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Impact of feeding by Neochetina weevils on pathogenicity of fungi associated with waterhyacinth in South Africa

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ABSTRACT

Feeding damage by arthropods is known to render waterhyacinth plants vulnerable to diseases. During this study, six South African fungal isolates (*Alternaria eichhorniae* Nagraj and Ponappa, *A. alternata* [Fr.] Keissler, *Acremonium zonatum* [Sawada] Gams, *Fusarium oxysporum* Schlecht, *F. solani* [Mart.] Sacc., and *Myrothecium roridum* Tode ex Fr) were tested for their disease-causing potential against waterhyacinth (*Eichhornia crassipes* [Mart.] Solms-Laubach; Pontederiaceae). They were applied to waterhyacinth in two treatments: on plants with feeding scars of weevils (*Neochetina* sp.; Coleoptera: Curculionidae; treatment W+) and on plants with no insect feeding damage (W–). The W+ plants were more prone to fungal infection as compared to W– waterhyacinth. A disease index (DI) of damaged plants varied significantly with different pathogens. By 45 d after treatment, DI was significantly higher in *F. oxysporum* (91.8 and 46.6%) and *A. eichhorniae* (87.6 and 65.8%) for W+ and W– waterhyacinth, respectively, followed by *A. zonatum* (56.6 and 50.6%), *F. solani* (43.6 and 27.0%), and *A. alternata* (26.6 and 12.6%). Lowest DI was observed in plants applied with *M. roridum* (21.8 and 10.0%). This study shows that to improve the biological control of waterhyacinth in South Africa, all available agents including native fungi should be released at all sites.

Key words: aquatic weeds, biological control, phytopathogenic fungi

INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms-Laubach; Pontederiaceae), is a free-floating, stoloniferous, perennial herb of South American origin and is known to be an aggressive invader of aquatic bodies throughout the world (Gopal 1987, Barrett 1989). It was introduced to South Africa as an ornamental plant in the early 1900s (Cilliers 1991). Since then it has established throughout the country and is now considered the most troublesome aquatic weed (Hill and Cilliers 1999). It is capable of developing impenetrable floating mats on the water surface, causing biodiversity loss (Midgley et al. 2006), impeding fishing and boat transport, as well as constituting a health hazard by sheltering disease-causing mosquitoes and snails, thus impeding all aspects of water utilization and threatening economic development. Problems are more severe in developing countries where human activities and livelihood are closely related to the water systems (Labrada 1995).

Biological control has been regarded as the only sustainable control option for this weed in many parts of Africa where there is resistance to the use of herbicides. The biological control program against waterhyacinth is well developed and has released seven arthropod species against it world-wide (Julien and Griffiths 1998, Coetzee et al. 2011). Since 1973 when the biological control program was initiated in South Africa (Cilliers 1991), six arthropods and one pathogen have been released in the country in an attempt to reduce infestations to a controllable level (Coetzee et al. 2011). Despite considerable resources allocated to the program in South Africa, the results have been variable, and more control agents are being considered for release (Center and Hill 2002) that could decrease the time required for effective control and increase the level of control.

Although considerable research has been undertaken using insect biocontrol agents of waterhyacinth, the role that native phytopathogenic fungi play has been neglected, especially in South Africa. Phytopathogens are often viable candidates in any biological control program because they are numerous and diverse, easily propagated and disseminated, host specific, and damaging to the host. In other parts of the world, several highly virulent fungal pathogens are known to cause waterhyacinth disease (Charudattan 1990), and biological control using plant pathogens has been shown to be highly effective against waterhyacinth under experimental and field conditions (Shabana et al. 1995a, Charudattan 2000). Among the known pathogens are *Acremonium zonatum* (Sawada) Gams, *Alternaria eichhorniae* Nagraj and Ponappa, *A. alternata* (Fr.) Keissler, *Bipolaris* spp., *Fusarium* spp., *Helminthosporium* spp., *Cercospora piaropi* Tharp. (= *C. rodmanii* Conway; Tessmann et al. 2001), *Myrothecium roridum* Tode ex Fr, *Rhizoctonia solani* Kuhn, and *Uredo eichhorniae* Gonz Frag and Cif. (Gopal 1987, Aneja and Singh 1989, Charudattan 1990, Martínez Jiménez and Charudattan 1998, Evans and Reeder 2001).

The impact of pathogen damage on the population dynamics of invasive species is often subtle (Boyetchko and Peng 2004, Hallett 2005, Sands and Pilgeram 2009, Gressel 2010); thus, the pathogen can be supplemented with the use of conventional herbicides (Gressel 2010, Peng and Wolf 2011) or arthropod agents (Moran 2005, Rayamajhi et al. 2010) to improve efficacy. Studies show that damage caused by insect feeding often weakens the plants and impairs their defense system, thus making the plant more susceptible to plant pathogens (Friedli and Bacher 2001, Kluth et al. 2001, 2002, Moran, 2005, Rayamajhi et al. 2010, Turner et al 2010).

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The waterhyacinth weevils *Neochetina bruchi* (Hustache) and *N. eichhorniae* (Warner) (Coleoptera: Curculionidae) are important biological control agents against waterhyacinth (Center et al. 1999, Julien et al. 1999). The adult weevils cause damage to waterhyacinth by feeding on the epidermal tissues of the laminae and petiole and removing the cuticle and part of the mesophyll tissue (Ray 2009). The larvae tunnel in the petioles and crown of the plant and pupate under water in a cocoon of fine root hairs attached to the root (Wright and Purcell 1995). Feeding by *Neochetina* spp. reduces plant height, biomass, and reproduction and increases shoot mortality (Center et al. 1999). Several studies have shown additive or synergistic effects of *Neochetina* feeding on fungi pathogenicity against waterhyacinth (Charudattan 1984, Moran 2004, 2005, Martínez Jiménez and Gómez Balandra 2007). In the present study, the disease-causing potential of six indigenous phytopathogens of waterhyacinth, *A. eichhorniae*, *A. alternata*, *A. zonatum*, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., and *M. roridum* were studied on weevil damaged (W+) and nondamaged (W–) waterhyacinth, under controlled conditions.

MATERIALS AND METHODS

Fungal culture and inoculum preparation

Cultures of *A. eichhorniae*, *A. alternata*, *A. zonatum*, *F. oxysporum*, *F. solani*, and *M. roridum* were isolated from diseased waterhyacinth collected from various parts of South Africa (Table 1; Figure 1). Leaf pieces of waterhyacinth (3 to 4 mm^2) were cut from the margins of necrotic lesions on diseased leaves of waterhyacinth, the surfaces disinfected with sodium hypochlorite for 3 to 4 min, and then placed on potato dextrose agar (PDA[1]); The isolates were cultured at 26 ± 2 C under a photoperiod of 14:10 h (light:dark) for 4 d. Aseptic cultures were obtained using a single-spore isolation technique (Choi et al. 1999), grown on PDA plates, and incubated in walk-in BOD incubators for 21 d. A mycoherbicidal formulation of each fungus was prepared in sterile distilled water. The spores of the fungi were harvested by flooding the petri plates containing the fungi with 10 mL sterile distilled water. The spores were concentrated by centrifugation, and the desired inoculum concentration (10^6 to 10^7 spores/mL) was prepared using a haemocytometer. The surfactant Tween 20 (oxysorbic polyxyethylene sorbitan monoleate) was added at the rate of 0.05 mL per 50 mL of spore suspension (Ray et al. 2008).

Plant culture

Young waterhyacinth plants were collected from local water bodies and grown in PVC tunnels in plastic tubs at Rhodes University, Grahamstown, South Africa. All the tanks containing waterhyacinth were fertilized with 15-3-12 N:P:K, slow-release fertilizer[2]. A commercial iron chelate (13% Fe) was also added to the water at a concentration of 2 g/23 L of water. The water was replenished when required and fertilized and changed every 30 d during the course of the 3 month experimental period. Plants were maintained in two separate tanks, one without *Neochetina* weevils and one with weevils. To obtain weevil-free cultures of waterhyacinth (W-), plants were sprayed with the insecticide Malathion[3] every 2 months. To obtain weevil damaged plants (W+) for the experiments, mixed cultures of *N. bruchi* and *N. eichhorniae* were released in the second set of tanks. For the experiment, individual plants were selected, and all dead leaves and stems and daughter plants were removed. These plants were grown individually in plastic tubs (30 cm diameter by 15 cm high) for about 15 d prior to their use in the experiment. For those treatments requiring weevil damage, two weevils were allowed to feed on each plant for 15 d. Before the experiment, the *Neochetina* weevils were removed manually; each weevil-damaged plant had two to six feeding scars per leaf.

Application of fungal inoculum to waterhyacinth

Spore suspensions of each of the fungi were applied to waterhyacinth until runoff occurred in each of the two treatments, W+ and W-; each treatment was replicated 5 times. Control plants were sprayed with sterile distilled water containing Tween 80 and kept in walk-in incubators at 60 to 70% relative humidity and 27 to 25 C under a 14:10 h light:dark photoperiod. The plants were individually enclosed in plastic bags for 24 h to create a dew effect conducive for fungal growth.

Disease intensity and severity was rated by visual observation at an interval of 24 h for 30 d. Disease intensity was determined visually on the basis of initiation of disease and increase in disease area every day after application of the inocula, using a score chart framed by Freeman and Charudattan (1984) that rated disease intensity as excellent (+++), good (++), poor (+), and no infection (–) at an interval of 4, 8, 15, and 21 d. Disease was scored using a 0 to 5 scale rating system where 0 = no symptoms; 1 = 1 to 10%; 2 = 11 to 25%; 3 = 26 to 50%; 4 = 51 to 75%, and 5 = ≥75% area covered by spots on leaves, until 30 d after fungal inoculation. Using this rating system, a disease index (DI) was calculated (Chaube

Table 1. Details on collection and isolation of fungal pathogens of waterhyacinth.

Fungal pathogen	Locality of collection of diseased waterhyacinth	GPS coordinate records	
		Latitude	Longitude
Acremonium zonatum	*Tongaat Sugar Estates, Tongaat, KwaZulu-Natal*	*S 29.27172*	*E 31.35584*
Alternaria alternata	*Rietondale, Pretoria, Gauteng*	*S 25.73142*	*E 28.22393*
A. eichhorniae	*Kluitjieskraal, Tulbagh, Western Cape*	*S 33.43628*	*E 19.17581*
Fusarium oxysporum	*Nseleni River, Empangeni, KwaZulu-Natal*	*S 28.74739*	*E 31.96890*
F. solani	*Goudini Road, Worcester, Western Cape*	*S 33.64420*	*E 19.29980*
Myrothecium roridum	*Nahoon River, East London, Eastern Cape*	*S 32.97392*	*E 27.92570*

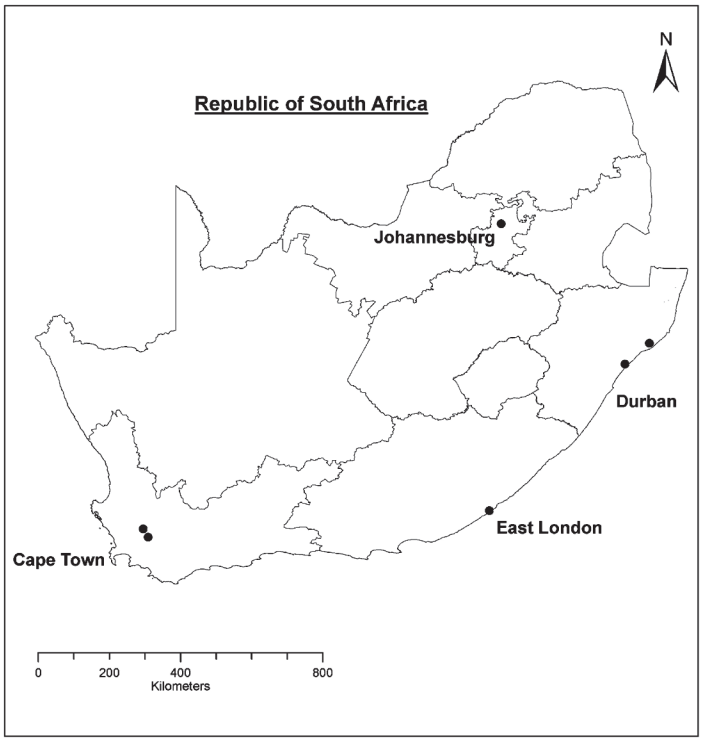

Figure 1. Collection locations in South Africa of diseased plant parts of waterhyacinth from which phytopathogens were isolated.

and Singh 1991) as per observations made 15, 30, and 45 d after treatment (DAT), with fungal inoculum taking into account individual leaf ratings using the following formula:

$$\text{Disease Index (DI)} = \frac{\text{Sum of all numberical ratings} \times 100}{\text{Total number of leaves measured} \times \text{Maximum disease index}}$$

where the sum of all numerical ratings = $(0 \times N_0) + (1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4) + (5 \times N_5)$; N_0 = number of leaves with score 0; N_1 = number of leaves with score 1; and . . . N_5 = number of leaves with score 5.

