Final Report

An Exploration of the Use of Parasitic Nematodes for the Biological Control of Variable Leafed Milfoil (*Myriophyllum heterophyllum*)

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SECTION 1

Introduction and Project Objectives

While much attention is paid to the more common nuisance Eurasian Milfoil (Myriophyllum spicatum) and its control (Cooke et al 1993; NALMS 2001) Variable Leafed Milfoil (*Myriophyllum heterophyllum*; also referred to as Variable Milfoil) has received less attention as it is only problematic in a handful of states and native or naturalized in most others (Crow and Hellquist 2000). With limited success using herbicide treatments alone, interest has turned to biological control agents. Recent work on the biological control of Eurasian milfoil has focused on the indigenous weevil Euhrychiopsis lecontei (Creed and Sheldon 1995) and the aquatic larvae of the moth Acentria ephemerella (Johnson et al 1998). These works suggests that E. lecontei is the better possible control agent for the Eurasian species but not for others including M. heterophyllum (Creed and Sheldon 1995, Creed 2000).

Nematodes have been considered in the control of invasive terrestrial plants and insects, yet, no documented work has been done to investigate their potential for use on aquatic plants. This is quite surprising given both the history and acceptance of nematode use (see below) and the complications involved with developing insect biological controls (costs, rearing, effective egg positioning, predators and others; see Cofrancesco 2000). Nematodes are arguably the most diverse and certainly the most numerous metazoans in terrestrial soil and aquatic sediments (Heip et al., 1985; Lambshead, 1993; Yeates, 1981). They parasitize virtually all plants in every habitat including weeds (Maggenti, 1981; Nickle, 1981). Some are migratory ectoparasites, that can cause a simple growth reduction of upper parts and roots, others are migratory endoparasitic that can damage roots, leaves, seeds, or stems and bulbs, depending on the nematode involved, and some are sedentary endoparasitic of which the most common are root parasites that cause damage ranging from dark spots (lesions) on roots to a significant amount of root decay. These plant-nematode associations are often very specific (Nickle, 1981; Verschoor, et al., 2002) and can result in a variable degree of plant damage ranging from a tolerable effect to that totally detrimental to the plant species in question depending on the density and pathogenecity of the nematode species or race involved. Furthermore, nematodes, primarily endoparasites, interact with other plant pathogens, such as fungi and bacteria, to form disease complexes with serious synergetic effects on plant health (Gowen & Quénéhervé, 1990).

Although free-living nematodes are present in all types of limnetic habitats including extreme conditions, current studies on freshwater nematodes globally show extreme regional bias; those from the southern hemisphere are extremely underrepresented, especially compared to European freshwater bodies. Discussion of their biogeographic distribution is hampered largely by the regionally biased surveys conducted so far. Some regions are well studied compared to others: for example, the Palearctic region (more specifically Europe and Russian territories) is the most sampled zoogeographic region for freshwater nematodes. Compared to Europe, North American freshwater bodies are relatively unexplored in the context of nematode biodiversity inventory and dynamics. The total number of species reported from the well-studied freshwater environments in Europe is

currently nearly 1000. Very few researchers are working on freshwater nematodes, but, many new species are added every year. There is no reason to expect a different trend in other continents. The current total number of documented freshwater species is primarily a reflection of sampling effort rather than any genuine differences in regional richness. Nematode communities of USA freshwater bodies are little studied. The total number of genera and species reported from the entire continent of North America until recently were 66 and 300, respectively (Poinar, 2002). This is comparable to what has been reported from a relatively well-studied geographically small single country (Eyualem-Abebe, 2002). Of these 66 genera reported from within North America, 20 belong to the insect parasitising order Mermithida, the remaining 46 included free-living and potential plant parasitic forms.

Nematodes are useful and accepted biological control agents:

Nematodes are already widely used in many countries as biological control agents against pests, especially insect pests (Rosales et al., 1999). The advantages of using nematodes over chemical control agents are that they are non-polluting, can be mass-produced in vivo or in vitro requiring little space, and can be stored under room temperature without loosing their infectivity (Grewal, 2000; Ostrec, 2001). In addition, nematode use is a widely accepted method for commercial and home landscaping and gardening integrated pest management in the U.S. and so would not be expected to cause as much concern or objections from the general public as compared to viral, bacterial or fungal control agents. The current level of knowledge clearly indicates that no single measure is generally sufficient to control established weeds; therefore management strategies should attempt to integrate a number of different approaches of weed control to reduce weed impact (Johnson, 1996). The tactic we propose is an integrated pest management approach that involves and requires an understanding of the biology of those organisms (parasitic nematodes) that are candidate for the biological control of the target weed or pest and the interaction between the two. Exploring the association between nematodes in the sediment and variable milfoil can potentially contribute to a long-term control of this weed in New Hampshire waters. Whether alone or in conjunction with other stressors (as is currently being investigated on Hydrilla using a fungal parasite and the herbicide Endothal, Shearer and Nelson 2002) parasitic nematodes may prove to be a useful and cost-effective addition to an integrated approach to *M. heterophyllum* management.

The objectives of this project were:

To compare and characterize the plant and nematode communities along the with water chemistry and sediment conditions, associated with variable milfoil in its native range (Midwest US) and in New Hampshire lakes using traditional, molecular and genetic tools, and discover possible plant-nematode association that can be of use in the biological control of variable milfoil. We also planned to document the native and invasive plant associations for a better understanding of additional management options

The following were our specific goals:

a) Find *Myriophyllum heterophyllum* occurring in its native range and investigate its co-associations and interactions with other native plants. Determine if there are any typical associations that act to keep it in check and document the water quality and sediment conditions in which it is found.

b) Identify plant feeding and parasitic nematode species within the stem and rhizome of milfoil and associated plants in the different lake sediment environments. Particular emphasis was to be placed on the identification of nematode species that have the potential to be biological control agents.

c) Analyze the physical, chemical and biological characteristics of the water and sediment at native or naturalized sites and introduced sites. Particular emphasis was placed on nutrients, anion/cations, pH, alkalinity, and organic matter, in the water and sediments, and coexisting plant species diversity and extent.

Study Sites Selection Strategy

The New Hampshire Department of Environmental Services provided a listing of lakes in the state that had confirmed *M. heterophyllum* and were a high priority for research. However, many of the lakes listed had recently or were scheduled for herbicide treatment or hand pulling control measures. A different suite of lakes were chosen with priority focused on exploring for aquatic nematodes: old vs new infestations, range of productivity from low to moderate, variations in sediment type, and disturbed areas not treated with herbicide but mechanical control measures to create plant stress. Available data on Midwest lakes with confirmed *M. heterophyllum* populations was reviewed and candidate lakes were chosen based on similarity to NH lake conditions (productivity and total alkalinity being used as major factors). Table 1-1 lists all of the lakes surveyed for the study as well as the target species for that lake and the lake Total Alkalinity. It was a challenge to find a Midwest lake that contained *M. heterophyllum* as well as had low to moderate productivity and low alkalinity.

Table 1-1 Location of Study Lakes for the project, their location information, targeted plant species and Total Alkalinity in mg/L $CaCO_3$

Study Lake	County	Lattitude	Longitude	Target Species	Confirmed Species	Total Alkalinity
Wisconsin Lakes						
Round Lake	Chippewa	N 45º 15.657'	W 90º 26.965'	*M. heterophyllum*	*M. heterophyllum*	8.0
Rock Dam Lake	Clark	N 144º 43.980'	W 90º 50.624'	*M. heterophyllum*	*M. heterophyllum*	15
Michigan Lakes						
Platte Lake	Benzie	N 44º 41' 05.8"	W 86º 03' 43.3"	*M. heterophyllum*	*M. heterophyllum*	369
Black River Lake Outlet	Cheboygen	N 45º 29.982'	W 84º 19.621'	*M. heterophyllum*	*M. heterophyllum*	182
Long Lake	Preque Isle	N 45º 13.271'	W 83º 30.281'	*M. heterophyllum*	*M. heterophyllum*	128
Bass Lake	Benzie	N 44º44'12.7"	W 86º03'54.9"	*M. heterophyllum*	*M. heterophyllum*	---
New Hampshire Lakes						
Back Bay, Winnipesaukee	Carroll	N 43° 35.145'	W 71°12.784'	*M. heterophyllum*	*M. heterophyllum*	6.8
Balch Pond	Carroll	N 43° 37.428'	W 70° 58.721'	*M. heterophyllum*	*M. verticillatum*	8.0
Crescent Lake	Carroll	N 43° 35.334'	W 71° 11.950'	*M. heterophyllum*	*M. heterophyllum*	7.0
Swain's Lake	Strafford	N43° 11.205'	W 71° 1.607'	*M. humile*	*M. humile*	2.3
Wentworth Heath Embayment	Carroll	N 43° 35.283'	W 71° 10.100'	*M. heterophyllum*	*M. heterophyllum*	7.3
Suncook Lake, Lower	Belknap	N 43° 22'23"	W 71°12.784'	treated for VWM previous year		4.1
Suncook River, Broads	Belknap	N 43° 20.394'	W 71° 16.642'	drawdown occurred		---
Squam Lake	Grafton	N 43° 46.563'	W 71° 32.921'	*M. heterophyllum*	*M. heterophyllum*	6.4
Squam River	Grafton	N 43° 42.690'	W 71° 37.324'	*M. heterophyllum*	*M. heterophyllum*	6.2

Note: some lakes were just sampled for Nematodes or used for plant community surveys. Due to the very recent infestations and an active control programs in place before we received approval to start our study the Squam Lakes sites were dropped. Nematode sampling on those two sites did not yield many species for study. While Balch Pond was initially targeted for *M. heterophyllum* we kept it in the study to allow for comparisons of Nematode communities between the invasive and native species of the same genus.

