State of Michigan's

Status and Strategy for European Frog-bit (*Hydrocharis morsus-ranae* L.) Management

Scope

Hydrocharis morsus-ranae L. (European frog-bit, hereafter EFB) is a free-floating aquatic plant native to Europe, Asia, and Africa and invasive in North America and parts of Asia (Cook and Lüönd 1982; Ganie et al. 2016). It was first detected outside of cultivation in Canada in 1939, was documented in the United States in 1974, and by 1996 was found in southeast Michigan (Minshall 1940; Roberts et al. 1981; Reznicek et al. 2011). European frog-bit has the potential to negatively impact the quality and use of waterbodies and is considered a high-risk invasive species by the Michigan Department of Agriculture and Rural Development (Weibert 2015). An earlier version of this document was a product of an Environmental Protection Agency – Clean Water Act Section 205(j) grant between the Michigan Department of Environmental Quality and Central Michigan University in 2014 (Hackett et al. 2014). It was significantly revised by Central Michigan University and reviewed by Michigan Departments of Environmental Quality and Natural Resources for the purposes of:

- Consolidating current science-based knowledge relative to the biology and ecology of EFB.
- Summarizing scientific literature and research efforts that inform management options for EFB in Michigan.
- Identifying future directions for research relative to successful EFB management in Michigan.

This document references peer-reviewed journals and publications. Any chemical, company, or organization that is mentioned was included for its involvement in peer-reviewed, published, publicly shared information, not to imply endorsement of the chemical, company, or organization.

Biology and Ecology

I. Identification

European frog-bit is an herbaceous, free-floating, freshwater aquatic plant. Its leaves are entire, cordiform (heart-shaped) or slightly orbicular (circular), and arranged in a floating rosette (Figure 1). Its leaves are 0.47 – 2.4 in (1.2 – 6 cm) long and 0.51 – 2.5 in (1.3 – 6.3 cm) wide. Some leaves may be emergent when it is growing in dense floating mats. Its petioles (leaf stalks) are slender and have two translucent stipules at their base (Figure 2).

Figure 1. European frog-bit (*Hydrocharis morsus-ranae* L.) in bloom. Photograph by "Petroglyph" [CC BY-NC 2.0 (https://creativecommons.org/licenses/by-nc/2.0/)], via Flickr

European frog-bit's roots hang below the rosette and are suspended in the water. In shallow water and on exposed muck, its roots may partially penetrate the substrate. Its roots are covered in fine root hairs and can be up to 19.7 in (50 cm) long (Cook and Lüönd 1982).

Figure 2. The two arrows point to the two transparent leaf-like stipules at the base of the petioles of European frog-bit (*Hydrocharis morsus-ranae* L.). American frog-bit (*Limnobium spongia* (Bosc) Rich. ex Steud.) has only one stipule. Photograph by Paul Busselen, courtesy of Go Botany, New England Wildflower Society

European frog-bit's flowers have white to greenish sepals, white petals, and a yellow center (Figure 1). The petals of female flowers may have a reddish tinge. Flowering is erratic in its native and invasive range and may be influenced by small fluctuations in temperature (Cook and Lüönd 1982). Its flowers are short-lived and bloom from June to September in North America (Gardner 2008).

European frog-bit's fruits are globose (spherical) berries that contain as many as 74 seeds (Scribailo and Posluszny 1985). Its seeds are dark brown, broadly ellipsoidal, 0.04 – 0.05 in (1 – 1.3 mm) long, and covered with blunt spiraling tubercles. European frog-bit also produces specialized vegetative reproductive structures, called turions (Figure 3), at the nodes of stolons. Turions are ellipsoidal, 0.20 – 0.28 in (6 –7 (–9) mm) long, and are produced in the late summer and early fall in its native and invasive range (Cook and Lüönd 1982; Catling et al. 2003).

