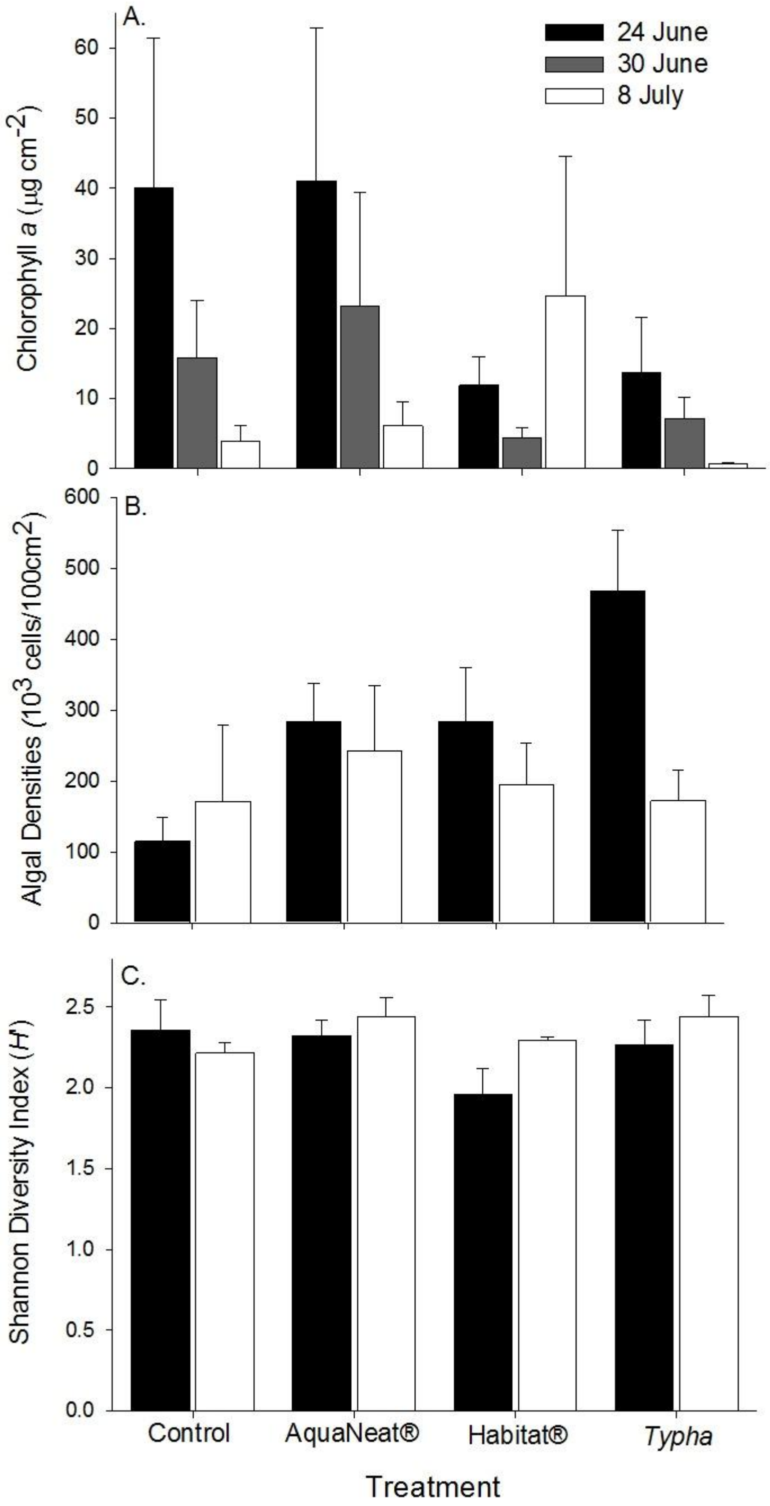

Figure 5. Mean (± 1 SE) A) chlorophyll *a* levels (µg cm^{-2}) on 24 and 30 June and 8 July, and B) densities (103 cells per 100 cm^{-2}), and C) Shannon-Weiner diversity indices (*H'*) of epiphytic algae on 24 June and 8 July.