SECTION 2

Plant Communities Associated with *Myriophyllum heterophyllum* (Variable Milfoil)

Approach:

Our intent was to compare and contrast at least 5 lakes in the Midwest as well as 5 in New Hampshire. Final field work interpreted here involved successfully sampling 5 lakes in the Midwest (2 in Wisconsin and 3 in Michigan) and 7 lakes/rivers in New Hampshire. Because correctly identifying native populations and collecting living plants with bottom sediments from the native range of *M. heterophyllum* for the nematode studies was critical to the study, field sampling took place in the Midwest in August during the prime time for flowering / fruiting. In order to assess whether or not the plant communities associated with *M. heterophyllum* in New Hampshire were similar with those in the native populations in the Midwest, data was collected to characterize associated plant communities in all lakes sampled. We also determined which associated aquatic plants in the Midwest native populations are also native to NH

Prior to taking the initial August 2005 trip to the Midwest, working with the Wisconsin Department of Natural Resources, the Wisconsin Biological Survey and using datasets from the Wisconsin LTER lakes program and the computerized records from the University of Wisconsin Herbarium a list of about 20 candidate lakes containing our target species were selected. When possible, DNR staff provided additional site location information. In August a preliminary survey trip to the candidate Wisconsin lakes was made by Schloss and Crow. Of twelve lakes surveyed, *M. heterophyllum* was only confirmed at two lakes: Rock Dam Lake and Round Lake. There were occurrences of other milfoil species which may have been mistakenly identified, or the site locations (if provided) were not accurate and we failed to find occurrences, or the populations reported may no longer occur. A second trip to Wisconsin was made by Schloss and Abebe to collect additional samples at Rock Dam and Round Lakes and to survey two additional lakes suggested by Wisconsin DNR staff. Unfortunately, no additional occurrences of our target species were discovered.

In the following year it was decided to visually check, in-person, the available voucher specimens from the two Michigan university herbaria and when possible confirm the existence of a *M. heterophyllum.* This approach worked well and allowed for a much higher success rate in finding the target species for the field work in Michigan. While additional lakes were sampled while in the state, only those lakes that allowed for a complete analysis are discussed in this report. For example, although we surveyed and even collected nematode samples from Bass Lake in Benzie County, as we were not able to secure the proper Park Service permits at the time no sediment pore water sampling could be done.

Field and Sampling Methods:

Plants were identified using *Aquatic and Wetland Plants of Northeastern North America* (Crow and Hellquist 2000a,b). Voucher specimens of *Myriophyllum heterophyllum* were collected in all lakes sampled, with specimens deposited in the Hodgdon Herbarium, University of New Hampshire as a permanent record documenting the study. Additionally, samples were sent to Dr. Michael Moody, University of Indiana, for confirmation of identity by DNA analysis. All plant species associated with the populations of *Myriophyllum heterophyllum* were recorded, along with relative abundance data. Abundance data was recorded for all species present in reference to density of colonies using the abundance scale for species in floras proposed by Palmer et al. (1995)—abundant, common, frequent, occasional, infrequent, uncommon, sparse (rare at the site). A more detailed overview of all of the methods used for this project can be found in the Quality Assurance Project Plan previously prepared (Schloss and Craycraft 2006).

Results and Discussion:

Table 2-1 summarizes all of the species found in association with the 12 lakes sampled in Wisconsin, Michigan and New Hampshire, and their relative abundance in each of the lakes. The more complete site field notes are included in Appendix A.

Of the 23 species encountered in the 12 populations sampled, none of the species showed a strong correlation of association with *Myriophyllum heterophyllum*. Although *Utricularia purpurea* (Purple Bladderwort) occurred in 4 of the 5 lakes in New Hampshire, it was abundant in only 2 of the lakes; however it occurred in only one Midwest lake sampled (Wisconsin), where the plant was infrequent. *Nymphaea odorata* ssp. *odorata* (White Waterlily) also occurred in 4 of the 5 New Hampshire lakes, but this common plant of New Hampshire was low in abundance (sparse to occasional) in those sites associated with *Myriophyllum heterophyllum*; this subspecies was not encountered in any of the Midwest sites sampled

The Wisconsin plants occurred in more shallow waters and were often found among floating leaved species when compared to NH plant communities. They occurred as a very small cluster or small patches along with other submerged and emergent plants. All of the Midwest communities except for

Five of the aquatic plant species that occurred in the Midwest are not typically found in, or are rarely encountered in, New Hampshire—but none of these showed a strong correlation with *Myriophyllum heterophyllum* in native Midwest populations. These species were:

1. *Elodea canadensis* (Canada Waterweed), a hard water species that is rare in New Hampshire because our waters are acidic; it occurs more commonly elsewhere in New England in alkaline waters of streams and ponds (Crow and Hellquist, 1982).

Table 2-1. List of all aquatic species associated with *Myriophyllum heterophyllum* in lakes sampled

Plant Species	WI-1 Rock Dam Lake	WI-2 Round Lake	MI-1 Platte Lake	MI-2 Black River Lake Outlet	MI-3 Long Lake	NH-1 Suncook River Broads	NH-2 Back Bay Winni.	NH-3 Crescent Lake	NH-4 Heath Brook Went-worth	NH Lower Suncook Treated Lake	NH Swains M. humile site	NH M. verticil-latum site (Balch)
Brasenia schreberi (Water-shield)		infreq							sparse		occas	sparse
Elodea canadensis (Canada Waterweed)			common	infreq								
Elodea nuttallii (Nuttall's Waterweed)							occas	infreq		1 plant seen		
Najas flexilis (Slender Water-nymph)			common	infreq						abund		
Nuphar variegata (Yellow Waterlily)												infreq
Nymphaea odorata ssp. *odorata* (White Waterlily)						infreq	sparse				occas	infreq
Nymphaea odorata ssp. *tuberosa (=N. tuberosa)* (White Waterlily)	occas				sparse							
Pontederia cordata (Pickerel-weed)		infreq				infreq					occas	
Potamogeton amplifolius (Large-leaved Pondweed)				infreq			abund	sparse	sparse			abund
Potamogeton bicupulatus (Pondweed)										1 plant seen		
Potamogeton crispus (Curly-leaved Pondweed)			infreq									
Potamogeton epihydrus (Leafy Pondweed)		infreq				infreq		abund	1 plant seen	abund		
Potamotegon oakesianus (Oakes' Pondweed)									sparse			sparse
Potamogeton natans (Floating-leaved Pondweed)											occas	
Potamogeton perfoliatus (Clasping-leaved Pondweed)				infreq								
Potamogeton zosteriformis (Flat-stemmed Pondweed)			abund	infreq								
Potamogeton sp. (Pondweed)									freq.			
Scirpus subterminalis (Water Bulrush)									abund			abund
Sparganium angustifolium (Narrow-leaved Bur-reed)							sparse		sparse	infreq	occas	sparse
Stuckenia (Potamogeton) pectinata (Sago Pondweed)			common	infreq								
Utricularia purpurea (Purple Bladderwort)		infreq				infreq		abund		frequent	abund	infreq
Utricularia radiata (Bladderwort)						infreq						
Utricularia resupinata (Bladderwort)		infreq										
Utricularia vulgaris (Common Bladderwort)									sparse			
Vallisneria americana (Tape-grass)			abund	infreq								

2. *Nymphaea odorata* ssp. *tuberosa* (=*N. tuberosa*; White Waterlily) is also a hard water species and it occurs mostly in the Champlain Valley, Vermont, and is scattered in New Hampshire essentially as an introduction—mostly planted sites (and frequently planted in mitigation wetlands). Our common White Waterlily is the soft water white waterlily, *Nymphaea odorata* ssp. *odorata* (Hellquist and Crow 1984; Crow and Hellquist 2000a).
3. *Potamogeton crispus* (Curly-leaved Pondweed) is a Eurasian weed that only recently has been reported as occurring in the state, having spread into southeastern New Hampshire in very few locations (Padgett and Crow 1993). This is the only plant that we found associated with *M. heterophyllum* that is non-native to North America.

4. *Potamogeton zosteriformis* (Flat-stemmed Pondweed/Eel-grass Pondweed) is also primarily a hard water species with scattered populations mostly in western New England and in northern Maine—but a few populations occur in New Hampshire, especially along the Connecticut River (Hellquist and Crow 1980), and is listed as "Endangered" in the state (New Hampshire Natural Heritage Bureau 2007).

5. *Stuckenia* (*Potamogeton*) *pectinata* (Sago Pondweed) is also a hard water species, but does sometimes occur in southeastern New England in somewhat polluted waters. It is on the New Hampshire rare plant list with a status of "Endangered" (Hellquist and Crow 1980; New Hampshire Natural Heritage Bureau 2007).

Of these, only *P. zosterformis* has been mentioned in the Wisconsin literature of being a "common associate" (Nichols 1999) to *Myriophyllum heterophyllum*. Other plant associates from that same study included *Ceratophyllum demersum*, *Potomogeton pusillus* and *P. richardsonii*.

In contrast, *Utricularia radiata*, a species common in New Hampshire, and was found in one NH site growing with *Myriophyllum heterophyllum*, is a coastal plain species—therefore it does not occur in the Midwest.

Native Species of Myriophyllum in New Hampshire

For comparison, 2 sites with the native species of Myriophyllum were examined and sampled for nematodes and plant community structure—*Myriophyllum humile* (from Swains Lake) and *M. verticillatum* (in Balch's Pond) Both of these species are rather rare in New Hampshire and have therefore been included on the New Hampshire "WATCH" list by the New Hampshire Natural Heritage Bureau (2007). It is most likely that both *M. verticillatum* and *M. heterophyllum* are expressing aggressively in Balch's Pond (Amy Smagula, personal communication). We just happened to sample an area of the community that was dominated by the former as the plants in that particular stand were highly variable in terms of morphology and no defining reproductive structures were present at the time of the survey.

Impact on/by Native Species and Aggressiveness of *Myriophyllum heterophyllum*

In as much as none of the aquatic plants observed growing in the 5 lakes examined in the Midwest showed a strong correlation with *Myriophyllum heterophyllum* in native

Midwest populations, we cannot state that the presence of this species as an aggresive plant has had a negative impact in displacing any one particular species. In addition, examining the Midwest communities we found no plants or plant associations that consistently dominated over the sparse growths of milfoil. Instead, since *M. heterophyllum* often grew in a monoculture as when it occurs in New Hampshire lakes (although typically limited in scope) the monoculture tended to either displace nearly all the native vegetation where the growth was dense or only *M. heterophyllum* was found in a limited area all by itself with just an occasional co-occurring species .

But to some degree, this is characteristic of most of the species of *Myriophyllum*, including even our native species. For instance, in the population of *M. verticillatum* in the lake sampled in Balch Pond in Wakefield (see Appendix 1) the Watermilfoil was extremely dense and crowded out other species of aquatic plants—just as it happens with *M. heterophyllum*. Likewise, in Swain's Lake, Barrington, NH, where *M. humile* is dense, other aquatics are sparse and rarely mixed in with the Watermilfoil although the extent of this occurrence tends to be limited over a long period of time even with the stands location very close to the boat ramp..

