First record of Eurasian Water-milfoil, *Myriophyllum spicatum*, for the Saint John River, New Brunswick

Meghann Bruce[1,*], Tommi Linnansaari[1], and R. Allen Curry[1]

[1]Canadian Rivers Institute, Biology, Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick E3B 5A3 Canada
[*]Corresponding author: meghann.bruce@unb.ca

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Abstract
Eurasian Water-milfoil (*Myriophyllum spicatum* L.) is regarded by conservation practitioners as one of the most challenging invasive aquatic plants to manage. Owing to its broad tolerance to environmental conditions, vegetative propagation, and rapid establishment and growth, *M. spicatum* introductions have the potential to drastically alter macrophyte species assemblages via a loss of native species and their respective ecosystem functions. Following the discovery of a single specimen of *M. spicatum* in the Saint John River, near Fredericton, New Brunswick (Canada) we further investigated the localized distribution of this non-indigenous species. Thirteen areas were identified as potential *M. spicatum* habitat and were surveyed by wading or snorkeling. Specimens of *M. spicatum* were collected and morphological identifications were verified through genetic analyses (ITS2; *rbc*La). The results of our investigation confirm the presence of *M. spicatum* at six different locations within the Saint John River. Here we discuss the implications of this discovery in the context of the contiguous aquatic habitats along a large river system.

Key words: Eurasian Water-milfoil; *Myriophyllum spicatum*; aquatic invasive; Saint John River

Introduction

Approximately 15% of non-indigenous plant species become invasive causing irreversible disruptions to ecosystem functions (Westbrooks 1998). In aquatic environments, not only do invasive plants alter floristic assemblages via loss of native species (Aiken *et al*. 1979; Boylen *et al.* 1999) and their respective ecosystem functions (Duffy and Baltz 1998; Thomaz and da Cunha 2010) and compromise habitat for many other species, but they also alter environmental flows, nutrient cycling, and can directly influence water quality (Zedler and Kercher 2004; Kovalenko and Dibble 2010; Villamagna and Murphy 2010). Additionally, invasive aquatic plants often grow to high densities and are detrimental to the economic, recreational, and aesthetic qualities of waterways (Newroth 1985; Eiswerth *et al.* 2000). Mitigation of the negative impacts of aquatic introductions requires active control measures and is costly (Pimental *et al.* 2004).

One of the five most noxious aquatic plant invaders of aquatic ecosystems is Eurasian Water-milfoil (*Myriophyllum spicatum* L.; Cronk and Fennessy 2001). Native to Eurasia and northern Africa (Sennikov 2016), *M. spicatum* is now present on every continent except Antarctica (Cook 1985). While the impacts of the introduction of *M. spicatum* vary in magnitude among different aquatic environments and in different regions (Smith and Barko 1990), it is generally acknowledged among scientists and conservation practitioners that this species frequently establishes dense, monospecific beds that outcompete local flora and reduce the diversity and abundance of native species (Grace and Wetzel 1978; Madsen *et al.* 1991; Boylen *et al.* 1999). In some instances, this species has outcompeted native flora in as little as 2–3 years (Aiken *et al.* 1979; Newroth 1985; Boylen *et al.* 1999). Considered the most widely managed invasive aquatic plant in the United States (Bartodziej and Ludlow 1998), *M. spicatum* is on several regional invasive species watch lists and is listed as one of the ten most unwanted species in Maine, USA (Hill and Williams 2007), and New Brunswick, Canada (New Brunswick Alliance of Lake Associations website: http://www.nbala.ca/new-page-1).

The vector and timing of introduction of *M. spicatum* to North America is not completely understood. While Couch and Nelson (1985) suggest *M. spicatum* was introduced to North America in the 1940s, Reed (1977) reviewed historical herbarium specimens and provided evidence that the earliest verified records of *M. spicatum* from North America are dated back to at least 1881 but acknowledged that the introduction was possibly as early as 1848. It is not uncommon for non-indigenous species to exhibit an initial lag in their growth before they become invasive, and many non-indigenous aquatic plant introductions go unnoticed until they are established as truly invasive. Thus, it is highly probable that *M. spicatum* was present in North America as early as 1848 and Couch and Nelson's (1985) report regarding introduction in the 1940s more accurately reflects the timing at which this species was first observed as invasive.

