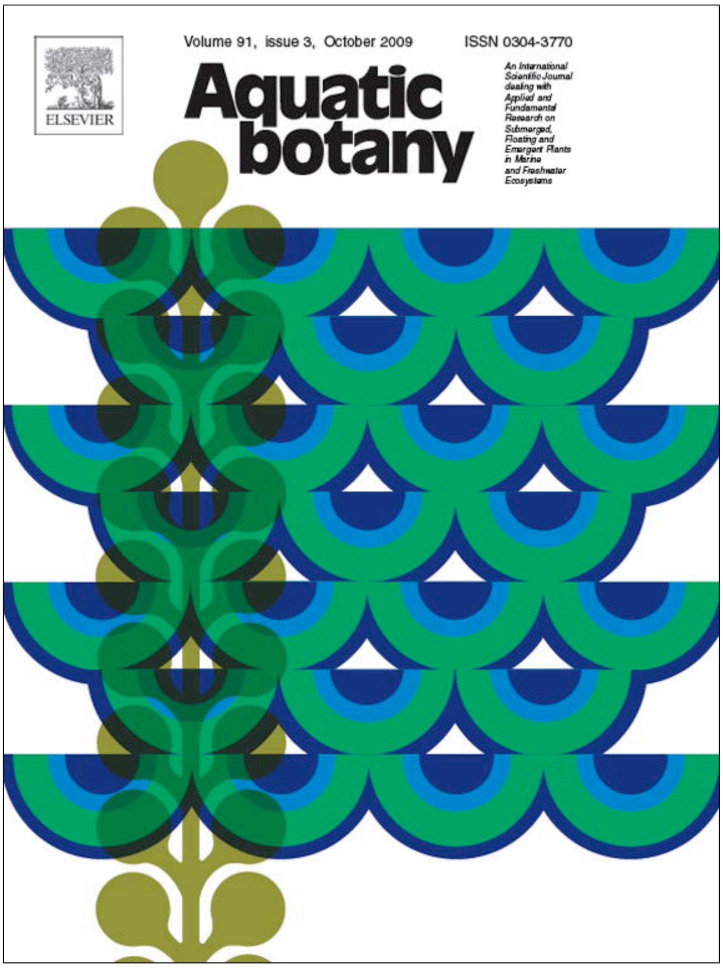
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Short communication

The effects of nitrate loading on the invasive macrophyte *Hydrilla verticillata* and two common, native macrophytes in Florida

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ABSTRACT

Rising nitrate concentrations in the water column and the spread of invasive, non-native macrophytes are two major threats to Florida's oligotrophic, freshwater ecosystems. We used a replicated mesocosm experiment to test the effects of elevated nitrate concentrations in the water on the growth of the invasive macrophyte *Hydrilla verticillata* and two common, native submerged macrophytes *Vallisneria americana* and *Sagittaria kurziana*. Results from this study indicate that nitrate concentrations of 1.0 mg L^{-1} NO_3-N in the water increased the final dry-weight biomass of *H. verticillata* by 2.75 times, while having no statistical effect on the growth of the two native species. Additionally, *H. verticillata* grew at a faster rate than the two native species in the low nitrate treatments accounting for 82% of the total biomass, indicating that it may have the capacity to invade relatively pristine communities. In waters where nitrate concentrations continue to rise, the cost of control efforts for *H. verticillata* may substantially increase in the future.

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1. Introduction

Rising nitrate concentrations and the spread of invasive species have been considered leading threats to Florida's oligotrophic, freshwater ecosystems (Langeland, 1996; Bacchus and Barile, 2005; Hoyer et al., 2005). The major source of freshwater for many of these communities is the Floridan aquifer which has been experiencing a rise in nitrate concentrations from non-point anthropogenic sources, including septic tanks, fertilizers, and sewage treatment spray-fields (Katz et al., 1999; Stevenson, 2000; Bacchus and Barile, 2005). Over the last 40 years, nitrate concentrations in the Floridan aquifer in some regions have steadily increased from historical concentrations which averaged $<$0.2 mg L^{-1} NO_3-N (Scott et al., 2004; Hoyer et al., 2005) to as much as a five-fold increase in nitrate concentrations in 13 first magnitude springs (average discharge $>$2800 L s^{-1}) where concentrations are now averaging near or greater than 1.0 mg L^{-1} NO_3-N (Katz et al., 1999; Stevenson, 2000; Scott et al., 2004; Bacchus and Barile, 2005).