Data analysis

The percentage data recorded for evaluating DI of different fungi were subjected to arcsine transformation prior to being compared using one-way analysis of variance (ANOVA; GenStat, VSN International Ltd). The treatment means were compared with Fisher's least significant difference (LSD) at 5% level of significance (Panse and Sukhatme 1957).

RESULTS AND DISCUSSION

Plants inoculated with different fungi were observed for the development of disease symptoms on both W+ and W– waterhyacinth. Disease caused by phytopathogens started as small necrotic spots and developed into a leaf blight that entirely covered the whole leaf by a maximum of 4 weeks post inoculation. For most of the fungal applications, symptoms appeared in 2 to 7 DAT from when the fungi were sprayed on the foliage, and disease progressed steadily over the following 2 to 5 weeks. The isolate of *A. eichhorniae* infected waterhyacinth by the fourth day in both W+ and W– waterhyacinth (Table 2). The plants treated with *A. alternata* developed disease symptoms on W+ and W– waterhyacinth leaves by 8 and 15 DAT, respectively; *A. zonatum* showed infection by 6 and 8 DAT, respectively; and *F. oxysporum* and *F. solani* both caused disease on waterhyacinth by 4 and 6 DAT, respectively. Disease symptoms caused by *M. roridum* appeared on W+ plants by the 10 DAT, and almost no symptoms had appeared on the W– waterhyacinth by 20 DAT.

Lesion diameters were visually much larger on leaves applied with *A. eichhorniae*, followed by *F. oxysporum* and *A. zonatum*. The lowest disease severity was observed in *M. roridum*-treated plants. *Fusarium oxysporum*, *F. solani*, and *M. roridum* caused infection uniformly on both young and old leaves while both *Alternaria* species and *A. zonatum* were ineffective on newly emerged leaves.

DI determined at 15 DAT on plants with fungal inoculation (Figure 2) indicated a significant difference in damage to waterhyacinth by various fungi ($F = 11.0$; df = 11, 48; $P < 0.0001$). The DI was significantly higher (66.0%) in W+ plants treated with *A. eichhorniae*, followed by DI of *F. oxysporum* (44.6%). The lowest DI was observed in *M. rodidum* applied to both W+ (3.0%) and W- (0.4%) plants. By 30 DAT ($F = 13.6$; df = 11, 48; $P < 0.0001$), DI was significantly higher for W+ waterhyacinth applied with *A. eichhorniae* (78.6%) and *F. oxysporum* (61.6%), respectively. By 45 DAT, disease spread was significantly higher on *F. oxysporum* W+ plants ($F = 23.5$; df = 11, 48; $P < 0.0001$). There was no significant difference between DI of *F. oxysporum* (91.8%) and *A. eichhorniae* (87.6%), respectively, on W+ waterhyacinth. The W– plants treated with *A. eichhorniae* (65.8%) and *F. oxysporum* (46.6%) were significantly lower than the W+ plants with same fungal treatmen, followed by *A. zonatum* (56.6 and 50.6% for W+ and W– plants, respectively), *F. solani* (43.6 and 27.0%) and *A. alternata* (26.6 and 12.6%). The lowest DI was observed in

Table 2. Disease initiation and intensity of infection caused by various fungi on *Neochetina* damaged and undamaged waterhyacinth.

Fungi (Days after application)	Waterhyacinth treated with fungi*	Disease intensity**			
		4	8	15	21
Acremonium zonatum	W+	–	+	++	+++
	W–	–	+	+	+
Alternaria alternata	W+	–	+	+	+++
	W–	–	–	+	+
A. eichhorniae	W+	+	++	++	+++
	W–	+	++	++	+++
Fusarium oxysporum	W+	+	++	++	++
	W–	–	++	++	++
F. solani	W+	+	++	++	++
	W–	–	+	++	++
Myrothecium roridum	W+	–	–	+	++
	W–	–	–	–	+

*W+ , weevil damaged plants; W–, plants with no weevil damage.
**Disease intensity: excellent (+++), good (++), poor (+), no infection (–).

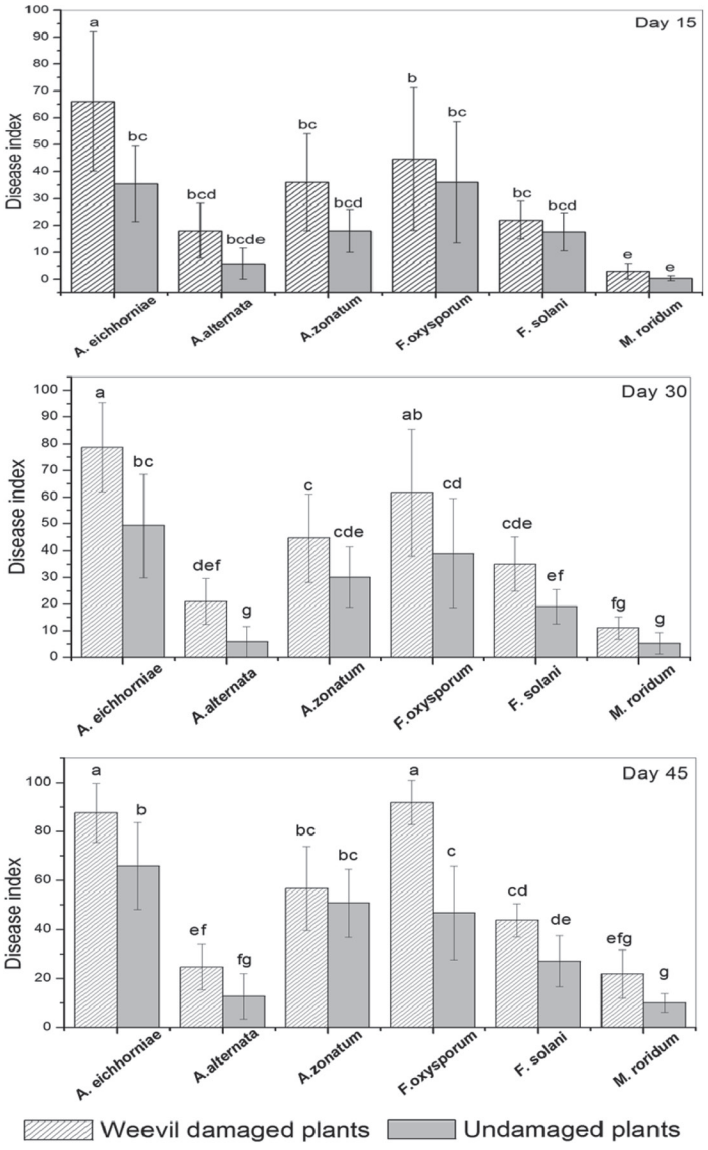

Figure 2. Pathogenicity of some potential fungal isolates from South Africa on waterhyacinth. One way ANOVA: Disease index of various fungi at 15 DAT: F = 11.01; df = 11, 48; P < 0.0001. 30 DAT: F = 13.64; df = 11, 48; P < 0.0001. 45 DAT: F = 23.53.01; df =11, 48; P < 0.0001. Mean values marked with same letters indicate no significant difference at p = 0.05.

plants applied with *M. roridum* (21.8 and 10.0%). The disease spread rate increased up to 15 to 20 d and then decreased in all pathogens except *F. oxysporum*. *Fusarium oxysporum* disease spread increased after 20 DAT, possibly due to accumulation of biologically active mycotoxins on the infected leaves, which accelerate cell death and leaf necrosis (Zhang and Watson, 2000). All plants except those treated with *F. oxysporum* and *A. eichhorniae* showed rapid regrowth 45 DAT.

In the present study, *F. oxysporum* and *A. eichhorniae* emerged as potential agents worth considering for integrated biological control of waterhyacinth in combination with insect agents. *A. eichhorniae* has already been extensively studied (Shabana et al. 1995a, 1995b, 1995c, Nag Raj and Ponappa 1970) and has been reported safe as biological control agent of waterhyacinth. Isolates of *F. oxysporum* have been identified as potential mycoherbicides of various weeds (McCain and Noviello 1985, Pandey et al. 1992, Boyette et al. 1993, Pilgeram et al. 1995, Sands et al. 1997) but is yet to be studied for waterhyacinth control. An isolate of *A. zonatum* used in the study caused 56.6 and 50.0% DI in W+ and W– treatments, respectively, 45 DAT. It has been reported to cause disease on water hyacinth in many parts of the world (Charudattan 2001). The other pathogens tested in the present study, *F. solani*, *A. alternata*, and *M. roridum*, caused <50% DI to waterhyacinth, thus suggesting they are weak pathogens with low potential for further studies.

Galbraith (1987) reported that feeding by *N. eichhorniae* increased infection by *A. zonatum*, but not because of feeding; rather, the spores were transported on the feet and digestive tract of the weevils. In the present study the weevils were removed prior to the experiment, but even then the W+ plants were more prone to fungal infection by all the phytopathogens. In various earlier studies (Charudattan et al. 1978, Galbraith 1987, Moran 2005, Martínez Jiménezand Gómez Balandra 2007), the disease-causing efficacy of *A. zonatum* and *C. piaropi* was considerably enhanced when applied to waterhyacinth in the presence of *Neochetina* weevils. The feeding by the weevils made way for the pathogens, thus facilitating infection on waterhyacinth. The occurrence of leaf spot disease on waterhyacinth by the fungus *Acremonium zonatum* has been observed on arthropod-damaged leaves (Charudattan et al. 1978). Moran (2005) reported that leaf scarring by the weevils *N. eichhorniae* and *N. bruchi* enhanced the disease-causing efficacy of the pathogen *C. piaropi* on waterhyacinth. Ajuonu et al. (2003) reported increase in disease caused by *M. roridum*, with an increase in feeding scars of adult weevils. The combined impact of arthropod and pathogenic fungi results in plants with smaller lamina, lower number of live petioles, and higher number of dead petioles per plant than noninfested plants. Studies on feeding damage by waterhyacinth mites (*Orthogalumna terebrantis* Wallwork) indicate it has also been known to be associated with fungal pathogens (Charudattan et al. 1978, Sanders et al. 1982). Although we did not investigate the effect of biological control agents on plant growth during field studies, Charudattan (1986) reported that arthropods alone reduced shoot height (by about 50%), whereas *C. rodmanii* (= *C. piaropi*) had only a slight effect in reducing plant height (by about 2%) but caused leaf necrosis, debilitation, and death of arthropod-damaged waterhyacinth. Plants treated with *C. rodmanii* and arthropods were more severely affected than those treated with the biocontrol agent alone.

The use of plant pathogens to control weeds is definitely an appealing concept but often the results obtained under field conditions are unpredictable and vary with time and location. Yet the use of phytopathogenic fungi as biocontrol agents can be valuable because pathogens can cause significant reductions in waterhyacinth biomass, especially following natural disease outbreaks, after severe insect attacks, or when used as inundative bioherbicide agents. They can be used to manage invasive weeds in natural areas and in situations where nonchemical alternatives to weed control are needed. Although bioherbicides can be used as the sole option for the management of certain weeds in several cases,

for a plant as invasive as waterhyacinth they need be supplemented with other control options (i.e., release of insect biocontrol agents or used as a major supplement to low doses of conventional chemical herbicides; Grant et al. 1990, Schnick and Boland 2004, Moran 2005, Martínez Jiménez and Gómez Balandra 2007, Mitchell et al. 2008, Peng and Wolf 2011). Note that in the present study, despite weevils being removed from the plants prior to starting the experiments, which resulted in low number of feeding scars (2 to 5/leaf), disease initiation and the DI of W+ plants was significantly higher compared to the W– waterhyacinth. This study provided additional evidence of the potential gains of deploying multiple biological control agents in providing additive or synergistic effects for controlling waterhyacinth. Thus with further studies on their host range, large-scale field trials and studies on better ways to formulate and implement these indigenous fungal pathogens can prove very useful in enhancing damage caused by the insect agents of waterhyacinth.