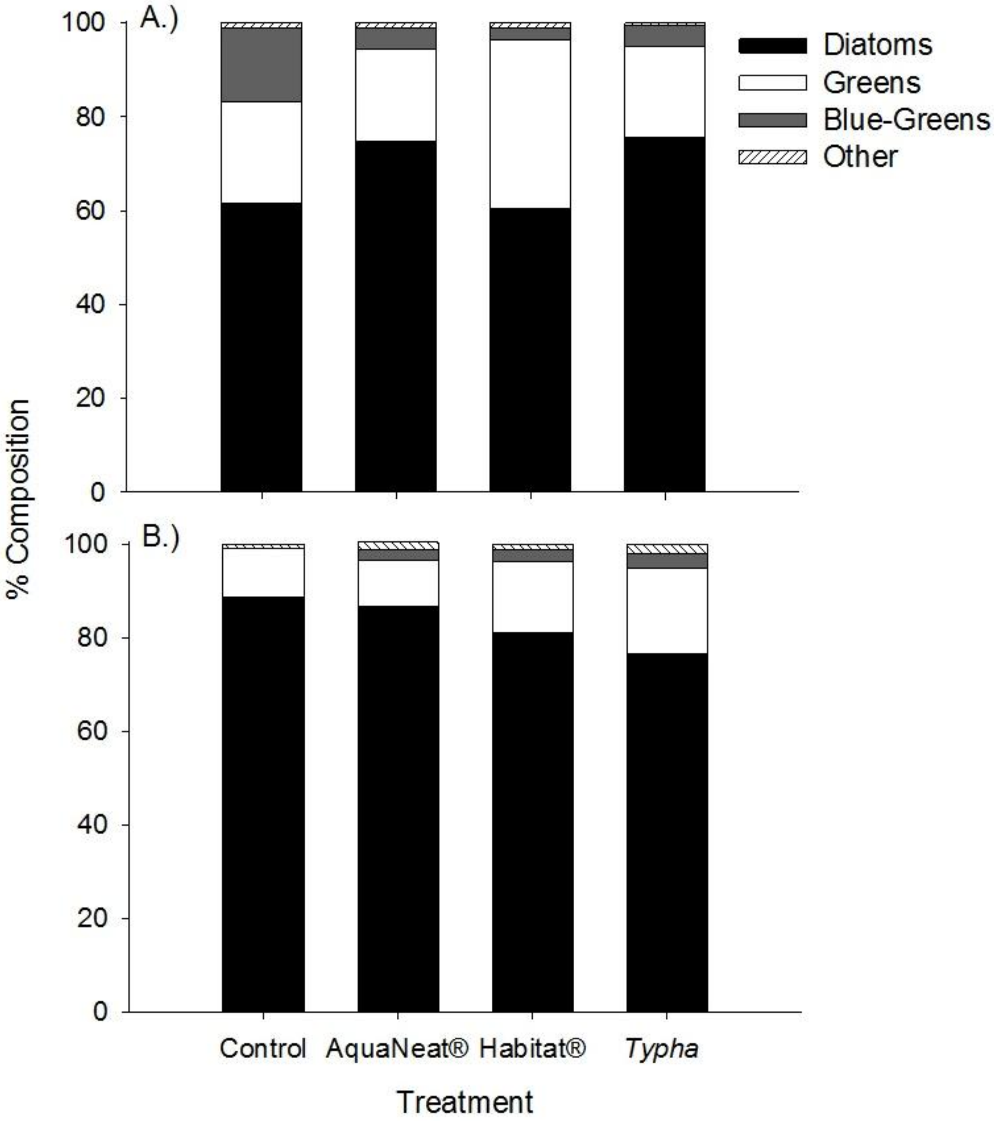

Figure 6. Algal composition in the four treatments on A) 24 June and B) 8 July. "Other" includes all other algae sampled, which predominately includes the groups Cryptophyta and Euglenophyta.