The Round Lake, Wisconsin and Black River Lake Outlet communities were of particular interest as the latter exhibited very aggressive growth at the outlet which was where the lake outlet continues on as the Black River. The location includes the main boat ramp as well as a marina just a few feet from the ramp that offers boat rentals. The increased chance for vegetative fragments to be generated by these activities most likely insures the typical large monospecific rosettes of Variable Watermilfoil, that are very similar to how the plant expresses itself in New Hampshire. In the case of Round Lake, Wisconsin, aggressive *Myriophyllum heterophyllum* populations appeared in the lake all of a sudden in 1991 (Konkel 2006). Boating activity was directed away from the few shallow cove areas where *M. heterophyllum* existed and by 2000 the population was in the minority. This may be a unique case where due to the water seepage from the Black Spruce (*Picea mariana*) bog the plant is only favored over the other plants in a very limited area of the lake restricted to the margins of the bog.

In other lakes in the Midwest it also was clear that Variable Watermilfoil was restricted to only a small section of a lake or pond. These areas were generally shallow fringes and/or embayments and in many cases areas where tributaries entered. If some part of the species life history is favored by more acidic conditions these areas may be the key to providing the optimum conditions to establish itself. At Rock Dam Lake (WI) the plants were restricted to the two tributary areas on the eastern shore. At Platte Lake (MI) where the milfoil associated with a mix of other species in sporadic clumps the distribution seemed to be limited to the large southern shallow backwater area separated by the outlet channel for the Platte River (see Appendix A for further details).

SECTION 3

Nematode Taxa Associated with *Myriophyllum heterophyllum* (Variable Milfoil)

As discussed in this report's introduction, global nematode biogeography is still in its infancy mainly because what we know of nematodes in freshwater habitats is limited both in extent and in geographic range. For instance, about 53% of the total global freshwater species are recorded from the Palearctic, more specifically from Europe and Russian territories. Based on these data, Eyualem-Abebe et al. (In Press) estimated a total global species count of 14,000 species. Consequently, studies of freshwater habitats in most parts of the unexplored world are most likely to result in many taxa unknown to science. Similarly, inventorying nematodes in the numerous unexplored freshwater lakes in the USA will undoubtedly reveal many new and unreported taxa as well as initiate an effort to explore the potential of discovering useful parasites for use in water resources management.

The primary goal of this part of the investigation was to compare and contrast the nematode communities associated with native and invasive Milfoils in the hopes of finding a potential species specific parasite that could influence *Myriophyllum heterophyllum* fitness and aggressiveness. With no previous investigations to refer to, the strategy was to examine the nematode community in the context of the plant communities. By attempting to compare communities from new and established populations, from native and exotic populations for the same species and from exotic and native populations of related species it was hoped that certain candidates would fall out for further research. The last section following describes the

MATERIALS AND METHODS

Milfoil Nematode Extraction and Mounting Procedures

For samples that included sediment and plant material the two were separated. Nematode extraction from the sediments was accomplished through the centrifugal floatation method employing Ludox as a density separator as described in detail in the project Quality Assurance Project Plan (Schloss and Craycraft 2006). A 500 µm sieve screened out the debris and a 38 um sieve captured the nematodes. A Baermann tray (Hopper, 1990) collected active worms in tissue paper attracted to the water in the tray escaping from the drying macerated plant tissues. We used a recently developed method (Yoder et al., 2006) to preserve the morphology and DNA of the extracted nematode specimens. DESS is composed of salt saturated EDTA and DMSO.

For preparing fixed nematodes for microscopic study, nematode specimens preserved in DESS were rinsed in tap water in a 38 µm sieve. Specimens from samples collected in 2005 were mounted in DESS for microscopic observation. Specimens from samples collected in 2006 were processed into glycerin because it was demonstrated that DESS preserved nematodes could be processed into glycerin and DNA can be extracted with ease from such specimens (Yoder et al., 2006). Each specimen was picked individually onto an embryo dish to be processed into glycerin. Specimens were transferred to glycerin

following Seinhorts (1959) and mounted in glycerin on slides for identification. Selected adult specimens that represented the diversity of the specific sample were imaged (De Ley and Bert, 2002; Eyualem-Abebe et al., 2004) and digested in worm lysis buffer to extract DNA for sequence-based molecular identification.

Selected adult nematode specimens that represented the diversity of the specific sample were imaged to capture their morphology in the form of multifocal video images (De Ley and Bert, 2002; Eyualem-Abebe et al., 2004). We used an IX81 Olympus inverted microscope with Differential Interference Contrast (DIC) optics. It is fitted with a CCD (Charge Coupled Device) Digital Camera that generates high-resolution images (1280 x 1024 pixels) and employs a high-speed low-noise system (12 frames per second full resolution in 8-bits). Such a high-resolution adds the flexibility of enlarging images after capture without a reduction in quality.

Results of extractions:

A total of 1535 nematode specimens were extracted from the 12 lakes studied, five from New Hampshire and seven from Midwestern states (Wisconsin and Michigan) over the period of two years. Both the rhizosphere (sediment area containing the root mass) and the deeper sediment were sampled when possible. A total of 325 specimens were picked and studied from four lakes in 2005, and a total of 1210 specimens were picked of which 899 were studied morphologically from eleven lakes in 2006 (Table 3-1). From these, 100 specimens were imaged, and digested for molecular analysis. Appendix B contains a list of the Nematode taxa collected through our study.

Results of morphological study:

New Hampshire Lakes

Lake Wentworth:

A total of 25 taxa were isolated from two samples collected approximately one year apart (August 2005 and October 2006). More nematodes (257 individuals) were isolated from the 2006 October samples than from samples collected in August 2005 (22 individuals). Nematode diversity in 2005 sample was low with 7 taxa identified. On the other hand the 2006 sample had 24 taxa. Five of the 7 taxa found in the 2005 sample were also recorded in the 2006 sample. Aside from the shared five taxa in the two samples, there is a clear difference in terms of the dominance/evenness profile. The 2005 sample harbored few individuals but was dominated (45%) by the plant parasitic genus *Hirschmanniella*, while the 2006 community was dominated by three genera contributing 74% of the individuals (*Laimydorus* 46%, *Mononchulus* 21% and *Westindicus* 7%) (Table 3-2, Figure 3-1). When we sampled in 2006 there had been hand pulling activity in the Health Brook Cove and that may have been a factor in the resulting nematode community. Nematodes isolated from the rhizosphere of the 2006 sample were not typical of a freshwater environment. Instead, the four taxa we found are typical of marine environment. Lake Wentworth shares 13 genera with either of the two lakes Swain's or Crescent. We have Imaged 36 nematode specimens from this site.

Table 3-1. Total number of nematodes isolated and identified categorized by sex and developmental stage, and the total number of taxa found from each site.

New Hampshire Lakes

Site		Isolated	Identified	Females	Males	Juveniles	Taxa
1. Lake Wentworth	2005 August sample	22	22	16	0	6	7
	2006 October sample	250	180	65	19	92	21
	Rhizosphere 2006 October sample	7	7	4	2	1	4
2. Swain's lake, 2006 sample	Sediment	96	81	26	11	44	16
	Rhizosphere	28	28	8	0	20	6
3. Crescent Lake, 2006 sample, Wolfeboro		97	97	41	18	38	12
4. Balch Pond, 2006 sample, Wakefield		39	37	13	2	22	8
5. Back Bay, 2006 sample, Wolfeboro		0	0	0	0	0	0

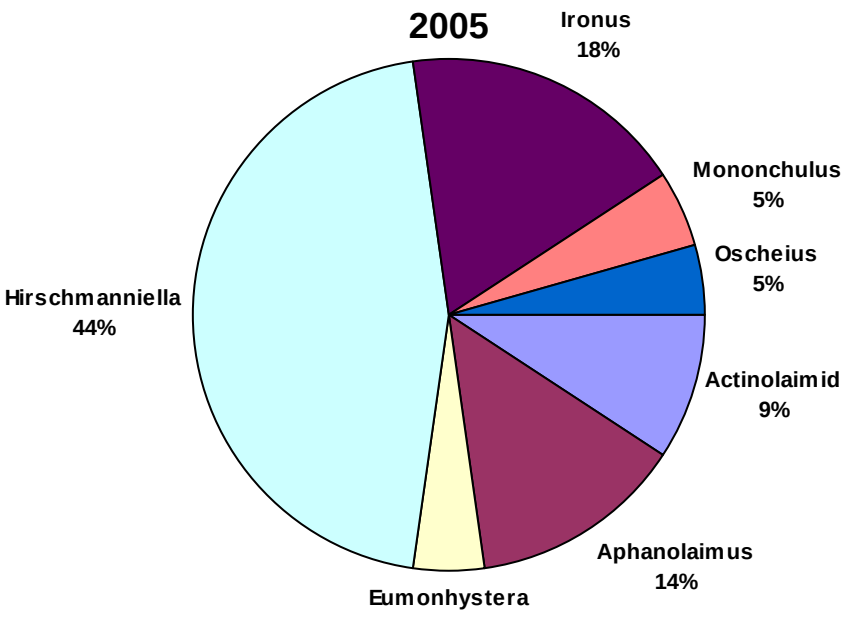
Midwestern lakes (Wisconsin and Michigan)

Site		Isolated	Identified	Females	Males	Juveniles	Taxa
1. Long Lake, 2006 sample (MI)		237	100	39	13	48	13
2. Bass Lake, 2006 sample (MI)		21	19	6	0	13	8
3. Rock Dam Lake, 2005 sample (WI)		195	167	66	7	94	26
4. Round Lake, 2005 sample (WI)		86	59	18	3	38	14
5. Black River Lake, 2006 sample (MI)	Shallow sandy bottom	20	20	6	0	14	8
	Sediment & Shallow Sand	124	80	42	9	29	13
6. Platte Lake, 2006 sample (MI)		184	100	17	2	81	13
7. Fifth Lake, 2005 sample (WI)		22	20	8	3	9	13

Table 3-2. Lake Wentworth: List of taxa from 2005 sample			
Taxa	**Males**	**Females**	**Juveniles**
1. Actinolaimid	1	1	2
2. Aphanolaimus	1	2	3
3. *Eumonhystera*	1	0	1
4. *Hirschmanniella*	9	1	10
5. *Ironus*	2	2	4
6. *Mononchulus*	1	0	1
7. Oscheius??	1	0	1
Total	16	6	22