SOURCES OF MATERIALS

[1]Biolab, Merck, Gauteng, South Africa
[2]Multicote 8, Controlled Release Fertilizer, Haifa Chemicals Israel, RSA Ptv Ltd
[3]Kombat Ptv Ltd

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Phytoparasitic nematodes associated with the rhizosphere of the aquatic weed *Hygrophila polysperma*

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ABSTRACT

Hygrophila (*Hygrophila polysperma* [Roxb.] T. Anders; Acanthaceae) is an invasive aquatic and riparian weed the southern United States. This rooted submerged or emergent plant is typically found in flowing fresh water channels and structured shorelines. In Florida, hygrophila interferes with irrigation, navigation, and flood control structures. To examine the diversity of nematode fauna associated with rhizosphere of this invasive weed, exploratory field surveys were conducted in the native (India, n = 19 sites) and invasive (Florida, USA, n = 7 sites) ranges of hygrophila during 2008–2009. Two core samples (10 cm diameter by 10 cm deep) containing moist soil and hygrophila roots were collected at each sampling site. Phytoparasitic nematodes were extracted, identified,

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and quantified to calculate diversity and evenness indices. Results showed that significantly higher densities of phytoparasitic nematodes are associated with hygrophila in India compared to Florida. In total, eight nematode species (representing seven genera), belonging to the order Tylenchida, were recorded from India. *Meloidogyne graminicola* Golden & Birchfield and *Helicotylenchus* sp. were the dominant species in the native range. In Florida, 10 phytoparasitic nematode genera were collected representing two orders, Triplonchida (n = 2 genera) and Tylenchida (n = 8 genera). *Helicotylenchus* and *Tylenchorhynchus* were the dominant genera of phytoparasitic nematodes collected across Florida. This study is the first report of phytoparasitic nematodes associated with the root zone of hygrophila.

Key words: *Hygrophila polysperma*, invasive weed, phytoparasitic nematodes, root zone

INTRODUCTION

Hygrophila (*Hygrophila polysperma* [Roxb.] T. Anders; Acanthaceae) is an invasive aquatic and riparian weed in the southern United States (US) and Mexico (EDDMaps 2010, Mora-Olivo et al. 2008). Introduced into the US as a popular aquatic plant (Innes 1947), this weed escaped cultivation and is now creating problems in warm water areas of the southern US and eastern Mexico (Cuda and Sutton 2000, Mora-Olivo et al. 2008). In the US, hygrophila is widely distributed across Florida (n = 13 counties; Cuda and Sutton 2000, EDDMaps 2010). In addition to Florida, its distribution in the US includes Alabama, South Carolina, and Texas (EDDMaps 2010). This plant is an Old World species, native broadly to Southeast Asia including India (Les and Wunderlin 1981, Cook and Cook 1996). It is listed as a Federal Noxious Weed (USDA 2006) and a Category-I invasive weed by the Florida Exotic Pest Plant Council (FLEPPC 2009). The dense stands formed by this herbaceous perennial weed interfere with irrigation, block flood control structures (Schmitz and Nall 1984, Sutton 1995), and also hinder navigation (Cuda and Sutton 2000).

Since 1990, a visible increase in the number of water bodies in Florida invaded by hygrophila suggests that current methods employed to control this weed are inadequate (Sutton 1995). The invasive characteristics exhibited by hygrophila as well as its biological and economic attributes make it a good candidate for classical biological control (Cuda and Sutton 2000). However, little information is available about the natural enemy complex associated with hygrophila in its native range. We recently undertook surveys in a range of habitats in India during 2008–2009 to collect and identify the plant's natural enemies (A. Mukherjee, unpubl. data). As a part of that survey, phytoparasitic nematodes present in the root zone of hygrophila also were extracted, enumerated, and identified. Similar surveys also were conducted in Florida, where hygrophila was introduced. Use of nematodes in classical weed biological programs is rare. For example, the leaf and stem gall nematode *Subanguina picridi* Kirja-nova (Nematoda: Tylenchidae) was released as a biological control agent of Russian knapweed (*Acroptilon repens*; Asteracee; Watson 1986, Ou and Watson 1993). However, the specific objective of this study was only to assess the diversity of phytoparasitic nematode fauna associated with the rhizosphere of this weed in its native and exotic range and to determine if hygrophila could act as an alternate host of important plant parasitic nematodes.

MATERIALS AND METHODS

Sampling and enumeration of nematodes

In September 2008, exploratory field surveys (n = 19) in India were undertaken in a range of locations in the states of West Bengal (n = 12, sites Ind-1 to Ind-12) and Assam (n = 7, sites Ind-13 to Ind-19; Figure 1), chosen for this study because they are climatically similar to the invasive range of hygrophila in the US (Mukherjee et al. 2011). Except for two sites in West Bengal (Ind-2 and Ind-8), all samples were collected from natural areas. For sites Ind-2 and Ind-8, samples were collected from irrigation channels in agricultural fields. Each survey site was geopositioned and assigned a unique accession number. Two soil cores containing hygrophila roots (10 cm diam by 10 cm deep) were collected at ~10 m intervals from each survey site. All samples were collected from shoreline ~2 m from the edge of the water and only from areas with established hygrophila plants. Cores (n = 2) collected from each survey site were pooled before extraction of nematodes. Nematodes were extracted following the sieving and specimen processing technique of Handoo and Ellington (2005). Identification of phytoparasitic nematodes to genus, and in some cases species, was performed at the Plant Health Diagnostic Laboratory, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

In August 2009, similar surveys were conducted in Florida (n = 7) to characterize the diversity of phytoparasitic nematodes associated with hygrophila in its exotic range (Figure 2). Samples were collected from natural areas in five counties, including Alachua (site Fl-7), Broward (site Fl-1), Dixie (sites Fl-5 and 6), Osceola (sites Fl-3 and 4), and Pinellas (site Fl-2). Using a metal trowel, two soil cores (10 cm diam by 10 cm deep) containing hygrophila roots were collected from each survey site at ~10 m intervals. Similar to native habitats, samples were collected from the shoreline to ~2 m from the edge of the water. Soil cores (n = 2) collected from each site also were pooled before extraction of nematodes. The geoposition of each survey site was recorded. Nematodes were extracted using aforementioned methods and identified to the genus level in the Nematode Assay laboratory, Entomology and Nematology Department, University of Florida.

Assessment of nematode dominance

To determine the dominant nematode taxa (genus or species) within the native or invasive ranges of hygrophila, a standardized index of prevalence (*Ip*, equation 1) was calculated following Zhou et al. (2003). Two criteria, density and frequency of a taxon for a given site were considered for calculation of *Ip*:

$$Ip = \left[\frac{N_i}{N} \times \frac{S_i}{S} \right], \quad (1)$$

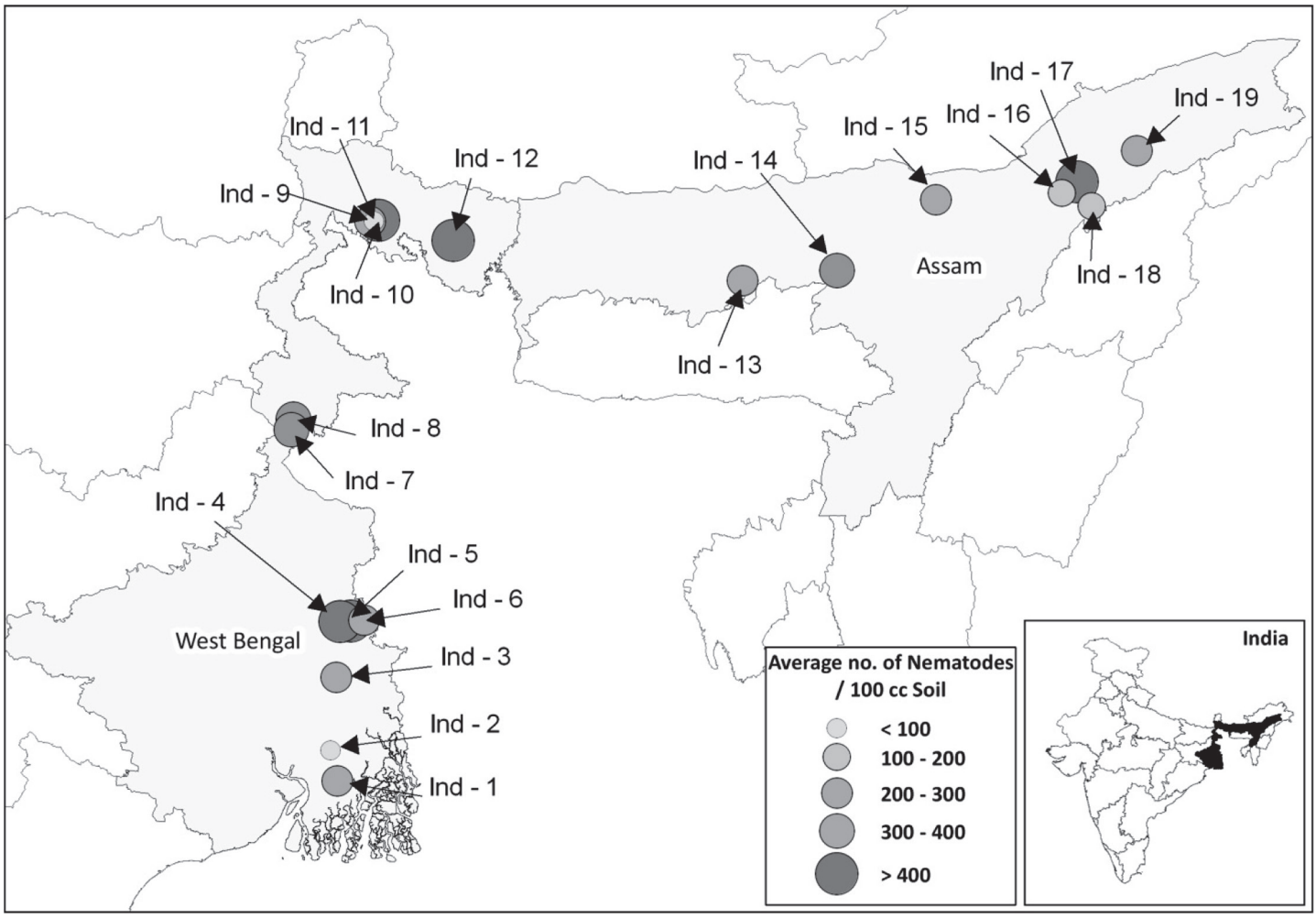

Figure 1. Survey sites in India (n = 19). Each site was assigned a unique accession number. Symbols are graduated based on average number of nematodes collected per core sample of soil.

where, N_i = total number of nematode taxon *i* collected across all sites within a range (native/invasive), N = total number nematodes collected from a given range, S_i = number of sites from which taxon *i* was collected, and S = total number of sampling sites within a given range.

A 95% confidence interval (CI) of the mean *Ip* was calculated using a technique by Buonaccorsi and Liebhold (1988). As emphasized earlier in Buonaccorsi and Liebhold (1988) and later by Beyene and Moineddin (2005), calculation of confidence interval of *Ip* is necessary because it is a product of two criteria. According to Zhou et al. (2003), a taxon was considered dominant if its *Ip* > upper limit of 95% CI, taxa with *Ip* intermediate between upper and lower limit of CI were considered common, and taxa with *Ip* < lower limit of CI were classified as occasional.

Assessment of nematode diversity

The diversity of the phytoparasitic nematofauna was assessed for each sampling site (n = 19 for native range, n = 7 for invasive range). In addition, data from all sampling sites within a given range (native or invasive) were pooled to calculate the overall diversity of the nematodes. Following techniques reported by Bernard and Schmitt (2005), Shannon diversity (H'; equation 2) and evenness (E_H; equation 3) indices were calculated to measure the α diversity (within site diversity) of each sampling site and habitat (Magurran 2004) using the following equations:

$$H' = -\Sigma p_i(\ln p_i), \quad (2)$$

$$E_H = H'/\ln S, \quad (3)$$

where p_i = relative abundance of each species, calculated as the proportion of individuals of the ith species (n_i) to the total number of individuals (N) in the community, or $p_i = \frac{n_i}{N}$; and S = total number of species present in the community or the species richness. The range of values for E_H is 0 to 1, with 1 being complete evenness.

For each geographical region sampled, the diversities and sample densities (number/100 cc soil) of nematode taxa were analyzed. The Kruskal-Wallis analysis of variance (here-

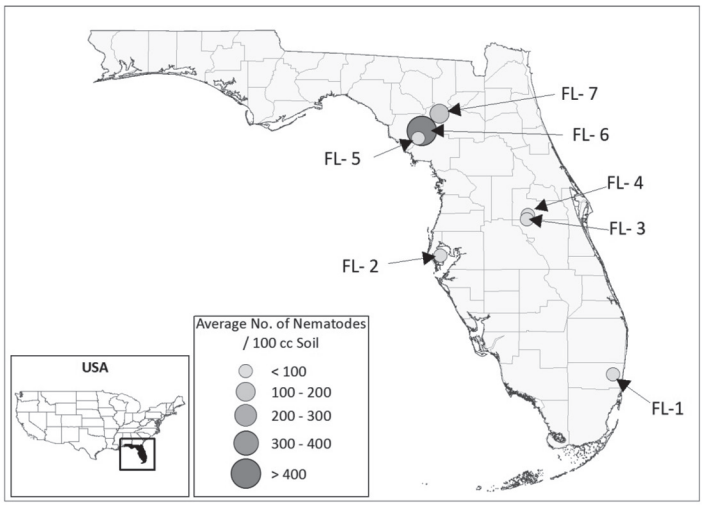

Figure 2 Survey sites in Florida (n = 7). Each site was assigned a unique accession number. Symbols are graduated based on average number of nematodes collected per core sample of soil.

after Kruskal-Wallis test; Corder and Foreman 2009) was used to test the difference in density and diversity of phytoparasitic nematodes between exotic and native ranges. Statistical tests were performed using the open source statistical software R (version 2.11.1) at $\alpha = 0.05$.