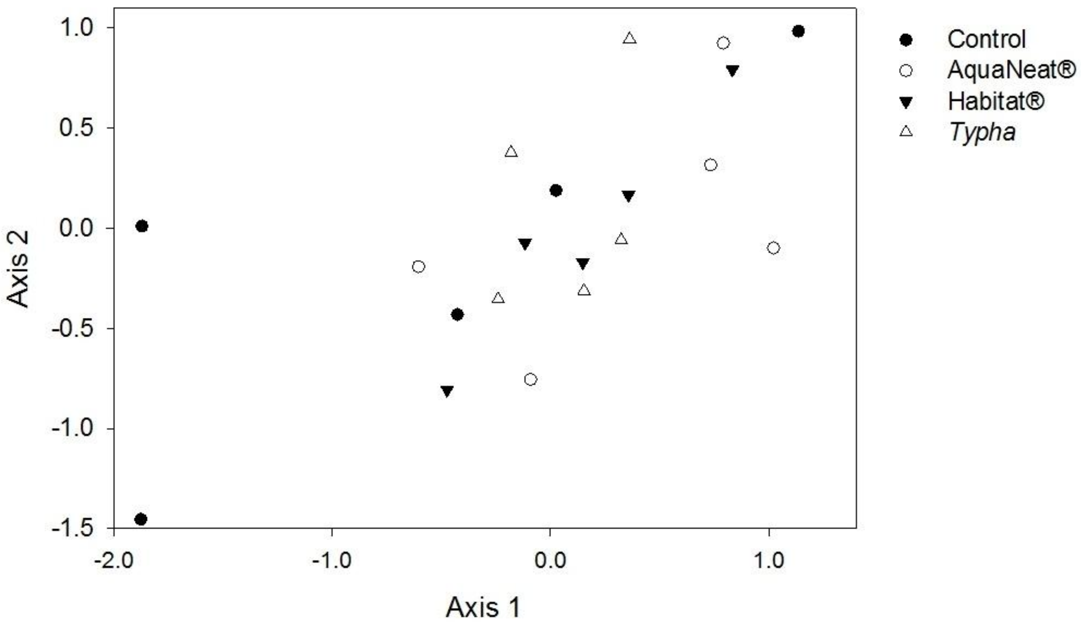

Figure 7. Non-metric multidimensional scaling ordination of algal assemblages from treatment plots on 8 July. Dominant epiphytes present in >75% of plots were examined.

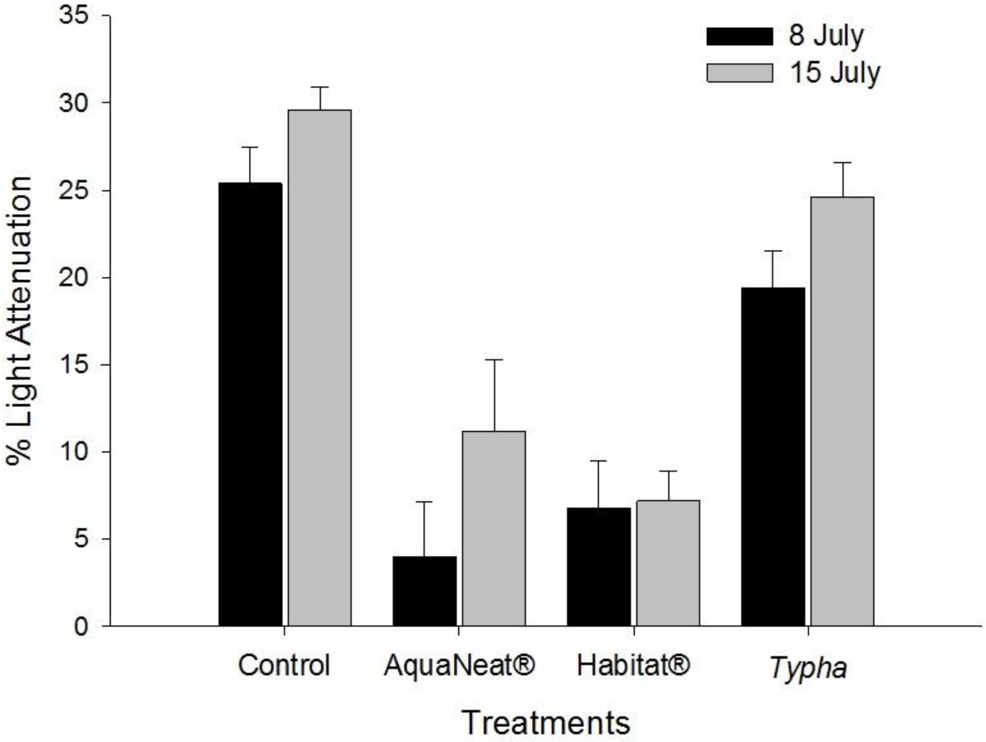

Figure 8. Mean (± 1 SE) light attenuation (%) for all treatments 8 July and 15 July.