List of taxa from 2006 sediment sample				
Taxa	**Males**	**Females**	**Juveniles**	**Total**
1. *Actinolaimus*	1	1	6	8
2. *Anonchus*	0	2	0	2
3. *Aphanolaimus*	1	0	1	2
4. *Aphanonchus*	2	1	0	3
5. *Aphelenchoides*	0	1	0	1
6. *Chronogaster*	0	1	0	1
7. Actinolaimid	2	0	0	2
8. Cryptonchus	0	0	1	1
9. Dorylaimus	0	1	2	3
10. *Ethmolaimus*	0	2	0	2
11. *Eumonhystera*	1	0	0	1
12. *Hirschmanniella*	0	1	0	1
13. *Ironus*	0	2	2	4
14. *Laimydorus*	8	15	57	80
15. *Paraphanolaimus*	0	1	0	1
16. *Paraphelenchus*	1	0	0	1
17. *Mononchulus*	0	24	13	37
18. *Paraphanolaimus*	0	1	0	1
19. *Paraphelenchus*	1	0	0	1
20. *Westindicus*	1	3	7	11
21. *Plectus*	0	8	1	9
22. *Prodesmodorsa*	0	2	0	2
23. Scleroactinolaimus	1	0	0	1
Total	19	65	90	174

List of taxa from 2006 rhizosphere sample				
1. Sphaerolaimid	2	2	0	4
2. Oncholaimid	0	0	1	1
3. Actinolaimid	0	1	0	1
4. Sabatieria	0	1	0	1
Total	2	4	1	7



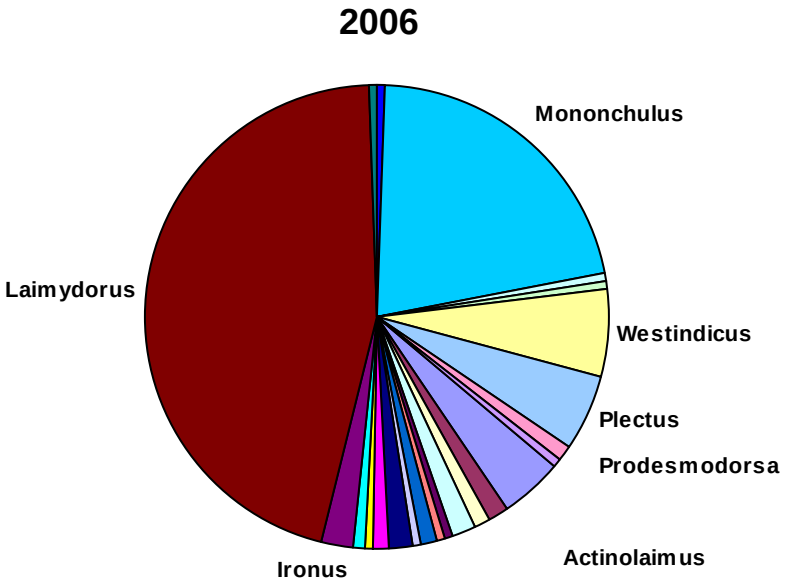


Figure 3-1 Pie diagram of the Lake Wentworth Nematode Community Dominance Patterns for 2005 and 2006

Swain's Lake:
Swain's Lake contains a well established community of M. humile. Noticably absent was the parasitic species Hirschmanniella. Sixteen taxa were found in the 2006 sample of which five are shared commonly with Crescent Lake and six taxa with Wentworth lake. Its nematode community is largely dominated by two genera: the genus Laimydorus (44%) followed by Mononchulus (24%).

Table 3-3. Swain's Lake: List of taxa from 2006 sample

Taxa	Males	Females	Juveniles	Total
1. Actinolaimid	0	0	2	2
2. *Actinolaimus*	0	0	2	2
3. Alaimid	0	0	1	1
4. *Aphanolaimus*	0	2	0	2
5. Doryiaimid	0	1	1	2
6. *Ironus*	0	2	2	4
7. *Laimydorus*	7	8	29	44
8. *Mononchulus*	0	9	0	9
9. *Noeactinolaimus*	2	0	0	2
10. *Paramononchus*	0	0	2	2
11. *Paraphanolaimus*	0	1	0	1
12. *Plectus*	0	1	1	2
13. Rhabditid	1	0	0	1
14. Tobrilid	0	1	4	5
15. Tylench	0	1	0	1
16. *Westindicus*	1	0	0	1
Rhizosphere sample				
17. *Ironus*	0	0	1	1
18. *Laimydorus*	0	1	3	4
19. *Mononchulus*	0	6	10	16
20. *Mononchus*	0	0	1	1
21. Tobrilid	0	0	3	3
22. *Tripyla*	1	0	2	3
Total	11	34	64	109

Crescent Lake:
The nematode community of Crescent lake was modestly diverse with a total of 12 taxa of which Crescent lake shares 10 taxa with Wentworth lake and five with Swain's lake. The three New Hampshire lakes commonly share five genera that are typical inhabitants of freshwater environments: Paraphanolaimus, Mononchulus, Laimydorus, Ironus and Actinolaimus. As in Wentworth and Crescent lakes, the genus Laimydorus contributed to community abundance significantly (27%) but unlike these lakes the dominant genus in Crescent lake was Ethmolaimus (30%). Another rarely dominant genus Anonchus also contributed significantly to the community abundance (14%). Most important the plant parasitic nematode Hirschmanniella that dominated the August 2005 sample in Wentworth was recorded in Crescent Lake, though represented by only one specimen.

Table 3-4. Crescent Lake: List of taxa from 2006 sample				
Taxa	**Males**	**Females**	**Juveniles**	**Total**
1. Actinolaimus	0	0	2	2
2. Anonchus	0	11	3	14
3. *Aphanonchus*	1	0	0	1
4. *Dorylaimus*	0	0	4	4
5. *Epitobrilus*	0	0	1	1
6. *Ethmolaimus*	12	14	3	29
7. Hirschmanniella	0	1	0	1
8. Ironus	0	2	3	5
9. Laimydorus	3	7	16	26
10. Mononchulus	0	5	3	8
11. Paraphanolaimus	0	1	0	1
12. Semitobrilus	2	0	3	5
Total	18	41	38	97

Balch Pond:
A total of eight taxa were identified. The plant parasitic genus Hirschmanniella dominated the community (35%) followed by the genus Laimydorus (27%). Balch Pond shares all its nematode community with Wentworth in that all genera found in the former are found in the latter. It also shares five of the eight taxa with Swain's Lake and Crescent Lake.

Table 3-5. Balch Pond: List of taxa				
	Males	Females	Juveniles	Total
Anonchus	0	0	1	1
Chronogaster	0	2	0	2
Hirschmanniella	0	6	7	13
Ironus	0	0	2	2
Laimydorus	2	1	7	10
Mononchulus	0	1	4	5
Paraphanolaimus/Paraphanonchus	0	2	1	3
Plectus	0	1	0	1
Total	2	13	22	37

Back Bay:
No nematodes were found in Back Bay. This bay was characterized by very high salt content and nutrient content (See next section).

Midwestern Lakes

Wisconsin Lakes:

Rock Dam Lake

Twenty-six taxa were identified from a total of 167 specimens studied and this makes Rock Dam Lake the highest in its number of taxa among the 12 lakes we studied. The community is dominated by mainly three genera (Aphanolaimus 19%, Ironus 16%, and Laimydorus 13%) none of which is plant parasitic. Members of the order Plectida are well represented in this site. Also, no plant parasitic nematodes were found in the samples. We imaged and digested 22 nematode specimens from this site.

Table 3-6. Rock Dam Lake: List of taxa from 2005 sample

Taxon	**Males**	**Females**	**Juveniles**	Total
1. Actinolaimid	0	2	0	2
2. Anatonchus	0	2	2	4
3. *Anonchus*	0	2	0	2
4. Aphanolaimid	0	1	0	1
5. *Aphanolaimus*	1	9	22	32
6. Aphanonchus	*3*	*2*	*2*	*7*
7. Cryptonchus	*0*	*3*	*2*	*5*
8. *Chronogaster*	0	0	2	2
9. *Laimydorus*	1	6	14	21
10. *Eumonhystera*	0	2	0	2
11. *Eutobrilus*	0	1	8	9
12. *Ironus*	0	12	14	26
13. *Miconchus/ Comiconchus*	0	1	8	9
14. *Monhystera*	0	11	2	13
15. Monhystrella	0	0	1	1
16. Mononchid (1)	0	1	3	4
17. *Mononchus* (2)	0	2	10	12
18. Mylonchulidae	0	1	0	1
19. *Paramononchus*	0	1	0	1
20. *Paraphanonchus/ Paraphanolaimus*	0	1	1	2
21. *Plectus*	0	1	1	2
22. *Prodesmodora*	0	4	1	5
23. *Rhabdolaimus*	0	1	0	1
24. Tobrilid	1	0	0	1
25. Tripyla	1	0	0	1
26. Tridentulus	0	0	1	1
Total	7	66	94	167

Round Lake:
The nematode community of Round Lake can be considered relatively diverse in light of the low number of identifiable adults encountered. It was an conspicuously actinolaimid-dominated community, the genus Westindicus contributing more than half towards total community abundance. Round lake shares 10 of its 14 taxa with Rock Dam Lake, six taxa with Fifth lake, six taxa with Long lake, five taxa with Black River lake, and four taxa with Platte lake. We imaged and digested 15 specimens from this site.

Table 3-7. Round Lake: List of taxa from 2005 sample

Taxa	Males	Females	Juveniles	Total
1. *Achromadora*	0	2	2	4
2. *Alaimus*	0	4	0	4
3. *Aphanolaimus*	0	1	0	1
4. *Cryptonchus*	0	0	1	1
5. Dorylaimid	0	0	3	3
6. *Epitobrilus*	0	1	1	2
7. *Ironus*	0	0	2	2
8. *Mononchus*	0	1	0	1
9. *Neotobrilus*	1	1	0	2
10. *Paramononchus*	0	1	0	1
11. *Prodesmodora*	0	1	0	1
12. Tobrilid	0	1	2	3
13. *Tripyla*	0	0	2	2
14. *Westindicus*	2	5	25	32
Total	3	18	38	59

Fifth Lake:
Fifth Lake is a soft-water lake that was sampled even though we could not find any stands of Variable Water Milfoil as expected. At the time it represented a low alkalinity Midwestern Lake.A total of 13 taxa were identified from a total of 20 identifiable individuals. The diversity of the community is not only in its relatively high number of taxa but also in the balanced number per taxa (evenness). Dominance was minimal and the site has a balanced abundance distributed among the taxa found. The major groups Tobrilids, Monhysterids and Plectids are represented by three taxa each.