Using the Morisita-Horn index of community similarity (C_{MH}; equation 4), cluster analysis of nematode assemblages was performed on all sampling sites within each region following the unweighted pair group average (UPGMA) method (Bernard and Schmitt 2005). C_{MH} is a measure of β diversity (between site diversity), which calculates the similarity in species composition between two sites. Walda (1981) investigated a number of similarity indices and recommended the use of C_{MH} because it is not influenced by the effects of sample size and species diversity. The limiting values of C_{MH} are 0 (completely dissimilar) and 1 (completely similar). The Morisita-Horn index of community similarity is calculated by the following equation:

$$C_{MH} = \frac{2\Sigma(a_i b_i)}{(d_a + d_b) \times (N_a \times N_b)} \quad (4)$$

where a_i and b_i are the i^{th} species of sites A and B, respectively; N_a and N_b represent the number of individuals collected, respectively, from site A and B; and d_a (and d_b) is calculated as $d_a = \Sigma a_i^2 / N_a^2$

RESULTS AND DISCUSSION

Nematode diversity

Native range: In total, eight phytoparasitic nematode species, representing seven genera were collected from India (Table 1; Figure 3A). The number of nematode species in the sampling sites varied between two (sites Ind-7, 8, 11, and 15) and seven (site Ind-12). Densities of nematodes extracted (number/100 cc soil) varied between 94 (site Ind-2) and 1130 (site Ind-12; Figure 1B), with an average (mean ± SD) of 609.3 ± 293.8 nematodes/100 cc soil (Figure 3B, black bar). The Shannon diversity (H') of nematodes in the native range (pooled data) was 1.82, with sampling sites ranging between 0.29 (site Ind-8) and 1.53 (site Ind-6; Figure 3C). Overall, a high evenness (E_H = 0.88, black bar Fig 3D) of nematode distribution was recorded across native habitats. The E_H value calculated among sampling sites ranged between 0.42 (site Ind-8) and 1.0 (site Ind-11).

Exotic range: In total, 10 phytoparasitic nematode genera were collected from Florida (Table 2; Figure 4A), with seven genera collected from site Fl-7 and one genus from site Fl-2. Nematode densities were found to be low in most of the sites, with an average density of 141.9 ± 307.7 nematodes/100cc soil, with the highest density (830 nematodes/100cc soil) recorded from site Fl-6, located in Dixie County, Florida (Figure 2 and 4A). The highest Shannon diversity index was calculated from site Fl-7 (H' = 1.27), with an overall habitat H' of 0.8 (Figure 4C). Evenness (E_H) of nematode distribution across exotic habitats was low (0.35; Figure 4D black bar). Because only a single genus was collected from site Fl-2 (Figure 4A), both calculated H' and E_H were zero (Figure 4C and 4D).

There were no differences in H' between exotic and native ranges (Kruskal-Wallis test, χ^2 =3.53, p = 0.06). Similarly, no difference was observed in E_H (χ^2 = 0.24, p = 0.62). In contrast, densities of phytoparasitic nematodes recorded from the na-

Table 1. Phytoparasitic nematode species associated with root zone of hygrophila in Assam and West Bengal, India.

Order, Taxon	Index of prevalence (Ip)†*	Sites collected
Tylenchida		
Meloidogyne graminicola Golden & Birchfield	0.18 [a]	Ind-1, 3, 5-7, 12, 14, 15, 18
Helicotylenchus sp. Steiner	0.17 [a]	Ind-1-11, 13-19
Meloidogyne incognita Chitwood	0.05 [b]	Ind-3, 5, 6, 13-15, 17, 19
Rotylenchulus reniformis (juvenile) Linford & Oliveira	0.05 [b]	Ind-2, 4-6, 8, 10, 12, 15, 16
Hirschmanniella oryzae Luc & Goodey	0.05 [b]	Ind-3, 5, 6, 8, 9, 11, 12, 19
Criconemoides sp. Taylor	0.03[b]	Ind-4, 5, 7-11, 14, 15
Tylenchorhynchus mashhoodi Siddiqi & Basir	0.02 [b]	Ind-1, 8, 9, 13
Hoplolaimus indicus Sher	0.002 [c]	Ind-1, 4, 5, 19

†Index of prevalence was calculated using equation 1.
*Upper and lower bounds of 95% confidence interval of Ip are 0.11 and 0.02, respectively.
[a]Dominant species, [b]common species, [c]occasional species (see methods for assessment of nematode dominance for classification criteria).

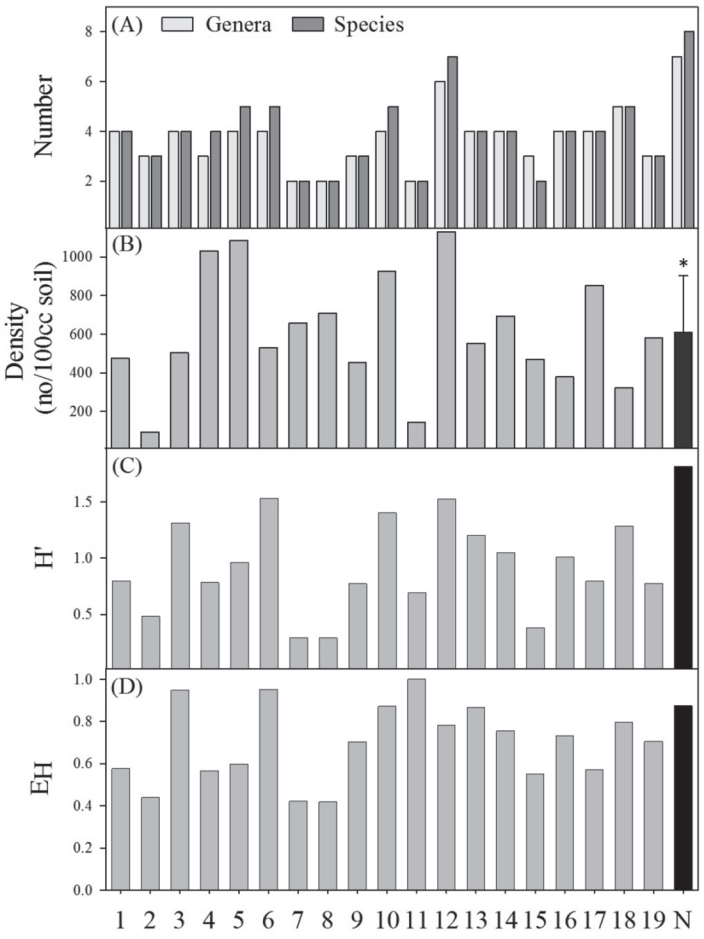

Figure 3. Nematode genera and species, density, Shannon diversity (H'), and evenness (E_H) calculated across sampling sites in India. Black bars represent values for native range (India, pooled data). Number labels on x-axis correspond to site numbers in Figure 1. N = native habitat. *For Figure B, black bar denotes the average number of nematodes/100 cc soil (609.3 ± 293.8), calculated across all sampling sites.

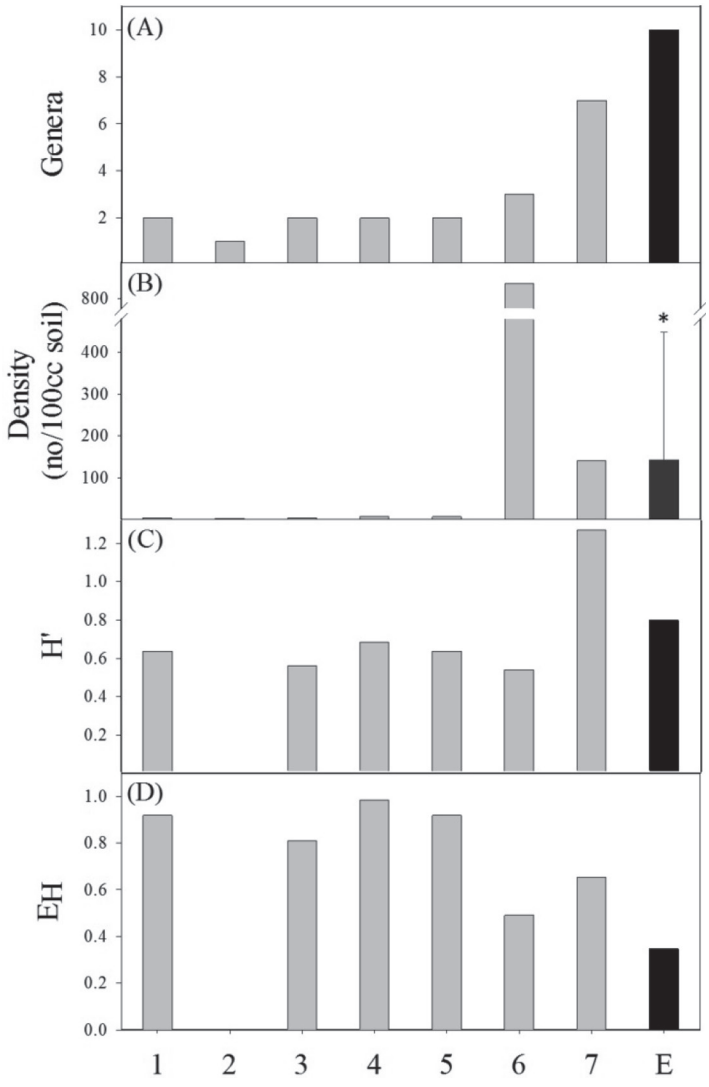

Figure 4. Nematode genera, density, Shannon diversity (H'), and evenness (E_H) calculated across sampling sites in Florida. Black bars represent values for exotic range (Florida, pooled data). Number labels on x-axis correspond to site numbers in Figure 2. E = Exotic habitat. *For Figure B, black bar denotes average number of nematodes/10 cc soil (141.9 ± 307.7), calculated across all sampling sites.

tive range were significantly higher than that of the exotic range ($\chi^2 = 8.86$, $p = 0.003$).

Phytoparasitic nematodes recorded

For both native and exotic ranges, the *Ip* of individual taxa (genus or species level for India, genus level for Florida) collected from the rhizosphere of hygrophila was recorded (Table 1 and 2, respectively).

Native range: In total, eight phytoparasitic nematodes species, all belonging to the order Tylenchida were collected from India (Table 1). The lower and upper limits of 95% CI of *Ip* were 0.02 and 0.11, respectively. Among all the taxa, the rice rootknot nematode *Meloidogyne graminicola* Golden & Birchfield (*Ip* = 0.18) and the spiral nematode *Helicotylenchus* sp. (*Ip* = 0.17) were recorded as dominant phytoparasitic nematode species (dominant species = *Ip* > upper limit of 95% CI) across native range samples (Table 1). The rootknot nematode *M. incognita* Chitwood, reniform nematode *Rotylenchulus reniformis* Linford & Oliveira, rice root nematode *Hirschmanniella oryzae* Van Breda de Hann, ring nematode *Criconemoides* sp., and stunt nematode *Tylenchorhynchus mashhoodi* Siddiqi & Basir were found to be common nematode species (*Ip* intermediate between upper and lower bounds of 95% CI) associated with root zone of hygrophila (Table 1). Using the criteria *Ip* < lower limit of 95% CI, *Hoplolaimus indicus* Sher was classified as an occasional species (*Ip* = 0.002).

Based on the Morisita-Horn index of similarity, sampling sites across India can be divided into two groups with low similarity in collected phytoparasitic nematofauna (<0.2; Fig 5). Among all sampling sites, highest similarity was observed between Ind-7 and Ind-15 (similarity index of 0.99; Fig 5). In both cases, two species of nematodes, *Helicotylenchus* sp. and *M. graminicola*, were collected with approximately equal densities. Overall, similarity indices across native range sampling sites documented wide variation in nematode fauna associated with hygrophila roots.

Table 2. Phytoparasitic nematode genera recorded from the root zone of hygrophila in Florida, US.