In an assessment of historical records for the distribution of *M. spicatum* in North America, Reed (1977) also observed a disjunct distribution with populations in eastern North America, southeastern North America, and an isolated region in California. He attributed this disjunction to independent introductions that were most likely a consequence of the release of aquarium plants, as various species of *Myriophyllum* were commonly cultured and distributed for the aquarium trade at this time (Reed 1977). While the release of aquarium plants may be the original source of introduction events across North America, introduction to new waters is now primarily attributed to fragments introduced by boats and their associated trailers (Johnson *et al.* 2001; Rothlisberger *et al.* 2010).

Successful eradication of recently established invasive species populations is highly dependent on rapid detection and prompt management actions (Willby 2007). Two factors may hinder the rapid detection of *M. spicatum*: difficulty in detection because it is primarily beneath the water's surface and difficulty in identification versus similar native congeners (especially the sister species Siberian Water-milfoil, *M. sibiricum* Komarov) because the key morphological features vary with phenotypic plasticity (Strand and Weisner 2001) and/or hybridization (Sturtevant *et al.* 2009).

While conducting macrophyte surveys for 171 sites along the Saint John River (SJR) as a part of a larger aquatic ecosystem study, the Mactaquac Aquatic Ecosystem Study (http://canadianriversinstitute.com/research/mactaquac-aquatic-ecosystem-study/), we discovered a single inconspicuous specimen of *M. spicatum*. Prior to our discovery of this species in the SJR, Hinds (2000) reported that this species had been collected from a small pond in Fundy National Park (Hinds 2000: 667). The introduction of *M. spicatum* to the SJR poses a threat to the submerged aquatic flora within the river and associated waterways. To assess the local distribution of this non-indigenous species, we used an active survey approach that involved snorkelling surveys of potential habitat and molecular approaches (DNA analyses) to verify our taxonomic identifications.

Methods

To identify potential *M. spicatum* habitat for this survey we looked for areas in the Fredericton region of the SJR (where the first specimen was initially collected) that were consistent with habitat conditions reported for this species (Aiken *et al.* 1979). Our survey emphasized sheltered cove environments or other low flow areas with soft substrate, as well as areas with frequent boat traffic (Figure 1). Where necessary, snorkelling surveys were conducted to ensure we could observe the submerged flora.

Apical portions of plants morphologically identified in the field as *M. spicatum* were collected and preserved as herbarium vouchers stored at the Connell Memorial Herbarium (UNB IH) at the University of New Brunswick (Table 1). Leaf tissue sub-samples were dehydrated in silica for subsequent genetic analyses (Fazekas *et al.* 2012). Dehydrated tissue was sent to the Canadian Centre for DNA Barcoding (CCDB) for DNA extraction, PCR amplification, and sequencing according to CCDB standardized protocols (Fazekas *et al.* 2012). To facilitate comparison of our genetic results with taxonomic data available in GenBank (NCBI Resource Coordinators 2016) and the Barcoding of Life Data System (Ratnasingham and Hebert 2007) we selected two standard land plant DNA barcode markers, *rbc*La and ITS2 (Fazekas *et al.* 2012).

Results and Discussion

Analyses of ITS2 and *rbc*La sequence data was consistent with the morphological-based identification of *M. spicatum* at six of 13 sites surveyed (Table 1). Four of the six sites where *M. spicatum* is present had only a few scattered plants (Table 1). The remaining two sites where this species was found had patches where it was clearly established as dense macrophyte beds (Figure 2). To assess the potential future impact of this introduction on the native aquatic flora, we reviewed what has been reported for the biology of this invasive species and considered what risks this may present for the aquatic environments along the SJR.