Table 3-8. Fifth Lake: List of taxa from 2005 sample				
Taxa	**Males**	**Females**	**Juveniles**	**Total**
1. *Anonchus*	0	1	1	2
2. *Aphanolaimus*	0	0	1	1
3. *Brevitobrilus*	0	1	1	2
4. Dorylaimid	0	0	3	3
5. *Ethmolaimus*	0	1	0	1
6. *Eumonhystera* sp1	0	1	0	1
7. *Eumonhystera* sp2	0	1	0	1
8. *Eutobrilus*	0	1	0	1
9. *Mohystera*	0	0	1	1
10. *Neotobrilus*	3	1	0	4
11. *Paraphanolaimus?*	0	0	1	1
12. *Rhabdolaimus*	0	1	0	1
13. Tobrilid	0	0	1	1
Total	3	8	9	20

Michigan Lakes

Platte Lake:
A total of 13 taxa were found in Platte Lake. The relatively low diversity compared to the total of 100 individuals studied could be a result of the dominance by juveniles (81%) whose morphological identification is uncertain due to the lack of adult characteristics. Juveniles often are lumped under the genus that is represented by adults in the sample.
Platte Lake has a unique nematode community compared to the nematode communities of the remaining 11 lakes we studied in that it is completely and overwhelming dominated by tobrlids. Five of the 13 taxa are tobrilids and four of these five genera, i.e. Semitobrilus (31%), Tobilus/Eutobrilus (25%), Neotobrilus (14), and an unidentified tobrilid (12), contribute 82% to the total community abundance. From the samples we studied, plant parasitic nematodes were not found in this community.

Table 3-9. Platte Lake: List of taxa from 2006 sample				
Taxa	**Males**	**Females**	**Juveniles**	**Total**
1. Brevitobrilus	0	0	1	1
2. Anonchus	0	2	0	2
3. Ethmoliamus	0	3	0	3
4. Ironus	0	3	1	4
5. Laimydorus	0	0	1	1
6. Monohystera	0	0	1	1
7. Neotobrilus	0	1	13	14
8. Paraphanolaimus	0	2	0	2
9. Plectus	0	1	0	1
10. Prodesmodora	0	2	0	2
11. Semitobrilus	1	1	29	31
12. Tobrilid	0	1	11	12
13. Tobrilus/ Eutobrilus	0	1	24	25
Total	1	17	81	99

Long Lake:
A total of 13 taxa were identified from Long Lake. Plectids, Anonchus (31%) and Paraplectonema (22%), dominate the community in abundance and this is unique among the remaining 11 lakes we studied. No plant parasite nematodes were observed.

Table 3-10. Long Lake: List of taxa from 2006 sample				
Taxa	**Males**	**Females**	**Juveniles**	**Total**
1. Anonchus	9	13	8	30
2. Aphanonchus	0	1	0	1
3. Cryptonchus	0	1	1	2
4. Ethmolaimus	0	1	0	1
5. Ironus	0	1	1	2
6. Laimydorus	0	0	1	1
7. Monhystera	0	3	1	4
8. Paraphanolaimus	3	1	4	8
9. Paraplectonema	0	9	12	21
10. Plectus	0	3	0	3
11. Prodesmodora	0	6	1	7
12. Semitibrilus	1	0	7	8
13. Tobrilus/ Eutobrilus	0	0	9	9
Total	13	39	45	97

Black River Lake:
A total of 17 taxa were identified from Black River Lake. The community was dominated by four genera: the dorylaimid genus Laimydorus (16%), the tylenchid genus Hirschmanniella (15%), and the two plectid genera Aphanonchus (13%) and Aphanolaimus (13%).

Table 3-11. Black River Lake – Shallow Sand Bottom: List of taxa				
	Males	**Females**	**Juveniles**	**Total**
1. *Anonchus*	0	2	0	2
2. *Dorylaimus*	0	0	1	1
3. *Ironus*	0	0	2	2
4. *Laimydorus*	0	2	3	5
5. *Mononchulus*	0	1	1	2
6. Tobrilid	0	0	4	4
7. Tylench	0	0	2	2
8. *Westindicus*	0	1	0	1
Total	0	6	13	19
Black River Lake Sediment & Shallow Sand: List of taxa				
1. *Anonchus*	0	2	1	3
2. *Aphanoliamus*	1	7	5	13
3. *Aphanonchus*	3	9	1	13
4. *Brevitobrilus*	0	0	1	1
5. *Chronogaster*	0	1	6	7
6. *Dorylaimus*	0	0	1	1
7. *Hirschmanniella*	3	8	4	15
8. *Laimydorus*	1	9	1	11
9. *Monohystera*	0	2	0	2
10. *Mononchulus*	0	1	0	1
11. *Paraphanonchus*	1	0	0	1
12. *Semitobrilus*	0	1	1	2
13. *Tobrilus*	0	2	7	9
Total	9	42	30	80

Bass Lake:
A total of eight taxa were identified from Bass Lake. The community was dominated by a tobrilid genus (42%) represented by juveniles only followed by the genus Cryptonchus (26%). Bass Lake shares six of its eight taxa with Black Lake and Fifth Lake as well as seven of its eight taxa with Lake Wentworth and Crescent Lake, four taxa with Swain's Lake and Round Lake, and three taxa with Balch Pond, Long Pond and Platte Lake.

Table 3-12. Bass Lake: List of taxa				
Taxa	Males	Females	Juveniles	Total
Anonchus	0	1	0	1
Aphanolaimus	0	1	0	1
Chronogaster	0	0	1	1
Cryptonchus	0	5	0	5
Dorylaimid	0	0	1	1
Epitobrilus	0	0	1	1
Laimydorus	0	0	1	1
Tobrilid	0	0	8	8
Total	0	7	12	19

Discussion

Nematode diversity:

We studied a total of 452 individuals from the five New Hampshire lakes and found 38 nematode taxa. From the total of 546 individuals we studied in the six Midwestern lakes, we identified a total of 41 taxa. Altogether we found 51 nematode taxa from the 12 lakes we studied from both the Midwestern region and New Hampshire. Of these 51 taxa, 29 (57%) are new geographic reports from within the USA. Certainly many of these genera are new at the species level, however it would require a significantly more time to make species level diagnosis of all the encountered taxa. We also strongly believe that one of the tobrilid genus we found in Lake Wentworth may represent a genus new to science. In light of the role of newly discovered nematode taxa in bridging the gap in phylogenetic and developmental studies, these findings could contribute significantly towards enhancing current research in the field of nematology.

Hirschmanniella

We found the plant parasitic genus *Hirschmanniella* in four lakes of which three are in New Hampshire (Wentworth, Balch Pond and Crescent lake) and one (Black River lake) in Michigan. All these water bodies harbor a dense population of the aquatic plant Myriophyllum heterophyllum. This nematode species is morphologically similar to the parasitic nematode that can cause damage to rice plants (a commercially important aquatic plant species). We found only one *Hirschmanniella* specimen in the Crescent lake sample. Due to the intensive removal of *M. heterophyllum* by divers that occurred before our sampling the lake, and the low densities of the plants remaining we were forced to sample at a deep ledgy area with just a few remaining plants. The area had a very shallow rocky bottom and the low density of the plant would not usually favor a high yield of this potential candidate for further study. However, associations of nematode population with plant density requires more data on and life cycles than we currently have available (see also below).

Dominance patterns and *Hirschmanniella*:

The plant parasitic genus *Hirschmanniella* dominated Lake Wentworth in the 2005 sample which was collected in August, but disappeared from the 2006 October sample except for a single individual. Currently there isn't any information on population dynamics and generation time of nematode species in freshwater bodies on the North American continent. Consequently, our initial sampling was designed based on projected generation time and assumptions on population dynamics that rely largely on studies in other continents. Furthermore, the two numerically dominant genera in Wentworth (*Laimydorus* and *Mononchulus*) from the 2006 October sample are widely recognized to be carnivorous/omnivorous in their feeding strategy (Bilgrami & Gaugler, 2005). Although, stochastic reasons could also play role in the observed differences, the fact that those carnivorous/omnivorous genera could have impacted the population of *Hirschmanniella* cannot also be ruled out altogether. As the population dynamics of prey-predator goes hand in hand, the peak population of the plant parasitic genus *Hirschmanniella* could be earlier in the year. It is notable that in all the lakes where we found *Hirschmanniella*, we also found a large population of the genus *Laimydorus*. Furthermore, the coupling of the two carnivorous/omnivorous genera *Laimydorus* and *Mononchulus* was also observed in Swain's Lake (NH) and in Black River Lake (MI).

Species level identification of *Hirschmanniella* in Wentworth

Species level identification of the genus *Hirschmanniella* requires comparison of population parameters (morphometric, morphological and genetic) with a closely related taxa. Our comparison of the Wentworth population showed that it is closest to *H. oryzae*.

The population dynamics of *Hirschmanniella*: a future continuum

It is important to understand the complete picture of population dynamics and generation time *Hirschmanniella* in New Hampshire lakes. A better accounting of the interplay and dynamics between the three genera, *Hirschmanniella* and *Laimydorus* and *Mononchulus*, would be critical in our knowledge and understanding of the potential impact of *Hirschmanniella* on plant communities in these water bodies. Documenting the annual population dynamics would provide critical information:

1. on the amplitude of population fluctuation of *Hirschmanniella* and relate it with environmental factors including the cycle of stages of the plant *Myriophyllum heterophyllum*, water temperature, degree days, mixing patterns etc.

2. with regard to the evaluation of the possible impact of the population of *Hirschmanniella* on the plant community.

3. on the generation time and possible relationship with food source and potential predator taxa such as Dorylaimids and Mononchids.

4. provide information on the population fluctuations specifically of *Laimydorus* and *Mononchulus* and its relationship with population fluctuation of the potential plant parasitic genus *Hirschmanniella*.

Other outstanding findings:

New geographic records and possible new taxa:

The fact that 57% of the taxa we recorded are new geographic reports to the USA and possible new to science, will certainly expand our knowledge and understanding of the biogeography of those nematodes. Furthermore, possible continued interest in the inventory of these taxa to the lowest taxonomic rank will produce many taxa new to science. As we stated above we have recognized a tobrilid genus we found in Wentworth may represent a genus new to science. New taxa, apart from providing insight into biological diversity and ecology, will have a role in expanding our knowledge and understanding of comparative phylogeny and interrelationship among other known taxa in the phylum Nematoda. This is even more critical in the context of the dearth of nematologists actively working on freshwater nematode biodiversity.

Distribution of Monhysterids:

Five of the six Midwestern lakes have representative monhysterids. where as apart from a single individual specimen of the genus Eumonhystera at Lake Wentworth, monhysterids are completely absent from five New Hampshire lakes. Monhysterids are generally considered opportunist r-strategist nematodes with the ability to flourish in disturbed habitats. Their presence may indicate some kind disturbance and their absence may indicate long term stability of the water body.

Platte Lake and the Tobrilids:

Platte Lake is unique among the 12 lakes we studied in that it was overwhelmingly dominated by tobrilids (82%). While Tobrilids are ubiquitous they have rarely been reported to dominate nematode communities numerically comparable to the one we observed in Platte Lake (Zullini, 2006).