Freshwater, oligotrophic communities may become degraded when nitrate concentrations approach or exceed 1.0 mg L^{-1} NO_3-N (Stevenson, 2000). For example, changes in limiting nutrients may be a key driving force for plant invasions (Elton, 1958; Romanuk and Kolasa, 2005). Some invading species are more capable than native species of increasing their growth rates in response to rising nutrient concentrations and can thus out-compete the latter under eutrophic conditions (Burke and Grime, 1996; Vitousek et al., 1997; Van et al., 1999; Davis et al., 2000). Competitors that are capable of higher growth rates may "crowd-out" and displace slower growing, native species.

Hydrilla verticillata (family: Hydrocharitaceae) was first introduced to Florida in the late 1950s and within 40 years spread to over 280 water bodies, covering over 56,600 ha (Langeland, 1996; Hoyer et al., 2005). Between 1992 and 1994 alone, *H. verticillata* infestations doubled in size from about 20,000 to 40,000 ha, requiring nearly $14 million for management (Schardt, 1997). As of 2005, *H. verticillata* still covered approximately 44,000 ha in 186 water bodies (Hoyer et al., 2005). *H. verticillata* has been called the "perfect aquatic weed" and has spread throughout the southeast as far north as the Potomac River, MD (Langeland, 1996). It is capable of growing 2.5 cm-day and has been known to form dense monotypic stands, reducing native vegetation and biodiversity

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(Langeland, 1996; Van et al., 1999). Changes in the physical properties of water including flow velocity, conductivity, turbidity, and dissolved oxygen have been associated with the presence of *H. verticillata* (Langeland, 1996; Van et al., 1999). Small fragments of *H. verticillata* containing only a single node are capable of growing in the water column and rooting in the substrate, and serve as the main vector for introductions. Previous studies by Van et al. (1999) have demonstrated that *H. verticillata* can out-compete native species under conditions of elevated nutrients in the sediment. Economically and environmentally, *H. verticillata* has been one of the most serious aquatic, exotic weed problems in the U.S. in the past 50 years.

Previous studies involving the effects of nutrient enrichment on the competitive ability of *H. verticillata* and other aquatic macrophytes have focused on manipulating nutrient concentrations in the sediment (McFarland et al., 1992; Sutton, 1993; Spencer and Ksander, 1995; Van et al., 1999; Mony et al., 2007; Thomaz et al., 2007) because nutrients in the sediment can positively affect growth rates (Barko and Smart, 1986). For example, Xie et al. (2005) demonstrated that nutrients in the soil were more important than nutrients in the water column for affecting the growth of *Vallisneria natans*. However, it is also necessary to understand how fast growing, non-native macrophytes may be affected by changes in nitrate concentrations in the water column (Shardendu-Ambasht, 1991). This is especially true for oligotrophic water's where dissolved nutrient concentrations have steadily increased.

For this study, we used replicated mesocosms to test whether elevated nitrate concentrations in the water column will affect the growth rates of three macrophytes; an invasive species *H. verticillata*, and two native species *Sagittaria kurziana* and *Vallisneria americana*. This is one of the first studies to investigate the effects of elevated nitrates in the water column on the growth rates of native and non-native macrophytes and will increase our understanding of the factors that may increase the susceptibility of oligotrophic, freshwater communities to invasions by non-native species.

2. Methods

2.1. Study organisms

Three macrophyte species were used for this experiment. The two native species, *V. americana* (family: Hydrocharitaceae) and *S. kurziana* (family: Alismataceae) were selected because they are common in many oligotrophic water bodies throughout the southeast USA. They are both rooted, aquatic perennials that can reproduce sexually. *S. kurziana* and *V. americana* produce long ribbon-like leaves and are capable of forming large, dense stands (Godfrey and Wooten, 1979). The third species, *H. verticillata* is a widespread invasive species that is capable of sexual and asexual reproduction. The macrophytes used for this study were collected from the Wacissa River (Jefferson County, Fl: 30°12′11.50″ N, 83°58′09.5″ W) in April 2003. The Wacissa River is relatively pristine and is fed by a series of small springs, with current nitrate concentrations averaging less than 0.3 mg L^{-1} (Stevenson, 2000).

2.2. Experimental design

For this study, 24 replicated mesocosms were used in a two-by-two factorial design at the Blandy Experimental Farm (Clarke County, VA) to test the growth of *V. americana* and *S. kurziana* in the presence or absence of *H. verticillata* in two nitrate concentrations. Each mesocosm was considered an independent experimental unit (Wilbur, 1987; Drake et al., 1996) and randomly assigned one of two nitrate treatments (low or high) and one of two *H. verticillata* treatments (present or absent) for a total of six replicates per treatment. The historical concentration of 0.2 mg L^{-1} NO_3-N was selected for our low (control) nitrate treatment and the current day nitrate concentrations for several natural springs of 1.0 mg L^{-1} NO_3-N were selected for our high nitrate treatment.