Reproduction

Myriophyllum spicatum shoots emerge and exhibit rapid growth from an overwintering rhizomatous mass in the early spring and throughout summer. As the growing season progresses, plant growth peaks at the water surface where stems are highly branched forming dense floating canopy (Titus *et al.* 1975). Vegetative portions of the plants break off throughout the growing season and in the fall when plants typically die back to the propagating rhizome crowns (Aiken *et al.* 1979).

Fragmented vegetative portions are the primary mode of reproduction and spread for *M. spicatum* within an aquatic ecosystem (Kimbel 1982). In the SJR, downstream spread of this species via vegetative fragmentation is naturally facilitated by peaks in hydrological flows, as well as seasonal ice scouring. The spread of *M. spicatum* between watersheds is largely attributed to vegetative material transported by boat motors and trailers (Johnson *et al.* 2001; Rothlisberger *et al.* 2010). In the Fredericton region of the SJR where we have confirmed the presence of *M. spicatum*, further spread by boat motors is a concern as this area is frequently used by recreational boaters. Consequently, this increases the potential of the species to move in larger, discontinuous jumps, enabling the species to spread upstream and to new water bodies.

Like many successful invasive species, *M. spicatum* has multiple modes of reproduction and frequently exhibits sexual reproduction in addition to vegetative fragmentation. Perhaps more concerning than the ability to undergo both asexual and sexual reproduction, is the ability of *M. spicatum* to hybridize with its native sister species *M. sibiricum* to produce plants that exhibit "hybrid vigor"—plants with competitive phenotypes that are superior to both parent species (Moody and Les 2002, 2007; Sturtevant *et al.* 2009). This hybridization, between an introduced invasive species and a native