Our finding of such a high density tobrilid community and with four separate genera can be appreciated in the context of model organisms and early embryonic developmental research. *Caenorhabditis elegans* is one of the most important model organisms in developmental biology. However the biology and development of *Caenorhabditis elegans* is being enriched by the comparative study of nematode taxa from major clades of the phylum. A recent study on early embryonic development of the genus Tobrilus (Schierenberg 2005) demonstrated that the development of *C. elegans* is not a phylum-wide phenomenon. Instead some groups such as tobrilids show a mechanism more similar to the rest of the animal kingdom. Comparative studies of nematodes is often hindered by inability to culture many groups of nematodes and inaccessibility to large amount of live specimens. Platte Lake could be an endless source to research on tobrilids, for they are large and dominate the nematode community numerically.

Nematode Ecology:

A more detailed analysis of nematode communities in relation to lake geomorphology, water chemistry, sediment characteristics etc. will contribute significantly to our understanding of the ecology of freshwater nematodes, something we currently lack (Eyualem-Abebe et al., 2006). We need to continue to collect and analyze additional data to generate meaningful conclusions and understand the interrelationship of the different taxa in the various lake communities.

SECTION 4

Pore Water and Sediment Conditions associated with *Myriophyllum heterophyllum* (Variable Milfoil)

Investigation into the lake, porewater and sediment conditions allows for the determination of similarities and differences between New Hampshire and the Midwest lakes that contained *M. heterophyllum.* It also allows for the characterization of the environment surrounding the nematodes during times in their life cycle.

Table 4-1 A and B display the pore water chemistry results for this study. Means are included as well as the quality assurance data. As can be seen, all field blanks were either below detection limits or insubstantial enough not to influence the assay outcomes. Field duplicate samples are also indicated and they all fall within the acceptable range.

With the limited number of lakes that were sampled due to the intensive field and sample processing nature of this study we were unable to provide an experimental design that allows for statistical analyses of major water quality differences between lakes with a range of occurrences of *Myriophyllum heterophyllum* or between any taxa of nematode. There are, however, some interesting general observations that can be made. The Michigan Lakes of this study tended to have a high mineral content in there waters as can be inferred by the high silicate, calcium and magnesium levels. Most pore water samples had very low soluble phosphorous levels except for Platte Lake which also had high total nutrient laden porewater with levels one or two orders of magnitude higher than any of the other lakes except for Back Bay, Winnnipesaukee in New Hampshire.

Back Bay was also notorious for extremely high sodium and chloride levels that prevented any nematode occurrences and even disrupted our analytical systems. The combination of the high salts and high ammonia nitrogen levels should b cause for concern. The sample site was very close to the boat ramp and street where slat laden runoff could easily make its way into the waters. It is amazing that this area is extremely productive for variable milfoil given the high levels of salts.

Round Lake in Wisconsin had the highest dissolved nitrate/nitrite levels which are not that much of a surprise as this seepage lake derives most of its water from groundwater flow and it is found in an area that is actively in agriculture. Swains lake also had relatively high sodium and chloride levels which would not be that surprising as that area is adjacent to the boat ramp and the road that could easily direct road runoff into the lake. Long Lake scored higher sulfate levels than most lakes and the Heath Brook site at Lake Wentworth showed an occasional high ammonia nitrogen level form some of the samples.

Table 4-1A. Pore Water Analysis Results for the Midwestern Lakes. Grey bars indicate field duplicates and blue bars indicate field blanks

Lake Sample_Name	Collection Date	SiO2 mg SiO2/L	PO4 ug P/L	NH4 ug N/L	Chloride mg Cl/L	Nitrate mg N/L	Sulfate mg S/L	Sodium mg Na/L	Potassium mg K/L	Magnesium mg Mg/L	Calcium mg Ca/L	Total Diss. N mg N/L	Total P ug P/L	Total N ug N/L	pH
Wisconsin Lakes															
Round Lake -1-1	8/25/2005	7.0	<2	671	1.9	0.032	0.29	0.9	1.39	1.32	6.10	1.14	16	1,806	5.6
Round Lake-2-1	8/25/2005	0.4	10	391	3.0	0.113	0.48	2.9	1.16	0.85	2.81	1.18	11	1,501	5.5
Round Lake-2-2	8/25/2005	0.4	9	450	1.0	0.104	0.51	0.9	1.10	0.86	2.97	1.25	40	916	
	Mean	**2.6**	**9**	**504**	**2.0**	**0.083**	**0.43**	**1.6**	**1.22**	**1.01**	**3.96**	**1.19**	**23**	**1,408**	**5.6**
Rock Dam Lake-1-1	8/27/2005	4.1	<2	40	2.4	0.022	1.02	2.0	1.02	1.94	4.85	0.39	62	873	6.1
RDL-1-2 (Field Duplicate of 1-1)	8/27/2005	4.4	<2	45	2.6	0.030	1.02	2.0	1.03	1.94	4.87	0.41	69	773	6.1
Rock Dam Lake-1-3	8/27/2005	4.6	<2	30	2.6	0.042	1.61	3.0	1.06	1.91	4.75	0.45	57	804	
Rock Dam Lake-1-4	8/27/2005	4.7	3	27	2.4	0.168	1.08	2.0	0.94	1.94	4.98	0.39	53	834	
	Mean	**4.5**	**2**	**33**	**2.5**	**0.078**	**1.24**	**2.3**	**1.01**	**1.93**	**4.86**	**0.41**	**59**	**820**	**6.1**
Michigan Lakes															
Platte Lake 1-1	8/20/2006	28.9	88	9,942	8.9	0.078	1.04	6.8	2.49	17.82	44.22	12.25	299	13,970	7.1
Platte Lake 1-2	8/20/2006	28.2	15	13,983	7.8	0.044	0.50	5.9	2.86	18.94	26.63	16.43	302	38,380	7.0
Platte Lake 1-4	8/20/2006	26.3	44	15,705	6.0	0.035	0.49	5.1	2.40	18.63	40.09	18.57	332	25,810	
	Mean	**27.8**	**49**	**13,210**	**7.6**	**0.053**	**0.68**	**5.9**	**2.58**	**18.46**	**36.98**	**15.75**	**311**	**26,053**	**7.1**
Black River Lake 1-1	8/21/2006	16.5	4	226	5.3	0.020	1.14	5.4	1.05	12.30	26.23	0.60	85	606	7.4
Black River Lake 1-3	8/21/2006	15.0	3	141	5.3	0.017	0.66	4.8	0.86	12.93	20.78	0.45	10	605	7.4
Black River Lake 1-4	8/21/2006	16.8	6	279	5.9	0.023	0.43	5.5	0.85	12.64	26.01	0.57	51	797	
	Mean	**16.1**	**4**	**215**	**5.5**	**0.020**	**0.74**	**5.2**	**0.92**	**12.62**	**24.34**	**0.54**	**49**	**669**	**7.4**
Long Lake 1-2	8/22/2006	4.7	<2	27	13.6	0.017	5.20	7.3	1.14	6.37	33.04	0.39	31	903	8.3
Long Lake 1-3	8/22/2006	4.7	<2	46	12.6	0.020	5.28	6.2	1.06	6.49	30.81	0.42	46	904	8.4
Long Lake 1- 4	8/22/2006	5.9	<2	29	12.7	0.018	5.45	6.1	1.00	6.44	27.22	0.44	35	670	
	Mean	**5.1**	**<2**	**34**	**13.0**	**0.018**	**5.31**	**6.6**	**1.07**	**6.43**	**30.35**	**0.42**	**38**	**826**	**8.4**
Field Blanks															
Rock Dam Lake-1-5	8/27/2005	< 0.3	<2	<20	<1.0	<0.005	<0.04	0.1	0.08	0.19	0.56	<0.1	<2	<50	
Platte Lake 1-3	8/20/2006	< 0.3	5	<20	<1.0	<0.005	<0.04	0.9	0.05	0.68	0.43	<0.1	<2	<50	
Black River Lake 1-2	8/21/2006	< 0.3	<2	<20	<1.0	<0.005	<0.04	0.6	<0.05	0.55	0.30	<0.1	<2	<50	
Long Lake 1-1	8/22/2006	< 0.3	<2	<20	<1.0	<0.005	<0.04	0.6	<0.05	0.57	0.33	<0.1	<2	<50	

Table 4-1B. Pore Water Analysis Results for the New Hampshire Lakes.
Grey bars indicate field duplicates and blue bars indicate field blanks