2.3. Establishment and initiation of experiment

Mesocosms were galvanized steel tanks, with 1.8 m^2 in surface area, a depth of 0.6 m, and a total volume of approximately 1100 L. Approximately 20 cm of washed, white sand was placed on the bottom and used to mimic the natural substrate of the native macrophytes. Well-water from a karst aquifer that had similar properties to the Floridan aquifer in terms of pH (7.2–7.5) and mineral content was the water source for the experiment. Nitrate and phosphorous concentrations at the start of the experiment were approximately 0.2 mg L^{-1} NO_3-N and 0.04 mg L^{-1} P, which was similar to historical oligotrophic nutrient conditions in Florida. Prior to planting, invertebrates were removed from the macrophytes. To ensure equal biomass and plant height at the beginning of the experiment, 30 individuals of each species were trimmed to a height of 10 cm (15.2 $\pm$ 2.1 mg dry wt $plant^{-1}$ for *V. americana* and 11.8 $\pm$ mg dry wt $plant^{-1}$ for *S. kurziana*). One-half of each mesocosm was randomly planted with *S. kurziana* and the other half with *V. americana*. Both native macrophytes were planted in the mesocosms on 22 April 2003 and given two months to establish before initiating the experiment.

The experiment began on 22 June 2003 with the addition of *H. verticillata*. *H. verticillata* is typically spread by the introduction of floating fragments. To mimic this process, 100 g wet wt of *H. verticillata* was added to each mesocosm to approximate the actual biomass and area covered by the native macrophytes. The fragments included various cut sections and apical stems. They were not planted, but were spread evenly around each mesocosm and were allowed to root naturally, mimicking introduction and rooting in a novel water body.

Since discharge from the Floridan aquifer would maintain relatively constant nutrient concentrations in a community, nitrate and phosphate concentrations were monitored every seven days using a spectrophotometer and sampling assays (CHEMetrics Inc., Calverton, VA) to maintain constant concentrations. Nitrate concentrations were maintained throughout the experiment at 0.2 mg L^{-1} NO_3-N for the low treatment and at 1.0 mg L^{-1} NO_3-N for the high treatment, by adding sodium nitrate ($NaNO_3$), as needed. Phosphorous concentrations were maintained at natural, historical concentrations of 0.04 mg L^{-1} P by the addition of water-soluble fertilizer. Micronutrients (including, potassium, iron, and magnesium) were added once every 10 days. Water was added to the tanks every two days to offset evaporative loss and to ensure nutrient mixing.

2.4. Data collection and statistical analysis of macrophytes

On 26 September 2003, all macrophytes were removed from the mesocosms and placed in a drying oven at 63 °C for eight days. Dry-weight biomass was used to estimate the growth of each species. In order to examine the combined effects of nitrates and the presence/absence of *H. verticillata* on the growth (final, dry-weight biomass) of the native plants (*S. kurziana* and *V. americana*), we performed a fixed-effect split-plot factorial ANOVA. Nitrate (low and high) and *H. verticillata* (present or absent) treatments were regarded as whole-plot effects since they were randomly assigned to each mesocosm. The species comparison (*S. kurziana* versus *V. americana*) was treated as a subplot effect since both species were assigned to each mesocosm. All pairwise and three-way interactions were included in the model. SAS proc mixed (Littlell et al.,

Table 1
Results from the mesocosm experiment indicating the final dry-weight biomass (g) of each species to the high and low nitrate treatments. The final dry-weight biomass (g) of the two native species, *S. kurziana* and *V. americana* in the presence and absence of *H. verticillata* is also reported.

Treatment	Species		
	Sagittaria kurziana	*Vallisneria americana*	*Hydrilla verticillata*
Hydrilla verticillata: present			
High	26.4 ± 7.9	28.5 ± 9.6	547 ± 20*
Low	19.2 ± 3.6	17.4 ± 3.2	199 ± 39*
Hydrilla verticillata: absent			
High	28.9 ± 9.4	26.2 ± 6.1	
Low	35.2 ± 7.9	5.4 ± 5.3	

The initial biomass for each species was <1% of the final biomass. Means are reported with one standard error. Nitrate treatments were one of two concentrations; high treatments were 1.0 mg L^{-1} and low treatments were 0.2 mg L^{-1}.
* Significant difference between the treatments ($P < 0.05$).