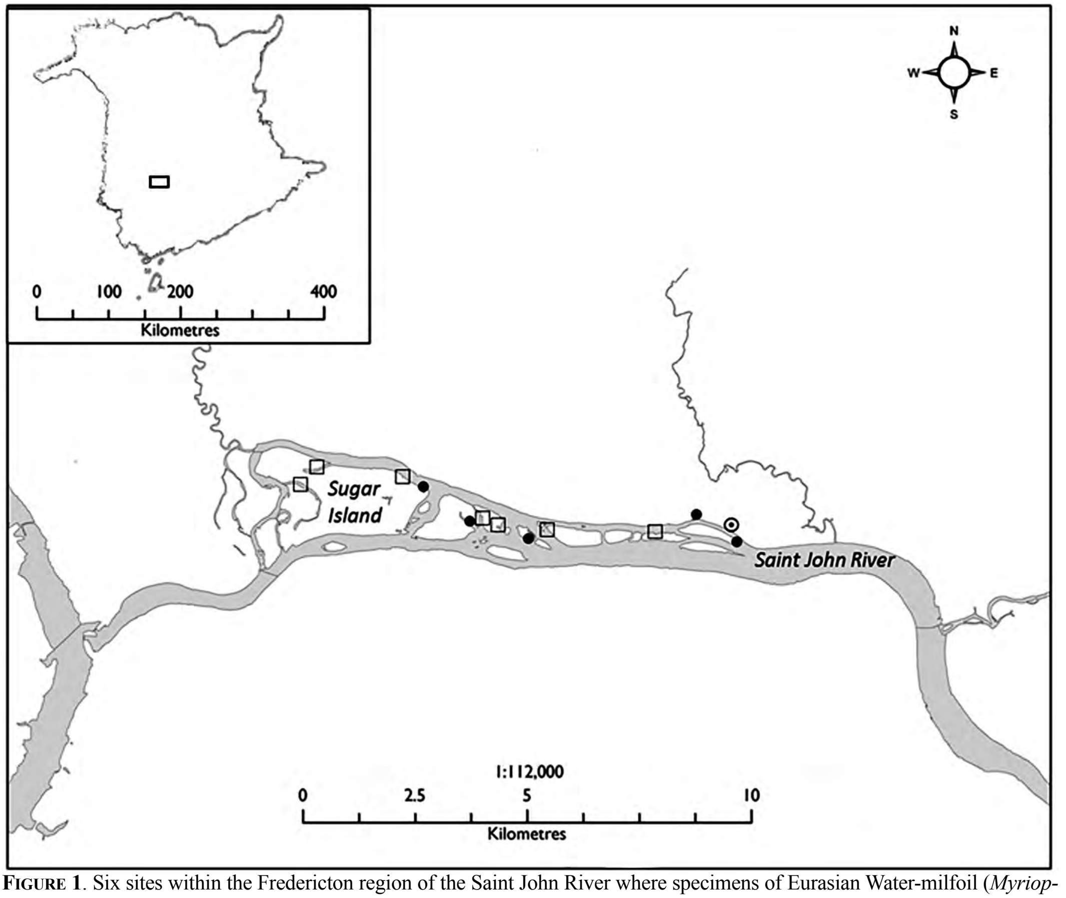

FIGURE 1. Six sites within the Fredericton region of the Saint John River where specimens of Eurasian Water-milfoil (*Myriophyllum spicatum*) were collected and identified (denoted by "●"). ⊙ = original site of collection; □ = potential *M. spicatum* habitat investigated but species was not present.

species, can result in "genetic pollution" introducing new alleles to the population and potentially wiping out locally adapted genotypes (Laikre *et al.* 2009). In New Brunswick, the native species *M. sibiricum* is classified as potentially vulnerable (S3/S4) and is widespread on the lower SJR system (S. Blaney pers. comm. February 2015). Thus, the ecological risks posed by the potential hybridization of *M. spicatum* and *M. sibiricum* are two-fold: hybridization may give rise to populations the exhibit hybrid-vigor and promote further colonization and populations of the native species, *M. sibiricum*, may be put at risk due to genetic pollution or competition pressure with *M. spicatum* or *M. spicatum* × *M. sibiricum* hybrids.

Habitat and area for potential colonization

Most commonly establishing in water with depth ranging 1–3 m, *M. spicatum* has been reported as deep as 10 m (Aiken *et al.* 1979), reaching 7 m high. Plants thrive in eutrophic lakes with soft organic substrates but persist in a wide range of substrates and environmental conditions (Nichols and Shaw 1986). With regard to water quality, *M. spicatum* is able to persist in a wide pH range (5.4–11), tolerate salinity up to 15 ppt, and tolerate various industrial pollutants (Aiken *et al.* 1979; Wang *et al.* 1996). When growing in shallow areas susceptible to drops in water level that may leave it exposed, *M. spicatum* assumes a terrestrial form allowing it to gradually become stranded and survive (Aiken *et al.* 1979). The broad environmental tolerance in this species enables it to colonize various types of lakes, wetlands and salt marshes, or river margins, coves, and inner island channels as observed in our surveys. Downstream of our confirmed *M. spicatum* population is approximately 130 km of river with extensive seasonal flood plain and contiguous habitat that has high potential for colonization by this species.

The Grand Lake Meadows (GLM), located approximately 40 km downstream from the sites of the *M. spicatum* occurrence, is the largest freshwater wetland and floodplain in New Brunswick. It includes the provincial Grand Lake Class II Protected Natural Area (GL PNA). Recent surveys of the flora in the area report 98 rare species that contribute 20% of the total flora (Papoulias *et al.* 2006). One of the taxa reported, Budding

TABLE 1. Eurasian Water-milfoil (*Myriophyllum spicatum*) specimens and associated collection and GenBank records.