Order Taxon	Index of prevalence (Ip)†*	Sites collected
Triplonchida		
Paratrichodorus sp. Siddiqi	0.0003[c]	FL-1
Trichodorus sp. Cobb	0.0001[c]	FL-1
Tylenchida		
Helicotylenchus sp. Steiner	0.33[a]	FL-5, 6, 7
Tylenchorhynchus sp. Cobb	0.09[a]	FL- 3, 6, 7
Hemicycliophora sp. de Man	0.02[b]	FL-3, 6, 7
Mesocriconema sp. Andrassy	0.003[c]	FL-2, 5, 7
Meloidogyne sp. Goeldi	0.003[c]	FL-7
Hemicriconemoides sp. Chitwood & Birchfield	0.0004[c]	FL-4
Pratylenchus sp. Filipjev	0.0003[c]	Fl-2
Hoplolaimus sp. Daday	0.0001[c]	FL-7

†Index of prevalence was calculated using equation 1.
*Upper and lower bounds of 95% confidence interval of *Ip* are 0.04 and 0.01, respectively.
[a]Dominant taxa, [b]common species, [c]occasional taxa (see methods for assessment of nematode dominance for classification criteria).

Exotic range: Genus level nematode taxa, representing two Orders (Triplonchida and Tylenchida), were collected from Florida (Table 2). With eight genera, the Order Tylenchida was found to be the most diverse across all the sampling sites in Florida (n = 7). The Order Triplonchida was represented by two genera, *Paratrichodorus* and *Trichodorus*.

The upper and lower limits of CI of *Ip* calculated from Florida samples were 0.04 and 0.01, respectively. Based on the criteria used to determine dominant taxon (*Ip* > upper limit of 95% CI), *Helicotylenchus* was the most dominant nematode genus (*Ip* = 0.33) followed by the genus *Tylenchorhynchus* (*Ip* = 0.09). The genus *Hemicycliophora* was classified as a common phytoparasitic nematode (*Ip* = 0.02). With *Ip* < the lower limit of 95% CI, all other phytoparasitic nematode genera collected across Florida were classified as occasional (Table 2).

The Morisita-Horn index of similarity among sampling sites across Florida was found to be generally low (Figure 6). Maximum similarity (~0.91; Figure 6) was recorded between sites FL-6 and FL-7. A somewhat lower similarity index (0.86) was recorded between sites FL-2 and FL-5. In contrast, site FL-1 was distinctly different than all other sites in Florida, with no similarity in phytoparasitic nematodes collected.

For both exotic and native ranges, the results of cluster analysis indicated that similarities among sampling sites were not correlated with between-site geographic distances. In India for example, high similarity was observed between sites Ind-7 and Ind-15, but similarities between sites geographically closer to these sites were low. Lack of correlation between similarity and geographic distance also was evident in Florida. Several factors, including soil characteristics, climatic conditions, proximity to agriculture fields, and surrounding vegetation can affect similarity of nematodes between sites. For example, Bernard and Schmitt (2005) found that site characteristics (bog, mesic, rain, and drier forests) influenced similarity of nematofauna in native plant communities in Hawaii. Based on our field observations, proximity to agriculture fields could explain the similarities of nematode fauna between geographically distant sites. For example, sites Ind-7 and Ind-15 were in close proximity to rice fields, and in both cases *M. graminicola* was the predominant species collected. Because no soil characteristics or vegetation data were collected during this study, no objective evaluation of why nematode similarities vary across sites was possible.

In Florida, highest nematode diversity was observed at site Fl-7 (Rum Island Springs; 29.83357, –82.67762). This is a heavily forested site, and large mats of hygrophila were

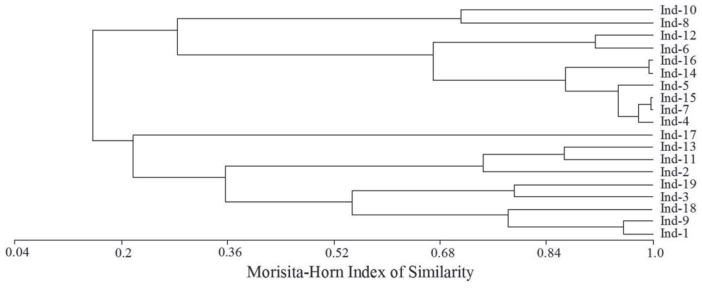

Figure 5. Cluster analysis of phytoparasitic nematode assemblage based on Morisita-Horn index of community similarity from sampled sites in India (see Figure 1 for site locations).

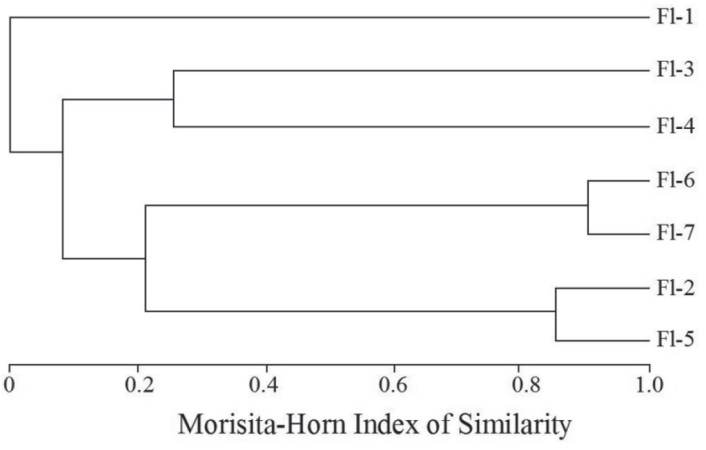

Figure 6. Cluster analysis of phytoparasitic nematode assemblage based on Morisita-Horn index of community similarity from sampled sites in Florida (see Figure 2 for site locations).

observed along the bank of the Santa Fe River. Perhaps soil conditions unique to the site and the presence of large mats of hygrophila could explain the high nematode diversity observed, but no soil data were collected to support this hypothesis. Interestingly, cluster analysis indicated high similarity in nematode assemblage between sites Fl-6 and Fl-7. Seven nematode genera were collected from Fl-7 and three from Fl-6 (Fig 4A); however, major nematode genera (genera collected in higher numbers) were the same, *Helicotylenchus*, *Tylenchorhynchus*, and *Hemicycliophora* (Table 2), explaining the similarity in nematode assemblage between these two sites.

Assessment of nematode assemblage across the native and invasive ranges of hygrophila demonstrated that significantly higher densities of phytoparasitic nematodes are associated with roots of this weed in its native habitat; however, no differences in nematode diversity were observed. This similarity of nematode diversities may indicate that in both exotic and native ranges, hygrophila can act as an alternative host to major phytoparasitic nematode genera. As mentioned earlier, except for two sites (Ind-2 and Ind-8), all samples in India were collected from natural areas; however, many of these sites were in close proximity to agriculture fields. In contrast, all sampling sites in Florida were in natural areas and not close to any agriculture sites. This observation suggests that proximity to agriculture fields may explain the higher density of nematofauna observed and also indicates that stable populations of phytoparasitic nematodes may be present in the root zone of hygrophila in India.

For both regions, the nematodes collected from the rhizosphere of hygrophila are considered pests of important agricultural and horticultural crops (Table 1 and 2). For instance, the rice blind root knot nematode *Hirschmanniella oryzae* Van Breda de Hann, lance nematode *Hoplolaimus indicus* Sher, and stunt nematode *Tylenchorhynchus mashhoodi* Siddiqi and Basir, collected in the native range of hygrophila are considered as major pests of rice (*Oryza sativa* L.) (CABI 2005). In Florida, species of the sheath nematode *Hemicycliophora* de Man, lance nematode *Hoplolaimus* Sher, as well as the stunt nematode *Tylenchorhynchus* Cobb are known to be important crop pests (Anderson et al. 1991, Fortuner and Nickle 1991, CABI 2005).

Previous studies have shown that invasive weeds can act as alternate hosts for important crop pests, including fungal pathogens (Wisler and Norris 2005) and insects (Seal 2004) as well as nematodes (Davis et al. 2006). In particular, Davis et al. (2006) demonstrated that the invasive weed tropical spiderwort (*Commelina benghalensis* L., Commelinaceae) can act as an alternate host for the peanut root knot nematode (*M. arenaria* [Neal] Chitwood). The high densities of phytoparasitic nematodes found in the root zone of hygrophila, particularly in its native range, suggest that this weed could act as an alternative host of these important plant parasitic nematodes. Further studies involving inoculation with phytoparasitic nematodes to assess performance of hygrophila as a susceptible host plan can provide further insight about its suitability as a transitional or alternative host. Overall, this study, demonstrated for the first time the root association of plant pest nematodes with the invasive weed hygrophila.

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Spatial and temporal variation in duckweed and filamentous algal levels in contiguous floodplain lakes of the Upper Mississippi River

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ABSTRACT

This study examined how free-floating macrophyte cover (principally composed of duckweeds [*Lemna* spp.]) and prevalence of floating filamentous algal mats (metaphyton) varied within and among lakes within three reaches of the Upper Mississippi River. Data were collected using standard sampling approaches over the period 1998 to 2008. Duckweed cover varied primarily within and among lakes; in comparison filamentous algae prevalence varied primarily among lakes and lake-years. Duckweed cover increased with submersed aquatic vegetation (SAV) abundance at within-lake and among-lake-year scales; in comparison, filamentous algae prevalence increased with SAV abundance at within-lake, among-lake and year scales. Given adjustment for SAV, filamentous algae prevalence decreased with increasing lake connectivity but was not statistically associated with annual changes in mean river discharge; duckweed cover was not associated with either connectivity or discharge. Documenting the relatively high levels of variation within lakes and of year-to-year variation in lake means improves our understanding of the dynamic nature of aquatic plant and algal communities in the Upper Mississippi River and will assist efforts to manage or control aquatic plants and nuisance algae in this region. In particular, this work explicitly characterizes sources of variability in free-floating macrophyte cover and filamentous algae prevalence, and highlights how this variation may complicate efforts to evaluate the short-term success of management and control efforts.

Key words: free-floating aquatic macrophytes, Lemnaceae, metaphyton, submersed aquatic vegetation, variance components

INTRODUCTION

High levels of free-floating aquatic plants, including *Lemna* and *Azolla* species and filamentous algal mats, may have profound effects on aquatic ecosystems and may substantially influence food web structure, biogeochemical cycles, and the recreational use of freshwater systems (Janes et al. 1996, Scheffer et al. 2003, Pinto et al. 2007, Saunders 2009, Fontanarrosa et al. 2010).

Filamentous algae often form conspicuous mats attached to substrates or submersed aquatic vegetation (SAV) or float below or near the water surface. Algal mats that originate beneath the water surface, referred to as metaphyton (Howell et al. 1990, Wetzel 2001), may become suspended by wind-induced circulation (Wetzel 2001) or when trapped gases accumulate and float them to the surface (Saunders 2009). The establishment of metaphyton and its subsequent accumulation in littoral or pelagic regions is common in temperate eutrophic or acidic lakes (Howell et al. 1990, Makarewicz et al. 2007). Algal mats and free-floating macrophytes like duckweeds have been associated with thermal characteristics of water bodies, decreased SAV growth, and decreased dissolved oxygen concentrations (Dale and Gillespie 1977, Phillips et al. 1978, Jones et al. 2002, Morris et al. 2003, Parr and Mason 2004, Hilton et al. 2006).

The ability of free-floating plants and algae to absorb insolation and reduce incident light likely causes the most dramatic impact on other portions of the aquatic plant community (Giorgi et al. 2005). Specifically, low light availability may decrease SAV growth and photosynthetic rates, biomass, richness, and alter community composition (Phillips et al. 1978, Jones et al. 2002). Decreased SAV photosynthesis may, therefore, lead to changes in dissolved oxygen and pH. Because photosynthetically active SAV may substantially increase water pH (Spencer et al. 1994), reductions in photosynthetic activity may influence aquatic chemistry and the activity of epiphytic microorganisms (Eriksson and Weisner 1999). Finally, decreases in dissolved oxygen associated with

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the decline of SAV can be detrimental to other aquatic life (Rooney and Kalff 2000, Morris et al. 2003) and may facilitate sediment phosphorus release (e.g., James et. al. 1995), promoting further epiphytic and metaphyton growth.

Despite their competitive interactions with and potential negative effects on SAV, the presence of filamentous algal mats in aquatic ecosystems is often dependent on SAV surfaces for establishment. Filamentous algae and SAV vary in composition and abundance in both space and time (Rooney and Kalff 2000, Ray et al. 2001), and these changes can be accelerated by cultural eutrophication (Cristofor et al. 2003, Rasmussen and Anderson 2005).