Lake Sample_Name	Collection Date	SiO2 mg SiO2/L	PO4 ug P/L	NH4 ug N/L	Chloride mg Cl/L	Nitrate mg N/L	Sulfate mg S/L	Sodium mg Na/L	Potassium mg K/L	Magnesium mg Mg/L	Calcium mg Ca/L	Total Diss. N mg N/L	Total P ug P/L	Total N ug N/L	pH
New Hampshire Lakes															
Back Bay 1-1	9/17/2006	8.6	<2	6,099	2,054	<0.005	0.04	969	<0.05	10.88	91.37	6.87	1233	>12,974*	5.7
Back Bay 1-2	9/17/2006	9.5	<2	7,168	1,754	0.012	0.23	816	<0.05	8.94	77.65	6.10	294	>13,279*	5.7
Back Bay 1-4	9/17/2006	10.0	<2	7,621	2,140	<0.005	<0.04	>1000	<0.05	10.73	92.13	6.62	131	>14,245*	
	Mean	**9.3**	**<2**	**6,963**	**1982.8**	**0.012**	**0.13**	**892.2**	<0.05	**10.18**	**87.05**	**6.53**	**553**	**>13,449**	**5.7**
Balch Pond 1-1	9/17/2006	4.3	5	152	7.6	0.005	0.78	5.8	0.60	0.82	5.35	0.41	114	623	5.9
Balch Pond 1-3	9/17/2006	5.4	8	341	6.3	0.009	0.50	4.3	0.68	0.81	6.75	0.64	93	612	5.8
Balch Pond 1-4	9/17/2006	6.2	10	263	5.4	0.023	0.35	3.9	0.56	0.68	7.04	0.59	88	1420	
	Mean	**5.3**	**8**	**252**	**6.4**	**0.012**	**0.54**	**4.6**	**0.61**	**0.77**	**6.38**	**0.54**	**98**	**885**	**5.9**
Crescent Lake 1-1	9/18/2006	3.0	16	74	8.9	0.011	0.91	6.5	0.62	0.51	3.10	0.33	289	439	6.2
Crescent Lake 1-2	9/18/2006	2.3	4	113	8.9	0.016	1.18	6.5	0.73	0.45	2.82	0.35	134	353	6.1
Crescent Lake 1-3 (1-2 Duplicate)	9/18/2006	2.7	4	117	8.9	<0.005	1.19	6.5	0.73	0.45	2.82	0.35	162	428	
Crescent Lake Root Zone	9/18/2006	1.9	17	79	12.4	0.003	1.03	8.9	1.06	0.50	4.79	0.54	172	804	
	Mean	**2.5**	**12**	**89**	**10.1**	**0.009**	**1.04**	**7.3**	**0.80**	**0.49**	**3.57**	**0.40**	**203**	**545**	**6.1**
Swain's Lake 1-1	10/6/2006	10.2	<2	4,484	385.4	<0.005	0.15	185	3.39	4.42	22.20	4.23	51	5,670	5.8
Swain's Lake 1-2	10/6/2006	7.6	<2	3,860	295.0	<0.005	0.18	134	2.63	3.29	16.10	3.30	79	3,940	5.8
Swain's Lake 1-3	10/6/2006	6.7	<2	2,773	236.4	0.008	0.31	105	2.57	3.05	16.23	2.52	111	2,932	
Swain's Lake 1-4 (1-3 Duplicate)	10/6/2006	7.0	<2	2,944	273.5	0.009	0.34	122	2.53	3.35	18.53	2.62	135	3,766	
	Mean	**7.1**	**<2**	**3,192**	**268.3**	**0.009**	**0.28**	**120.3**	**2.57**	**3.23**	**16.95**	**2.81**	**108**	**3,546**	**5.8**
Wentworth Heath PS	10/9/2006	7.4	<2	166	2.7	<0.005	0.52	3.2	0.62	0.27	2.01	0.49	SVI	SVI	6.0
Wentworth Heath -1	10/9/2006	12.5	19	620	3.9	<0.005	0.11	5.2	0.94	0.67	6.32	0.95	222	1,296	5.9
Wentworth Heath -2	10/9/2006	1.2	11	322	4.2	<0.005	0.47	4.2	0.74	0.27	1.97	0.98	27	2,193	
Wentworth Heath -3	10/9/2006	13.8	15	1,044	3.1	<0.005	0.23	4.6	0.98	0.90	7.47	1.26	157	1,466	
Wentworth Heath -4 (Heath 3 dup.	10/9/2006	13.7	14	1,099	2.7	<0.005	0.20	4.2	0.95	0.94	7.74	1.36	110	1,758	
	Mean	**8.7**	**12**	**545**	**3.4**	**<0.005**	**0.33**	**4.2**	**0.82**	**0.53**	**4.48**	**0.93**	**127**	**1,700**	**6.0**
Field Blanks															
Back Bay 1-3	9/17/2006	< 0.3	<2	27	<1.0	<0.005	<0.04	<0.1	<0.05	<0.1	0.27	<0.1	<2	<50	
Balch Pond 1-2	9/17/2006	< 0.3	<2	<20	<1.0	0.005	<0.04	0.3	<0.05	<0.1	0.50	<0.1	<2	<50	

SVI= Sample Volume Insufficient for Total Nutrient analysis

Note: Back Bay Samples could not be run for Total Nitrogen as the high salt values interfered with the derivative spectroscopy method.

Sediment digestion and analysis results are listed in table Table 4-2. Percent organic results correlated well to those sites with peat and humic material lining the lake bottom and percent moisture followed along for the most part. The pattern of loosely sorbed and Iron bound phosphorous yield did not seem to show a consistent trend for any possible grouping. As for total phosphorus, Back Bay again displayed some very productive levels of nutrient and in the second tier all those lakes including Platte, Balch, Wentworth/Heath and Swain's were up there.

Table 4-2. Sediment Analyses for New Hampshire and Midwestern Stidy Lakes

Lake Sample_Name	Collection Date	% Organic	% Moisture	Loosely Sorbed P mg/g	Iron Bound P mg/g	Total P mg/g	Comments
Wisconsin Lakes							
Round Lake 1-1	8/25/2005	26.8	55.0				Peat over
Round Lake 2-1	8/25/2005	33.9	53.6	110	100	215	Sand with Silt
Round Lake 2-2	8/25/2005	34.4	54.9	100	80	260	
Rock Dam Lake 1-1	8/27/2005	8.1	43.6	125	60	340	Sandy
Rock Dam Lake 1-3	8/27/2005	9.2	44.1	95	80	325	
Rock Dam Lake 1-4	8/27/2005	7.8	46.3				
Michigan Lakes							
Platte Lake 1-A	8/20/2006	26.0	52.2	140	230	555	Mixed Sand and Silt
Platte Lake 1-B	8/20/2006	31.8	50.1	240	200	490	
Platte Lake 1-C	8/20/2006	30.0	56.3				
Black River Lake 1-A	8/21/2006	2.7	21.7	160	50	260	Sandy
Black River Lake 1-B	8/21/2006	3.5	20.1	160	20	290	
Black River Lake 1-Cha	8/21/2006	6.2	28.2	----	----	----	
Long Lake 1-A	8/22/2006	4.3	39.9			300	Clay and Gravel
Long Lake 1-B	8/22/2006	3.8	30.4			390	
Long Lake 1-C	8/22/2006	4.4	32.5	----	----	----	
New Hampshire Lakes							
Back Bay 1-A	9/17/2006	43.6	70.8	490	800	1300	Very organic
Back Bay 1-B	9/17/2006	44.1	72.2	430	420	990	
Back Bay 1-C	9/17/2006	46.3	74.7				
Balch Pond 1-A	9/17/2006	30.2	65.6	120	410	640	Organic over sand
Balch Pond 1-B	9/17/2006	30.9	62.5	110	560	780	
Balch Pond 1-C	9/17/2006	30.0	71.6	----	----	----	
Crescent Lake 1-A	9/18/2006	6.3	44.5	100	70	480	Very Sandy
Crescent Lake 1-B	9/18/2006	7.4	43.9	200	90	430	
Crescent Lake 1-RZ	9/18/2006	11.8	46.4	----	----	----	
Swain's Lake 1-A	10/6/2006	9.9	63.4	180	330	570	Organic over gravel
Swain's Lake 1-B	10/6/2006	9.5	56.9	220	290	690	
Swain's Lake 1-RZ	10/6/2006	16.2	53.8				
Wentworth Heath -A	10/9/2006	10.1	46.3	210	170	540	Thin organic over
Wentworth Heath -B	10/9/2006	9.3	42.6	160	80	320	sand/silt over
Wentworth Heath -C	10/9/2006	9.2	49.4	----	----	----	sand/ smallgravel

SECTION 5

Conclusions and Summary

These investigations have provided a better understanding of how *Myriophyllum heterophyllum* occurs in its native range, its common associations, and its aggressiveness and displacing nature under the right conditions anywhere. We also learned which plants tend to return when controlled in its invasive range as the Suncook situation has informed us. It also opens up the possibility that contrary to our original hypothesis, that we would find a potential parasite operating in its native range there to make a case for bringing it back here, we have discovered that in lakes with long-term established population of the plant we have a hopeful candidate that has been here all along to devote further study to.

To best proceed on that front will require more intensive life cycle studies as well as continued investigation into the various nematode plant associations that exist. The good news is that we can do these studies closer to home. The challenging element is the finding of our candidate in Balch Pond among the *M. verticillatum* and, hopefully the *M. heterophyllum*. Samples collected throughout the year should inform us on the nematode life history, potential predator prey cycles and critical times in nematode population development.

In any case we have provided for managers more complete information on the ecology of our problematic Milfoil and in the process we have greatly advanced the science of nematology.

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Appendix A

Plant Community Field Notes:

New Hampshire and Midwest Plant Communities Associated with
***Myriophyllum heterophyllum* (Variable Milfoil)**

Plant Communities Associated with
***Myriophyllum heterophyllum* (Variable Milfoil)**

Midwest Lakes (see Table 1):

WI-1
WISCONSIN: Clark County: ca. 31 mi. east of Eau Claire; Rock Dam Lake. T26N, R4W, sec.15, se ¼. N 144º 43.980', W 90º 50.624'. Depth near center of lake 8.7 ft.
Secchi disk: without scope—85 cm; with scope—94 cm; Water color: darkly stained.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9913*) plants were fertile, rooted, ca. 0.6 – 0.8 m depth, growing in a near monoculture. The sediments were sandy. Associated plants observed: *Nymphaea odorata* ssp. *tuberosa*.

A second site on the lake was also observed: *Myriophyllum heterophyllum* plants growing along margin of streamlet entering lake in shallow water with a nearly pure sandy shore and bottom. The associated plants observed included some *Utricularia vulgaris* intermixed at stream margin. Other plants were found growing along margin, but not intermixed with *Myriophyllum heterophyllum* included: *Potamogeton epihydrus,Nymphaea odorata* ssp. *tuberosa* and *Utricularia geminiscapa.*

WI-2
WISCONSIN: Chippewa County, Round Lake, Ca. 11 mi. n. of Bloomer.
T32N, R9W, sec. 14. N 45º 15.657', W 90º 26.965'.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9914*) plants were growing long boggy side of lake. The bottom was sandy-gravelly. The plants were sparse, short, rooted in water 2.2 ft. deep; none of the plants reaching the surface, and many fragments were observed floating. Associate plants observed: *Potamogeton epihydrus, Utricularia purpurea, Brasenia schreberi, Pontederia cordata* (juvenile submersed form) and *Utricularia resupinata*; all vegetation was very sparse. The adjacent boggy vegetation along the shore was dominated by Black Spruce (*Picea mariana*).

A second site on the lake was also observed (45º 15.556'N, 91º 27.074'W): *Myriophyllum heterophyllum* plants were found growing more dense, healthy plants. Water depth 2.8 ft.; Water color: darkly stained. *Utricularia purpurea* was very abundant—more so than *M. heterophyllum;* the *M. heterophyllum* was usually in areas with *Nymphaea odorata* ssp. *tuberosa*. Associate plants observed: *Potamogeton gramineus, Nuphar variegata* (very sparse), and *Brasenia schreberi* (sparse).

MI-1
MICHIGAN: Benzie Co., Benzonia Township: Platte Lake, along County Rd. 708 ca. 2 mi. northwest of village of Honor; southernmost small bay at south end of lake. T26N, R15W, Sec. 1 se. ¼. N 44º 41' 05.8", W 86º 03' 43.3" [at parking area on nw side of bay].