UNB IH accession (research specimen number)	Collectors	Location	Collection date	Habitat	Abundance	GenBank accession number	
						ITS 2	*rbc*La
66290 (MRB000031)	M. Bruce C. Brooks Z. Compson S. Andrews	45.97577ºN, 66.68854ºW	20 August 2015	Edge of back channel, soft bottom, water ~60 cm at time of collection	Single small plant	MG648683	MG648689
66921 (MRB000034)	M. Bruce H. Johnson G. Filloramo	45.97711ºN, 66.69473ºW	4 July 2016	Sheltered, soft substrate	Dense patch (~4 m × 5 m) surrounded by several peripheral plants	MG648684	MG648690
66292 (MRB000035)	M. Bruce S. Andrews	45.97552ºN, 66.75940ºW	16 July 2016	Sheltered island inlet, soft substrate, water ~120 cm at time of collection	Numerous scattered plants	MG648686	MG648692
66293 (MRB000036)	M. Bruce H. Johnson G. Filloramo	45.98299ºN, 66.77400ºW	16 July 2016	Sheltered island inlet, soft substrate, water ~180 cm at time of collection	Dense patch in center of cove, ~2 m × 3 m	MG648685	MG648691
66293 (MRB000037)	M. Bruce B. Pardy	45.972010ºN, 66.68262ºW	4 July 2016	Small cove on back channel, soft substrate, water ~90 cm at tie of collection	A few plants	MG648687	MG648693
66294 MRB000039	M. Bruce S. Andrews Z. Compson	45.97290ºN, 66.74338ºW	4 September 2015	Edge of island, mixed substrate, water ~60 cm at time of collection	Single small plant	MG648688	MG648694

FIGURE 2. Eurasian Water-milfoil (*Myriophyllum spicatum*). a. Overall habit, plants growing in a dense patch with a high degree of branching, forming a canopy at the water surface. b. Specimen of *M. spicatum* showing leaves in whorls of four along stem. c. Single pectinate leaf with 15 pairs of pinnae. Photos: M. Bruce.

Pondweed, *Potamogeton berchtoldii* subsp. *gemmiparus* (J.W. Robbins) Les & Tippery, is the only known record of this species for New Brunswick and it is rare on the national level (Papoulias *et al.* 2006). In the spring, the SJR floodplain spills into the GLM and GL PNA, downstream of our confirmed populations of *M. spicatum*. The GLM and the GL PNA is thus an area of special concern that should be monitored for a potential M. *spicatum* invasion.

Options for controlling further spread

Early detection of *M. spicatum* and minimizing risk of further spread of early introductions hold the most promise for aquatic ecosystem management (Willby 2007). For individual plants, or small stands of *M. spicatum*, shading with a black cloth that inhibits photosynthesis can kill the plants (Bailey and Calhoun 2008). This could be an option for the plants that were found in this study, or small isolate populations in other areas. Another option that may be useful (for at least this area of the SJR) is to reduce water flow when air temperatures drop to freezing in early winter. Exposing the crown of the plant to freezing temperatures has shown some success in managing populations of this species in other areas (Bates and Smith 1994; Wagner *et al.* 2008). Considering that our observed plants were all in shallow areas below the Mactaquac Hydrogeneration Station (MGS) which have been observed to be exposed at times when the MGS retains water (M.B. pers. obs. early August 2015 and 2017), this could be an option for managing the small populations in this area. Where *M. spicatum* has established as invasive, raking of vegetation helps to temporarily reduce biovolume; however, reproductive fragments render application of this method as high-risk for further spread. Herbicides and the introduction of natural pests have also shown some promise, although the previous studies do not assess potential negative impacts to non-target native species within the aquatic ecosystem (Creed 1998; Cock *et al.* 2008).

Conclusions and future investigations

With knowledge of the presence of this non-indigenous species within the SJR, we intend to quantitatively assess the amount of potential downstream habitats available for colonization by developing spatial models of potential habitat. Spatial models have been used in a number of ecological and biological studies to identify links between the abiotic and biotic environment (e.g., Milhous *et al.* 1981; Milhous 1999). Such models use environmental variables, such as velocity, substrate composition, temperature, etc., to explain the presence and spatial distribution of biota of interest (e.g., Dunbar *et al.* 2011). We intend to build a spatial model to (a) identify habitat utilized by *M. spicatum* and (b) apply our model to the SJR to identify areas that may potentially be available for colonization.

Unfortunately, we currently cannot ascertain when or how this species arrived, or the full extent of this species' range in the SJR. Our immediate priority is to extend our survey coverage and to determine if *M. spicatum* is present beyond the range we have observed. Prior to the recreational boating season, we will engage local conservation practitioners and develop an action plan to educate and engage the public as to the presence of *M. spicatum* in this region in an effort to minimize the further spread of this species and mitigate the negative effects of already established occurrences.

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