A natural choice for studying scales of variation of filamentous algae and duckweed at small to medium spatial scales is that of contiguous floodplain lakes (hereafter backwater lakes or lakes). Backwater lakes of large floodplain rivers often differ substantially in limnological properties, including in depth, water clarity, trophic status, and vegetation biomass. For example, comparative studies of backwater lakes have shown that these properties may be influenced by connectivity with channels and local morphometry (Heiler et al. 1995, Knowlton and Jones 1997, Van Geest et al. 2003). Roozen et al. (2003) studied associations between the dependent variables vertical light attenuation, inorganic suspended solids and chlorophyll *a* and the potential predictors of SAV cover, presence or absence of floating vegetation, and lake depth using data from 93 lakes from the lower Rhine River.

This study evaluated variation in duckweed cover (family Lemnaceae) and filamentous algae occurrence at multiple spatiotemporal scales in backwater lakes in three reaches of the Upper Mississippi River (UMR). We estimated variation for both taxa groups within lakes, among lakes and among lake-years. Associations between filamentous algae and SAV at multiple scales were also investigated.

The scale-related focus of this study has implications for aquatic plant management. Artificial manipulation of filamentous algae (metaphyton) or free-floating macrophyte levels is logistically most efficient when applied at moderately large spatial scales (e.g., addressing loading and considering management considerations at the spatial scale of backwater individual lakes; Zohary et al. 1998, Makarewicz et al. 2007). However, selecting lakes with, for example, high and persistent metaphyton or free-floating macrophyte levels using data may be challenging when metaphyton or macrophyte levels vary substantially either within lakes or across years (for a given lake) or both. Further, evaluation of the success of any such intervention will need to acknowledge and address both sources of variation.

MATERIALS AND METHODS

Study regions. Vegetation data were collected by the Long Term Resource Monitoring Program (LTRMP; Johnson and Hagerty 2008) from backwater lakes in three reaches of the UMR. Backwater lakes were defined based on enduring geomorphic and physical features (Wilcox 1993) and quantified from 1989 aerial photography using a geographic information system (GIS). Lakes not connected to channels during typical summer water surface elevation were not sampled. The reaches represent Navigation Pool 4 below Lake Pepin, Minnesota (river mile 753 to 765); Navigation Pool 8 located near La Crosse, Wisconsin (river mile 679 to 702.5); and Navigation Pool 13 located near Bellevue, Iowa (river mile 522.5 to 557; Figure 1). Lower Navigation Pool 4 and Navigation Pools 8 and 13 are hereafter denoted reaches 1, 2, and 3, respectively.

For this study, individual backwater lakes represent subregions within each backwater region of the three reaches, with lakes defined as units separated by channels and terrestrial areas (Figure 2) and delineated using Arc/Info Grid command "regiongroup" (ESRI 1991). Backwater lakes defined using this method may include very small bodies of water (e.g., 0.01 ha) that are well connected to channels. While such water bodies may be better described as bays or even channel edges, we use "lake" throughout to describe the full range of backwater units.

Sampling design. The sampling frame for each reach was defined by laying a square north-south and east-west grid over a reach-specific GIS coverage of backwater lakes; the grids had spacing of 50 m on each side. Each grid intersection represented a member of the population of possible sampling sites from which actual sampling sites were selected at random. The probability of sampling a given backwater was proportional to the area of the backwater. Sampling began in 1998 and continued through 2008. With the exception of years 2001 to 2004 in reach 2 (when sites were revisited), sampling sites were reselected each year. Sampling events were completed within 20 to 59 days, beginning as early as 15 June and ending as late as 31 July. Sampling plots were defined as a 2 m ring around the boat used for sampling (Figure 3); such plots represent the rectangular analogue of the square doughnut plot defined by Thompson (2002, p. 280).

Duckweed. Duckweed levels represented the estimated proportion of the above-mentioned 2 m ring that was covered by duckweed species. Cover assignments were categorical, with scores 0 through 5 denoting covers of 0%, 1 to 20%, 21 to 40%, 41 to 60%, 61 to 80%, and 81 to 100%, respectively. Duckweed is used here to generally describe the free-floating macrophyte community and included common duckweed (*Lemna minor*), star duckweed (*L. trisulca*), common duckmeat (*Spirodela polyrhiza*), and Columbian watermeal (*Wolffia columbiana*). Rare occurrences of Carolina mosquitofern (*Azolla caroliniana*) and slender liverwort (*Riccia fluitans*) were also included in this description of the free-floating assemblage of macrophytes we refer to as "duckweed." Taxonomic nomenclature used here follows that in the PLANTS database (USDA-NRCS 2011). Further vegetation sampling details are provided by Yin et al. (2000).

Filamentous algae. Filamentous algae were recorded using visual and rake methods at six approximately equidistant locations (subsites) located within the sample plot (Figure 3). The visual method was implemented prior to the use of the rake method and consisted of visual inspection from the boat of the intended rake location. Visual inspections were scored as either "present" or "not detected" (failure to detect algae may result from absence of algae or from a false negative). Rake surveys consisted of sweeping the substrate within an area of approximately 1.5 m by 0.36 m using a modified garden rake (Yin et al. 2000). The tines of the rake were marked to create five categories, with successive categories denoting increased proportions of rake teeth filled by biomass. These

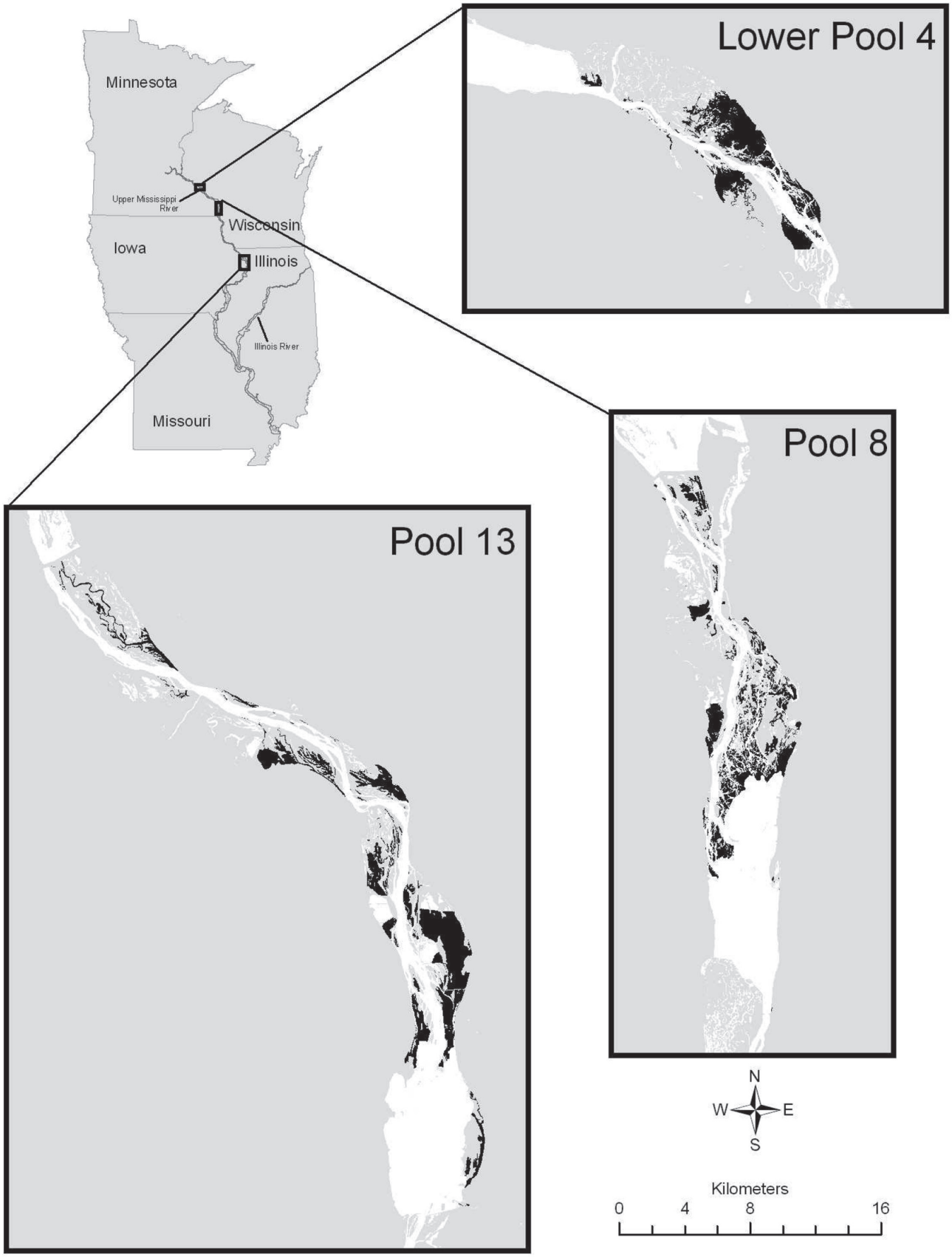

Figure 1. Location of study reaches for duckweed and filamentous algal levels in backwater lakes of the Upper Mississippi River, USA. Backwater lakes are depicted in black.

Figure 2. Illustration of typical backwater lakes (black) with respect to channels (gray) in the Upper Mississippi River floodplain.

proportions, along with their corresponding categorical scores, correspond to those listed above for duckweed cover (i.e., the "0" category corresponds to no algae observed on the rake). We analyzed filamentous algae as an indicator variable, with the indicator denoting present when algae was observed visually or by raking or as not detected, because only 0.3% of rake scores exceeded 1, and because visual scores were confined to present and not detected only. Filamentous algae were not identified at the species level during our surveys, but work in 2009 and 2010 from study reaches revealed filamentous algae assemblages composed of species from *Cladophora*, *Spirogyra*, *Oedogonium*, *Mougeotia*, *Lyngbya*, *Hydrodictyon*, *and Microspora* genera; *Cladophora* species were most common in both years (S. Giblin, Wisconsin Department of Natural Resources, 29 June 2010, pers. comm.). We use filamentous algae synonymously with metaphyton because some filamentous algal mats originate below the water surface on benthic or SAV substrates (Saunders 2009).

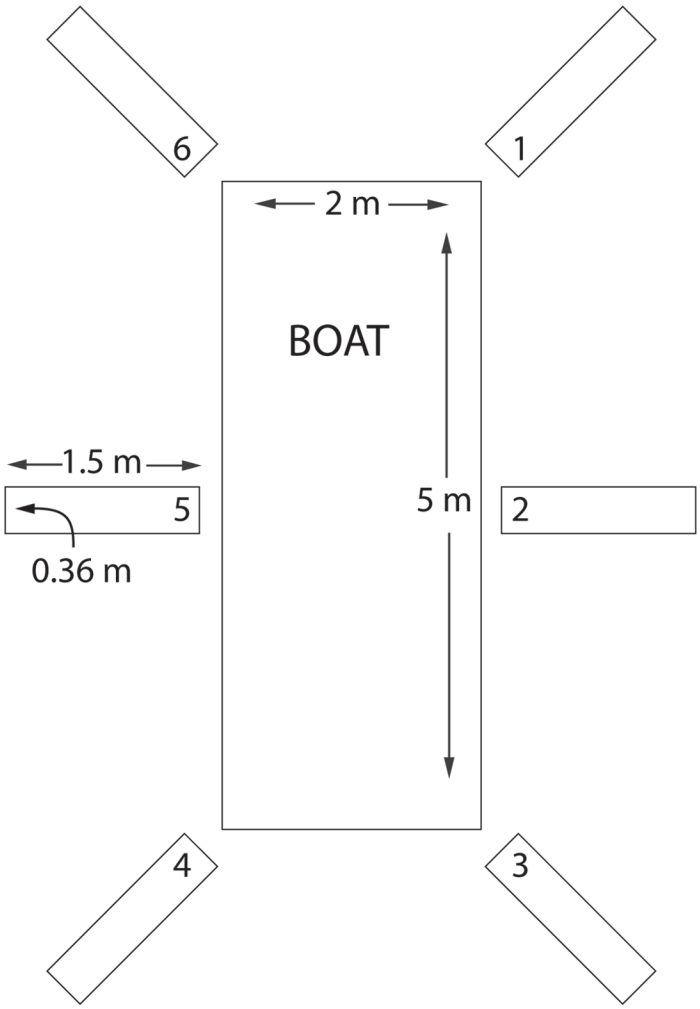

Figure 3. Diagram of sampling plot used by the Long Term Resource Monitoring Program for vegetation measurement. Six surveys were taken at roughly equal distances within a rectangular "doughnut," with "doughnut hole" defined by the boat.

Submersed aquatic vegetation (SAV). SAV was surveyed using the same rake method and ordered rake scores as those described for algae. We estimated SAV species richness at each site from the SAV rake survey and visual measurements at each subsite.