2007) was used to analyze the split-plot model using log-transformed biomass of each native species from each mesocosm as the dependent variable. Log-transformed data had homogeneous variances based on an *F*-max test and satisfied the ANOVA assumption of normality. Log-transformed biomass of *H. verticillata* from the low and high nitrate treatments was compared using a one-way ANOVA using data only from those 12 mesocosm inoculated with *H. verticillata*. All analyses were performed using the SAS general linear model procedure (proc glm) (SAS Institute, 2001).

3. Results

All of the macrophytes grew substantially during the course of the experiment. The initial wet-weight biomass of each species was less than 1% of their final dry-weight biomass. Both *S. kurziana* and *V. americana* increased in the total number of plants, spreading to cover most of the area of each mesocosm with their leaves often reaching the water's surface. In all cases, *H. verticillata* rooted in the sandy substrate and grew to reach the water surface. However, turions were not produced during the course of this study.

The two native species did not differ significantly from one another in final biomass (Table 1), with means for *S. kurziana* and *V. americana* across all treatment averaging 22.4 g and 20.4 g, respectively. The biomass of the native species did not significantly differ between low and high nitrate treatments ($F_{1,23} = 0.43$, $P = 0.52$). However, the final biomass of the native macrophytes was lower in the presence of *H. verticillata*, the results were not significant ($F_{1,23} = 0.05$, $P = 0.83$; Table 1). The lack of significant two-way ($F_{1,23} = 0.77$, $P = 0.39$) and three-way ($F_{1,23} = 0.14$, $P = 0.72$) interactions indicated that each of the native species responded similarly to both *H. verticillata* and nitrate concentrations.

H. verticillata differed significantly in biomass based on the nitrate treatment ($F_{1,10} = 62.9$, $P < 0.0001$; Table 1). The mean dry-weight biomass of *H. verticillata* was 2.75 times higher in the high nitrate treatment. Additionally, it became the dominant species based on biomass, accounting for 82 ± 3% and 91 ± 2% of the total, final biomass in the low and high treatments, respectively.

4. Discussion

Nitrate enrichment in the water column had differential effects on the macrophytes used in this study. Perhaps most notable was the lack of any significant difference in growth of the two native macrophyte species, regardless of the nutrient treatment, indicating the reliance on nutrients in the sediments for growth, as demonstrated by previous studies (Barko and Smart, 1986; Sutton, 1993; Van et al., 1999; Mony et al., 2007; Thomaz et al., 2007). However, *H. verticillata* grew faster in higher nitrate concentrations. These findings are similar to other studies involving other species indicating the generalizable point that non-native species often have accelerated growth rates with increased nutrient loading when compared to native species (Burke and Grime, 1996; Hofstra et al., 1999; Van et al., 1999). However, *H. verticillata* also grew at a much faster rate than the native plant species, even in low nutrient conditions.

The results from this study differ from other important studies investigating the growth of macrophytes under conditions of elevated nutrients. For example, Van et al. (1999) showed that *H. verticillata* was actually out-competed by *V. americana* under conditions of lower nutrients in the sediment. Although, the properties of sediments are very important for the growth of macrophytes (Barko and Smart, 1986; McFarland et al., 1992), nitrate concentrations are rising in the water column as a result of anthropogenic effects (Stevenson, 2000; Bacchus and Barile, 2005). Exotic species that are capable of exploiting this nutrient input have the power to have severe, negative effects on community composition. In our study, where nutrient concentrations in the water column were maintained at a constant level, *H. verticillata* was capable of producing more biomass than the two native species combined in both low and high nitrate treatments. When nitrate concentrations were elevated, *H. verticillata* had more than twice the biomass than was present in the low nitrate treatment. The differential ability of *H. verticillata* to utilize nutrients in the water column may be essential in determining species dominance in freshwater, oligotrophic communities.

Our results indicate the possibility that *H. verticillata* likely has the capacity to establish and become invasive in pristine, oligotrophic communities where *V. americana* and *S. kurziana* are the dominant macrophytes in a native community. In our experiment, *H. verticillata* readily established from vegetative propagules in low nutrient conditions. In the many communities where nitrate concentrations have increased recently, *H. verticillata* may spread even more rapidly. In regions where nitrate concentrations continue to rise in Florida, the cost of maintenance of *H. verticillata*, given its ability to outgrow native species, will continue to rise to prohibitive levels.

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