Small population of *Myriophyllum heterophyllum* (UNH voucher specimen *Crow 9923, 9924*) with large scattered plants with several upright stems from a single rooted stem; mostly submerged, some plants fertile. Water shallow, ca. 0.5-1 m deep.
Associated plants: *Vallisneria americana* (very abundant), *Stuckenia (Potamogeton) pectinata* (common), *Potamogeton zosteriformis* (abundant), *Elodea canadensis* (common), *Najas flexilis* (common), *Potamogeton crispus* (sparse here, but abundant at mouth of stream).
Alkalinity: 362; 374
Date sampled: 19-20 August 2006

MI-2
MICHIGAN: Cheboygan Co., Grant Township: Black River outflow from Black Lake at marina; ca. 13 mi. se. of Cheboygan; ca. 11 mi. w. of L. Huron. T36N, R1W, sec. 21 nw ¼. N 45º 29.982' W 84º 19.621'.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9925*) plants were growing in sand in shallow water, submersed; except for fragments chopped up by motor boats, all plants were submersed—the tips not reaching the surface. Only a few fragments found with bracts subtending flowers. The plants were very abundant with the growth very lush—densest growth at depth of 2-5.2 ft (along both margins of river). Growth was very sparse in deeper areas of channel and none were observed growing in deepest portion of river channel (8.2 ft deep). Additionally the terrestrial growth form of *M. heterophyllum* was developing on stranded fragments along sandy margin. Associated plants: *Vallisneria americana,*

Potamogeton amplifolius, Potamogeton zosteriformis, Potamogeton perfoliatus, Najas flexilis, Elodea canadensis, Stuckenia (Potamogeton) pectinata.
Water pH: 8.2 at surface; sediment pH 7.4
Alkalinity: 182
Date sampled: 21 August 2006

MI-3
MICHIGAN: Presque Isle Co., Presque Isle Township: Long Lake, northwest side. Ca. 5 mi. west of L. Huron; ca. 14 mi. north of city of Alpena. T33N, R8E, sec. 31 ne ¼. N 45º 13.271' W 83º 30.281'. Small bay at DNR boat launch. Lake bottom of limestone rocks and silt.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9925*) plants were sparse—only 6 multi-branched plants seen, growing in water ca. 2 ft. deep. No other plants were growing directly associated with these plants, but *Nymphaea odorata* ssp. *tuberosa* was growing nearby (indicating quiet water, on a large lake with much wave action). Numerous fragments of *M. heterophyllum* were seen washed up on shore and the terrestrial growth form of the plant was seen developing on stranded plants.
Surface pH 8.35; sediment pH 8.33
Alkalinity: 128
Date sampled: 22 August 2006

<u>New Hampshire Lakes (see Table 2-1):</u>

NH-1
NEW HAMPSHIRE: Belknap Co., Town of Barnstead. Ca. 1 mi. west of Center Barnstead. Suncook River, cove along Parade Rd. N 43º 20.394', W 71º 16.657'. Shallow backwater long river.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9928*) plants were observed to be growing abundantly and extremely dense in shallow water. All the plants were submerged and vegetative, with winter buds beginning to form at base of the plants. Associated plant growth was generally sparse compared with the *M. heterophyllum*: *Potamogeton epihydrus* (infrequent), *Utricularia purpurea* (infrequent), *Utricularia* cf. *radiata* (infrequent), *Pontederia cordata* (infrequent), *Nymphaea odorata* ssp. *odorata* (infrequent).
Date sampled: 13 September 2006

NH-2
NEW HAMPSHIRE: Carroll Co., Town of Wolfboro, Backbay, at boat slips by Railroad Station. N 43° 35.145' W 71°12.784'.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9931*) population of extremely dense growth, nearly a monoculture; few other species growing with it. Plants growing in water ca. 2-4 ft. deep, the bottom sedimens deep and extremely fine. Associated plants: *Potamogeton amplifolius* (locally abundant), *Sparganium angustifolium* (sparse), *Elodea nuttallii* (occasional), and *Nymphaea odorata* ssp. *odorata* (sparse).
Date sampled: 17 September 2006

NH-3
NEW HAMPSHIRE: Carroll Co., Town of Wolfboro, Crescent Lake, cove near dam. N 43° 35.334', W 71° 11.950'.

Myriophyllum heterophyllum (UNH voucher specimen *Crow 9932*) population was very sparse, mostly occurring in long deep channels (original river channel), in water ca. 4.1 m deep. The bottom was very rocky, with little sediment. (Lake was searched in several coves with very few plants of *M. heterophyllum* found.) Associated plants: *Potamogeton epihydrus* (abundant), *Utricularia purpurea* (abundant), *Elodea nuttallii* (infrequent), and *Potamogeton amplifolius* (sparse).
Date sampled: 18 September 2006

NH-4
NEW HAMPSHIRE: Carroll Co., Town of Wolfboro, Wentworth Lake, Heath Brook Cove.

Myriophyllum heterophyllum (no voucher specimen collected) was found growing very densely in the sediments of Heath Brook Cove, forming a near monoculture. Associated plants: *Scirpus (Shoenoplectus) subterminalis*, a plant with thread-like stems, was growing very abundantly adjacent to the population; and submersed stems were found sparsely intermixed with M. heterophyllum; *Potamogeton oakesianus*, a pondweed with floating leaves was also growing, but sparsely, adjacent to the population, and rarely intermixed with *M. heterophyllum*.

Info added 7-11-07
From field notebook dated 24 June 2005
NH, Wolfboro, Wentworth Lake.
Small stream on western side connecting with Crescent Lake.

Small, but very dense patch of Myriophyllum heterophyllum. Almost pure stand.
Other plants within stand dwarfed, except where there are small pockets without M. heterophyllum/or thin growth:
Potamogeton amplifolius, very dense adjacent to M. h. patch; Brasenia schreberi, few underwater leaves, but not reaching surface with M. h. patch—common outside patch; Utricularia vulgaris, sparse within patch, common outside patch; Potamogeton sp. (narrow-leaved species), somewhat frequent within patch, but very thin plants/scrawny, not reaching surface; Potamogeton epihydrus, one plant collected, submersed phase only (probably too

early for floating leaves to develop); Sparganium cf. angustifolium, a few plants seen—ribbon-like submersed leaves only).

Lake treated to kill *M. heterophyllum*

NEW HAMPSHIRE: Belknap Co., Town of Barnstead. Lower Suncook Lake. N 43° 22'23", W 71°15'45". Southern portion of lake adjacent to Ridge Rd.

Lake treated with herbicide in July 2005 to kill *Myriophyllum heterophyllum*. Area examined for plants presently growing submerged in the area where *M. heterophyllum* had been common by using underwater camera and dragging bottom with grapple hook (modified garden hand rake).
Plants seen: *Potamogeton epihydrus*—abundant; most common plant on the bottom, growing over much of the area. *Najas flexilis*—abundant; growing commonly throughout much of the area. *Utricularia purpurea*—frequent; in some areas locally abundant.
Sparganium angustifolium—infrequent in the deeper areas (mostly growing in shallow areas at edge of lake where leaves float on water surface). *Elodea nuttallii*—one plant seen (probably occurs very infrequently in deeper areas, could be expected occasionally in shallower water near margin of lake). *Potamogeton bicupulatus*—one plant seen (brought up on rake; probably occurs infrequently in deeper areas, but could be expected occasionally in shallow water along shallow areas at edge of lake.
Date sampled: 13 September 2006

Native Species of *Myriophyllum*

For comparison, 2 sites with the native species of Myriophyllum were examined and sampled for nematodes and plant community structure—*Myriophyllum humile* and *M. verticillatum*. Both of these species are rather rare in New Hampshire and have therefore been included on the New Hampshire "WATCH" list by the New Hampshire Natural Heritage Bureau (2007).

***Myriophyllum humile* site (a native species)**

NEW HAMPSHIRE: Strafford Co., Town of Barrington. North end of Swain's Lake at Young Rd. public boat launch. N 43° 12' 01", W 71° 02' 48.1".

Myriophyllum humile (UNH voucher specimen *Crow 9927*) plants in population were growing abundantly along a channel in the lake from the culvert under Young Road out into the lake. The plants were submerged, with reddish tips, the axillary flowers just beginning to develop underwater. Associated plants: *Utricularia purpurea* was very abundant, and formed a monoculture when growth was dense; other plants included *Potamogeton*

epihydrus, *Potamotegon natans*, *Pontederia cordata*, *Sparganium angustifolium*, *Brasenia schreberi*, and *Nymphaea odorata* ssp. *odorata*.
Date sampled: 8 September 2006

<u>*Myriophyllum verticillatum* site (a native species)</u>

NEW HAMPSHIRE: Carroll Co., Town of Wakefield, just east of East Wakefield at Balch Hill Rd. Balch Pond (also known as Stump Ponds), at Loon Cove. N 43° 37.428', W 70° 58.721'.

Myriophyllum verticillatum (UNH voucher specimen *Crow 9930*) plants were very dense, of tall, ascending submersed plants, all vegetative, growing in quiet water ca. 2-5 ft. deep. The population was dense with relatively little other vegetation growing mixed among the *M. heterophyllum*. Bottom sediments were deep and very fine. Associated plants: *Utricularia purpurea* (some mixed within the population), *Scirpus subterminalis* (abundant, with only some mixed within the population), *Sparganium angustifolium* (sparse), *Nuphar variegata* (infrequent, at edge of population), *Nymphaea odorata* ssp. *odorata* (infrequent, at edge of population), *Brasenia schreberi* (sparse), *Potamotegon oakesianus* (sparse), and *Potamogeton amplifolius* (abundant at edge of population).
Date sampled: 17 September 2006

NOTE: this population was originally identified as *M. heterophyllum*, but was not in fertile condition; DNA analysis revealed the population to be *M. verticillatum*.

Appendix B

A List of Nematode Taxa Discovered to Occur with *M. heterophyllum*

Achromadora
Actinolaimid
Actinolaimus
Alaimid
Alaimus
Anatonchus
Anonchus
Aphanolaimid
Aphanolaimus
Aphanonchus
Aphelenchoides
Brevitobrilus
Chronogaster
Comiconchus
Cryptonchus
Doryiaimid
Dorylaimus
Epitobrilus
Ethmolaimus
Eumonhystera
Eutobrilus
Hirschmanniella
Ironus
Laimydorus
Miconchus
Monhystera
Monhystrella
Mononchid
Mononchulus
Mononchus
Mylonchulidae
Neotobrilus
Noeactinolaimus
Oscheius??
Paramononchus
Paraphanolaimus
Paraphanonchus
Paraphelenchus
Paraplectonema
Plectus
Prodesmodora
Rhabditid
Rhabdolaimus
Scleroactinolaimus
Semitibrilus
Tobrilid
Tobrilus
Tridentulus
Tripyla
Tylench
Westindicus
Sphaerolaimid
Oncholaimid
Sabatieria