While SAV should be presumed to be sampled by the rake method with classification errors, the effects of those errors on inferences from SAV rake data are not wholly clear. While vegetation rake score data have been treated using means of scores (e.g., Kenow et al. 2007), ordinal data in general are often presumed to best be modeled under ordinal multinomial assumptions (Fielding et al. 2003). Despite this, methods for elaborating multinomial models to accommodate classification errors (Royle and Link 2005, Holland and Gray 2010, Holland et al. 2010) make assumptions that the current study's data may not fulfill. These assumptions are that individual surveys represent replicates on site, that the highest rake score is observed without error, and that published methods for singly nested data may be extended for use with data from cross-classification (e.g., lake-year) designs. Hence, where a single rake value was required at a scale larger than that at which SAV was measured, we followed conventional methods by using mean rake scores.

Lake connectivity and discharge. Lake connectivity was defined as the percentage of each lake's perimeter (including channel connections) that was channel. This surface connectivity measure does not address actual discharges of water into and out of a lake but is expected to explain a substantial fraction of the variability in water exchange rates among lakes. Large values of this metric denote highly connected lakes.

Discharge represents the mean Mississippi River discharge prior to each annual sampling period at the gauging station closest to each study reach. For reaches 1, 2, and 3, these were Prescott, Wisconsin (US Geological Survey [USGS] 05344500); Winona, Minnesota (USGS 05378500); and Clinton, Iowa (USGS 05420500), respectively.

Statistical analyses. Binary algal data were modeled using logistic regression while the ordered duckweed cover and SAV rake data were modeled using cumulative logistic regression (Hosmer and Lemeshow 2000). For variance components and covariate analyses (but not for descriptive statistics), the 7% of SAV rake scores ≥2 were treated as 2s. SAV richness was modeled as a Poisson-distributed random variable with log link. Covariate associations with metaphyton were assessed using data from reach 2, with the forms of those associations (such as linear and quadratic) inferred from reach 1 data. Due to small sample sizes, the covariate associa-

tions at the lake and lake-year scales estimated for duckweed cover and possibly for filamentous algae may be too small in magnitude (Grilli and Rampichini 2011). Models were fitted using maximum likelihood (Vonesh 1996, Givens and Hoeting 2005), with random site, lake, year, and lake-year effects treated as normally distributed on the log (richness) or logit (else) scales. Lake-year variance estimates for duckweed cover incorporated not only lake-year variation but also site-year variation because sites were not typically revisited, duckweed cover was measured at the site but not subsite scale, and sampling variation of duckweed cover was assumed constrained to that of a standard logistic random variable. Estimates of "among-site variation" represented not only spatial variation among sites but also variation at spatial scales that were intermediate between site and lake scales. For brevity, however, we treated among-site variation and within-lake variation as synonymous. Variance components from logistic regression models were estimated under a latent logistic assumption (Snijder and Bosker 1999), while variance components for SAV species richness were estimated on the log scale. Spatial correlation within sites, lakes, and lake-years was broadly addressed by treating each as random. Dataset limitations precluded more extensive treatment of spatial correlation. Models were fitted using SAS' generalized linear mixed modeling procedure (GLIMMIX procedure; SAS 2009).

The nature of dependency is not always clear when estimating associations between metaphyton and SAV. As our models imply, filamentous algae and duckweed are dependent on SAV to provide substrate and protection from wind and current, respectively. However, the opposite may also be true; filamentous algae and duckweed compete with SAV for light and, for some SAV species, with nutrients, and such competition will increase with cover and density of filamentous algae and duckweed. For the study sampling periods of our study data, however, levels of filamentous algae and duckweed typically reached their maxima subsequent to LTRMP vegetation sampling. Hence, we treated SAV as a predictor of filamentous algae presence and duckweed cover.

RESULTS AND DISCUSSION

Descriptive statistics. Study data contained relatively large numbers of lakes and lake-year combinations, and for reaches 1 and 3, mean numbers of sites sampled per lake (Table 1). However, median numbers of sites per lake and mean and median numbers of sites per lake-year were low (<8) in all reaches. Fewer sampling sites per lake in reach 2 reflect, in part, smaller lakes in that reach. Correlations between number of sites per lake and lake area were high for all reaches (r = 0.96, 0.81, and 0.93; n = 39, 150, and 46; and $p < 0.0001$ for reaches 1, 2 and 3, respectively).

Mean duckweed cover, filamentous algae, SAV rake cover score, and SAV species richness values were typically low (Table 2). For example, mean and median duckweed cover proportions fell in the 1 to 20% category and the 0% category, respectively.

As may be expected from the subsite statistics (Table 2), the proportion of sites without algal detections was high. When filamentous algae was detected at a site, however, it was more frequently detected at all rather than only some subsites (Table 3), suggesting clustering of filamentous algae within sites. An example of clustering is when the probability of algal presence at a site is low, but if present has a higher than expected probability of being present at most or all locations within that site. This topic may be addressed by comparing observed probabilities of algal detection at sites with those calculated from subsite probability estimates. The site-level estimates were 0.24, 0.32, and 0.28 for reaches 1, 2, and 3, respectively; however, the corresponding estimates from the subsite estimates (Table 2) are much larger: 0.60, 0.74, and 0.67, respectively (obtained under assumptions of independence and identical distribution by $1 - (1 - \hat{p})^6$, where $\hat{p}$ denotes the subsite scale probability estimate from Table 2 and "6" the number of subplots per site). The differences among these sets of values seems to be explained by mismatches between observed and expected relative count frequencies. Given the relatively small subsite-level probability estimates and properties of the binomial distribution, we would expect roughly equal proportions (~35%) of 0s and 1s, roughly 20% 2s, fewer than 2% 4s, and <0.2% of counts of 5 or 6. However, the proportions of sites where algae was detected 1, 2, or 3 times were considerably lower than these expected proportions while the proportions of sites where algae was detected more than 3 times was considerably higher than expected (Table 3). These findings may reflect a conditional process. Filamentous algae is rarely found at sites but, when present it is often found at a majority of locations within that site. We did not model this conditional process and suspect that not doing so accounted for our failure to successfully model unexplained variation in algae at the site scale (see next section).

Mean lake connectivity was highest in reach 2 whereas discharge increased downriver and, hence, with reach number (Table 2). The finding of higher connectivity in reach 2 is related to the finding that lakes in that reach are typically smaller (Table 1) and that many of these small lakes are channel border-like or bay-like.

Variance component estimates. Duckweed cover varied most among sites in reaches 1 and 2 and among lakes and sites in reach 3 (Table 4). Contributions of year, lake-year, and lake (reaches 1 and 2 only) effects to variation in duckweed cover levels were modest. From an across-reach perspective, duckweed cover varied most among lakes in reach 3, while lake levels of duckweed cover were most stable among years in reach 1.

Table 1. Sample sizes by spatial unit and study reach for a study of duckweed and filamentous algal levels in backwater lakes of the Upper Mississippi River.

Number of	Reach 1	Reach 2	Reach 3
Lakes sampled	39	150	46
Lakes sampled per year (range)	13-23	44-62	18-30
Unique lake × year combinations	182	567	249
Sites per lake (all years; mean/median)	37.1/4	10.3/3	37.4/5.5
Sites per lake per year (mean/median)	7.9/2	2.7/1	6.9/3
Total sites	1446	1544	1722
Area (mean [SE[a]]/median) (ha)	46 [24]/4.8	12 [3]/2.6	60 [22]/8.2

[a]Standard error.

Table 2. Descriptive statistics for vegetation variables and their potential covariates by reach from a study of duckweed and filamentous algal levels in backwater lakes of the Upper Mississippi River.[a]

Variable	Unit	Scale of variation	Reach 1		Reach 2		Reach 3	
			Mean (SE[b])	Median	Mean (SE)	Median	Mean (SE)	Median
Duckweed	Mean percent cover[c]	Site	13 (0.7)	0	15 (0.7)	0	6 (0.4)	0
Filamentous algae	Mean probability of detection	Subsite	0.14 (0.004)	0	0.20 (0.004)	0	0.17 (0.004)	0
Submersed aquatic vegetation (SAV)	Mean percent rake teeth filled[c]	Subsite	10 (0.2)	10	13 (0.2)	10	10 (0.2)	0
		Site	10 (14[d])	8	13 (16[e])	10	10 (15[e])	5
		Lake	13 (15[d])	9	9 (8[e])	8	5 (8[e])	2
		Year	11 (7[d])	9	14 (4[e])	13	10 (3[e])	10
		Lake-year	11 (13[d])	8	11 (11[e])	8	7 (12[e])	2
SAV species richness	Number species detected	Subsite	1.55 (0.02)	1	1.74 (0.02)	1	1.08 (0.01)	0
Connectivity	% channel	Lake	17.6 (3.1)	6.8	29.7 (2.1)	25.0	14.4 (2.8)	5.0
Discharge	1000 cfs	Year	21.1 (3.2)	18.4	35.4 (6.3)	29.4	54.2 (6.9)	48.4

[a]Not adjusted for group effects. [b]Standard error. [c]Means of midpoints of cover classes or rake score classes. [d]Standard deviation.

Filamentous algae varied most among lake-years, subsites, and possibly sites in reaches 1 and 2 and most among lakes and subsites in reach 3. Failure to adequately model variation among sites for filamentous algae (Table 4, footnote "d") ensured that a fraction of the estimates of among lake-year variation for filamentous algae arose from the site scale. As with duckweed cover, contributions from year effects to variation in algae detection were modest for all reaches.

Patterns in relative variance components of SAV rake scores were qualitatively similar to those seen for duckweed cover: highest at the site scale in reaches 1 and 2 and highest at the lake scale in reach 3 (Table 4). Patterns for SAV species richness were broadly similar to those seen with SAV rake scores.

Covariate associations (reach 2). Duckweed cover was positively associated with SAV levels and, specifically, with mean SAV rake score at the site and lake-year scales, based on comparison of -2 log likelihood and Akaike Information Criterion (AIC) values (Table 5) and inspection of point estimates with confidence intervals (Table 6). For example, the odds of a higher observed duckweed cover class at a typical site increased by approximately 300% for each 1-unit increase in mean SAV rake score. This value may be derived by subtracting 1 from the odds ratio point estimate of 4.18 and multiplying by 100% [i.e., 100 × (4.18 - 1)]. The corresponding estimate for a unit increase in mean lake-year rake score was 73%. In contrast, duckweed cover was not clearly associated with connectivity or discharge (Table 4 and 5).

The odds of detecting filamentous algae at the subsite scale increased when SAV was present at that same scale (Table 6). Specifically, the odds of detecting filamentous algae at the subsite scale given failure to detect SAV at that scale (i.e., of rake score = 0) was 73% lower than the corresponding odds given SAV rake score = 2 [i.e., 100 × (0.27 - 1)]. The odds of detecting algae appeared similar at rake score = 1 and at rake score ≥ 2.

The odds of detecting filamentous algae also increased substantially as mean SAV rake score increased at site, lake and year scales. Confidence intervals for the algae-SAV site-scale association were probably too narrow, owing to our failure to adequately model variation in algae at that scale (Table 4). The odds of algal presence decreased with lake connectivity but were not clearly associated with discharge (Table 5 and 6). SAV rake score was not clearly associated with either lake connectivity or discharge.

Duckweed cover considerations. The relatively high levels of unexplained variation in duckweed cover among sites in reaches 1, 2, and, to lesser extent, reach 3 suggest efforts to identify sources of that variation (Table 4). Given important associations between duckweed cover and SAV (Table 5 and 6), factors contributing to the abundance of SAV (e.g., sediment organic matter content; Makkay et al. 2008) may represent useful predictors of duckweed cover. Other factors associated with variation in duckweed cover include water velocity, local wind patterns, and recreational water use (Portielje and Roijackers 1995, Mumma et al. 1996, Rooney and Kalff 2000, Makarewicz et al. 2007, Makkay et al. 2008).

Efforts designed to manage nuisance levels of duckweed must consider the range and scale of factors that contribute to the distribution and abundance of plants within lakes.

Table 3. Proportions of sites with given number of filamentous algae detections by reach as measured in backwater lakes of the Upper Mississippi River.

	Proportion of sites with given number of detections per subsite (maximum = 6)						
Reach	0	1	2	3	4	5	6
1	0.761	0.057	0.036	0.025	0.021	0.018	0.082
2	0.676	0.076	0.042	0.034	0.029	0.037	0.105
3	0.725	0.060	0.038	0.034	0.023	0.036	0.084

While our results do not preclude the possibility of successful whole-lake interventions to control duckweed cover in reaches 1 and 2 in lakes with high cover levels, our results do suggest that efforts to document that success in typical lakes may be hampered by relatively high levels of within-lake variability and, for reach 2, of among-year variability (Table 4). The relatively high among-lake variance estimate for duckweed cover in reach 3 suggests that efforts to document the effects of duckweed management in typical lakes in that reach will, on average, be more successful than will similar efforts in reaches 1 and 2.

Duckweed cover in reach 2 was associated with mean SAV rake score not only at the site scale but also at the lake-year scale (Table 6). The latter association, given failure to observe a duckweed cover-SAV association at the lake scale, may have arisen from annual fluctuations in the lake-specific importance of SAV levels (which might occur, for example, if the prevalence of strong winds varied among study years and fetch varied among lakes) or by annual fluctuations in lake-specific nutrient and other limnological characteristics that might affect both duckweed cover and SAV. Another plausible explanation is related to the sampling design; sites were revisited in only a minority of years (2000-2003) and in only reach 2. Consequently, the observation of lake-year effects will reflect to at least some degree the selection of different sites within lakes in different years.

A final comment about duckweed addresses commonality among patterns of variation. Correlation between variance components for duckweed and SAV rake scores among reaches, as seen with duckweed cover and SAV rake scores, for example, does not necessarily imply correlation at smaller scales (Table 4). If duckweed coverage, for example, increased with SAV levels at the within-lake scale but SAV levels varied more among than within lakes (perhaps because nutrient levels were broadly constant within lakes), then duckweed levels will be observed to vary primarily among lakes, even if dependence primarily occurred at the within-lake scale (Table 4 and 6).

Filamentous algae considerations. Filamentous algae was seen to vary most at the lake-year scale in reaches 1 and 2 and at the lake scale in reach 3 (Table 4). As with duckweed cover, variation among lake-year means probably reflected some contribution of site selection. The patterns in variance components in algae did not mirror those seen with duckweed, possibly because filamentous algae mats may have been adhered to SAV and were submersed during sampling and/or there were low levels of internal gases inside the mats (Saunders 2009).

Table 4. Variance component percentages from intercept-only models of vegetation variables as measured in backwater lakes of the Upper Mississippi River.

	Variance percentages by scale [point estimate (standard error[a])]				
Reach	Subsite	Site	Lake	Year	Lake-year
			Duckweek cover		
Reach 1	na[b]	59% [3.29[c]]	25% [1.37 (0.66)]	15% [0.82 (0.40)]	1% [0.06 (0.06)]
Reach 2	na[b]	53% [3.29]	13% [0.77 (0.22)]	21% [1.31 (0.60)]	13% [0.79 (0.20)]
Reach 3	na[b]	32% [3.29]	42% [4.27 (1.70)]	14% [1.40 (0.71)]	12% [1.20 (0.36)]
			Filamentous algae		
Reach 1	32% [3.29]	na[d]	17% [1.74 (1.14)]	9% [0.97 (0.61)]	41% [4.25 (1.11)]
Reach 2	24% [3.29]	na[d]	20% [2.70 (0.88)]	14% [1.94 (0.90)]	42% [5.83 (0.82)]
Reach 3	25% [3.29]	na[d]	47% [6.29 (2.77)]	7% [0.87 (0.50)]	21% [2.86 (0.71)]
			SAV rake score		
Reach 1	9% [3.29]	50% [18.37 (1.27)]	28% [10.38 (4.24)]	10% [3.65 (1.83)]	4% [1.37 (0.66)]
Reach 2	16% [3.29]	53% [10.71 (0.65)]	20% [4.10 (0.94)]	7% [1.33 (0.62)]	4% [0.79 (0.32)]
Reach 3	8% [3.29]	25% [10.72 (0.67)]	54% [23.06 (7.60)]	na[d]	13% [5.62 (1.15)]
			SAV species richness[e]		
Reach 1	[0.24]	[1.63 (0.10)]	[0.99 (0.40)]	[0.13 (0.09)]	[0.26 (0.10)]
Reach 2	[0.23]	[0.83 (0.05)]	[0.53 (0.13)]	[0.12 (0.06)]	[0.10 (0.04)]
Reach 3	[0.29]	[0.91 (0.06)]	[4.96 (1.60)]	[0.30 (0.15)]	[0.40 (0.10)]

[a]Asympotic or large sample estimates. [b]Duckweed cover not measured at the subsite scale. [c]Variance of a logistic random variable (i.e.,$\pi^2/3$). [d]Models containing this term did not converge or yielded invalid estimates. [e]Log scale; sampling variance estimate derived after Gray and Burlew (2007), with multiplicative adjustment for under-dispersion with respect to a Poisson distributional assumption.

Table 5. Vegetation-covariate model statistics by vegetation type from a study of duckweed and filamentous algal levels in backwater lakes of the Upper Mississippi River. Smaller Akaike Information Criterion (AIC) values indicate models with greater support from the data; −2 log LL and AIC values cannot be compared across vegetation types.

Covariate(s)	Scale at which covariate(s) vary	Number of parameters	−2 log LL	AIC
	Duckweed cover			
None	na	4	3608.0	3624.0
SAV	All (site, lake, year, lake-year)	10	3396.6	3420.6
Connectivity	Lake	5	3606.6	3624.6
SAV, connectivity	All, lake	11	3396.3	3422.3
Discharge	Year	5	3605.6	3623.6
SAV, discharge	All, year	11	3394.8	3420.8
Connectivity, discharge	Lake, year	6	3604.2	3624.2
SAV, connectivity, discharge	All, lake, year	12	3394.5	3422.5
	Filamentous algae[a]			
None	na	4	6822.3	6830.3
SAV	All (subsite, site, lake, year, lake-year)	10	5915.2	5935.2
Connectivity	Lake	5	6814.8	6824.8
SAV, connectivity	All, lake	11	5906.9	5928.9
Discharge	Year	5	6821.9	6831.9
SAV, discharge	All, year	11	5915.0	5937.0
Connectivity, discharge	Lake, year	6	6814.3	6826.3
SAV, connectivity, discharge	All, lake, year	12	5906.7	5930.7
	Submersed aquatic vegetation rake scorea			
None	na	8	15608.6	15618.6
Connectivity	Lake		15608.1	15620.1
Discharge	Year		15608.3	15620.3
Connectivity, discharge	Lake, year		15607.8	15621.8
	Species richness (SAV)			
None	na	5	26073.6	26083.6
Connectivity	Lake	6	26073.5	26085.5
Connectivity×yr	Lake, lake×yr	7	26067.6	26081.6
Discharge	Year	6	26073.1	26085.1
Connectivity, discharge	Lake, year	7	26073.0	26087.0

[a]Excludes an among-site variation in intercept term (cf., model presented in Table 4).

Associations in reach 2 between filamentous algae at the subsite scale and SAV rake score were seen at all study scales, other than possibly that of lake-year (Table 6). Associations at scales other than the subsite scale imply contextual effects, suggesting that algal levels would depend not only on SAV at the measured or subsite scale but also at larger or "context" scales. The implication is that the probability of the presence of algae increased not just when SAV was present at the scale at which algae was measured but also when SAV was present at other locations: other subsites within the same site and other sites within lakes, years, or lake-years. SAV at other locations within a site and at nearby sites may affect hydraulics (principally water velocities; Gregg and Rose 1982, Wilcock et al. 1999) and nutrient levels (Scheffer et al. 2003) at the measurement or subsite scale.

Contextual effects may be better understood by considering year and lake effects. Previous investigations have noted associations between variation in SAV and among-year variation in growing season temperature, hydrologic condition, nutrient loading and, to lesser extent, recreational use (Mumma et al. 1996, Rooney and Kalff 2000, Biggs et al. 2005, Makarewicz et al. 2007). Similar associations have been seen for lake-scale potential predictors, including lake morphometry, connectivity, and size (Rooney and Kalff 2000, Ray et al. 2001). However, absent contextual effects, we would expect these reported year and lake associations to be primarily expressed at the spatial scale at which filamentous algae attaches to SAV because filamentous algae often uses SAV surfaces for establishment (Wetzel 2001). However, the lake- and year-scale effects found in the current study (Table 6) reflect associations that were adjusted for such local-scale effects (at subsite and site scales). Hence, metaphyton levels appear to have changed not only locally with SAV but also when SAV changed at larger scales. As mentioned earlier, such changes might be associated with changes in hydrology that were associated with changes in SAV abundance at those larger scales.

Filamentous algae decreased with increasing connectivity but was not clearly associated with discharge (both adjusted for SAV rake score levels; Table 6). Understanding how connectivity affects the different growth forms of the aquatic plant community may be important when devising management options for nuisance levels of filamentous algae or free-floating macrophytes (Barko et al. 1986). For example, artificially manipulating connectivity between lakes and channels

Table 6. Estimated covariate associations by vegetation outcome (from full or largest model defined in Table 5) from a study of duckweed and filamentous algal levels in backwater lakes of the Upper Mississippi River.

Covariate(s)	Scale at which covariate varies	Units	Odds ratio estimate (95% CI[b])
		Duckweed cover	
SAV	Site	Mean rake	4.18 (3.17, 5.51)
	Lake	Mean rake	0.73 (0.34, 1.59)
	Year	0.25 mean rake	2.07 (0.89, 4.84)
	Lake-year	Mean rake	1.73 (1.01, 2.96)
Connectivity	Lake	10 points	0.97 (0.87, 1.08)
Discharge	Year	1000 cfs	0.99 (0.97, 1.01)
		Filamentous algae	
SAV	Subsite (rake = 0)	Rake	0.27 (0.17, 0.41)
	Subsite (rake = 1)	rake	0.93 (0.74, 1.17)
	Subsite (rake > 2)	na	Reference
	Site	Mean rake	5.26 (4.03, 6.85)
	Lake	Mean rake	4.48 (1.36, 14.76)
	Year	0.25 mean rake	5.44 (2.44, 12.12)
	Lake-year	Mean rake	1.58 (0.72, 3.47)
Connectivity	Lake	10 points	0.79 (0.67, 0.93)
Discharge	Year	1000 cfs	1.004 (0.986, 1.022)
		Submersed aquatic vegetation rake score	
Connectivity	Lake	10 points	0.958 (0.850, 1.081)
Discharge	Year	1000 cfs	0.996 (0.979, 1.013)
		Species richness (SAV)	
Connectivity	Lake	10 points	1.005 (0.940, 1.075)
Discharge	Year	1000 cfs	0.997 (0.988, 1.006)

[b]95% confidence interval; degrees of freedom estimated as number of units at which covariate varied (from Table 1) less the number of covariates that varied at that scale.

may lead to decreases in metaphyton prevalence without detrimentally affecting SAV levels.

Conclusions. The study data and methods seem broadly suitable for identifying lakes with free-floating macrophyte (e.g., duckweed) and metaphyton levels that are typically high. We recognize that managers interested in implementing duckweed or algae control strategies may be interested in identifying lakes that attain not only minimum average duckweed or metaphyton levels but that also exhibit relatively low interannual variation in those levels (Howard and Harley 1998). Such low levels of among-year variability, if persistent following a lake-level intervention (e.g., drawdown or herbicide treatment), would permit more rapid inference on the success of the intervention. Unfortunately, our design did not typically specify resampling of sites within years and therefore will typically preclude direct inferences on among-year variability within lakes.

A concern with this study is that we treated lakes as ecologically and statistically equivalent. Given the multiple ways in which lakes might vary and the small number of lakes in at least two of the three reaches (Table 1), the validity of these assumptions is not easily addressed. We did adjust for connectivity effects and, for free-floating macrophytes and algae, for SAV levels among lakes; however, lakes varied not only by connectivity and SAV levels but also by sample size, area, depth, contributions from springs, proximity to tributaries, and other variables. Given that the study data derived from few large lakes (Table 1), subsetting the data (e.g., by large and small lakes) will lead to further imprecision in variance component estimates. Hence, we were unable to fully address this concern.

The study's long-term variance component and scale-related foci make the study unique and yield findings of interest to aquatic plant managers. For example, whole-lake manipulations or herbicide applications are often considered the most efficient and cost-effective way to control nuisance or invasive macrophytes, restore native SAV stands, or enhance recreational use (Zohary et al. 1998, Makarewicz et al. 2007, Kovalenko et al. 2010); however, selection of management approaches for macrophyte or algal control requires a thorough understanding of the variation of macrophyte or algal levels across space and time. Here, we documented substantial levels of variation in free-floating macrophyte cover and filamentous algae at within-lake and, for filamentous algae, at lake-year scales. Given these results, we recommend that efforts to quantify and document factors that underlie variation in duckweed and algal cover (e.g., associations with SAV cover and lake connectivity) be completed prior to initiation of management actions. Failure to plan for spatial and temporal sources of variation in macrophyte or algal cover may compromise efforts to evaluate the success of management actions.

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