Species that can be mistaken for EFB include: American frog-bit (*Limnobium spongia* (Bosc) Rich. ex Steud.), American white waterlily (*Nymphaea odorata* Aiton), and water-shield (*Brasenia schreberi* J.F. Gmel). *L. spongia* is not found in Michigan and has only one stipule on its petioles compared to EFB's two (Figure 2; Gardner 2008). *L. spongia* also has larger aerenchyma (spongy tissue) spaces on the undersides of its leaves (Catling and Dore 1982). *N. odorata* has circular leaves that are 8 – 12 in (20.3 – 30.5 cm) long and have a distinctive slit on one side compared to EFB's 0.47 – 2.4 in (1.2 – 6 cm) long heart-shaped leaves. *B. schreberi* can be distinguished from EFB by its maroon flowers and oval leaves that have a coating of gelatinous slime on their underside.

Figure 3. A turion of European frog-bit (*Hydrocharis morsus-ranae* L.). Photograph by Christopher Fischer

European frog-bit seedlings can be difficult to distinguish from duckweed species: common duckweed (*Lemna minor* L.) and greater duckweed (*Spirodela polyrhiza* (L.) Schleiden). The roots of duckweed species arise from the underside of their leaves while the roots of EFB arise from the base of a rosette or leaf petiole (Catling et al. 2003).

II. Detection

European frog-bit is typically found in calm to slow moving waterbodies in areas protected from wind and wave action (e.g., shorelines, wetlands, inlets). In the Great Lakes region, EFB occurs across the major wetland vegetation zones (i.e., emergent, floating, submerged vegetation). Dense floating mats of EFB are typically found in the floating vegetation zone or in sheltered openings of the emergent vegetation zone (Figure 4; Halpern 2017; Wellons 2018). European frog-bit detection efforts are best conducted from early summer to early fall when its leaves are floating on the surface of the water (Catling et al. 2003). European frog-bit plants have been documented on the surface of the water beginning in May in the Lower Peninsula of Michigan and in late June to early July in the Upper Peninsula (Cahill et al. 2018). When growing in the floating and submerged vegetation zones, EFB can typically be detected via visual searches from a boat or land. More intensive sampling may be required for detection when EFB is growing among emergent vegetation.

Figure 4. Floating mat of European frog-bit (*Hydrocharis morsus-ranae* L.) in a canal on Harsens Island, Michigan. Photograph by Blake Cahill

Remote sensing technology can be used to detect and distinguish EFB. In the South Nation River in Ontario, EFB mats were distinguished at an overall accuracy of 72.8% (Kappa 66.0%) for unsupervised fuzzy and object-based image analysis (Proctor et al. 2012). The unsupervised analyses occasionally mistook EFB for other free-floating and floating-leaved plants (i.e., *N. odorata*, *L. minor*, yellow water-lily (*Nuphar lutea* (L.) Sm.), coontail (*Ceratophyllum demersum* L.), floating pondweed (*Potamogeton natans* L.)) and sometimes for mixed forest along the shoreline. Supervised processing performed better (overall accuracy 87.4%, Kappa 84.3%), but required image processing experts to develop

classification rules at every step. Proctor et al. (2012) did not report the time required for supervised or unsupervised processing of imagery.

Typically, a patch of EFB would have to be 5 pixels in size to be detected with remotely sensed imagery. Unmanned aerial systems would likely be required to gather imagery at a resolution fine enough to detect smaller EFB patches or individual EFB plants. It is also difficult to detect EFB interspersed among emergent wetland vegetation (e.g., cattails (*Typha* spp. L.), common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), bulrush (*Schenoplectus* spp. (Rchb.) Palla)). Research led by Loyola University Chicago (Lishawa et al.) is currently evaluating the use of aerial imagery gathered after emergent vegetation has senesced for EFB detection (Cahill et al. 2018).

Many studies have demonstrated the utility of genetic material shed by organisms into the environment for biodiversity and early detection monitoring in aquatic systems (e.g., Bakker et al. 2017; Gingera et al. 2017; Wittwer et al. 2018). Genetic markers have been developed for detecting genetic material shed by EFB into the environment and these markers have been used to successfully identify EFB from laboratory-generated water samples (Scriver et al. 2015). Given the near shore habitat that EFB occupies and its easily distinguishable features, it may not be efficient to utilize this approach for EFB detection. However, it could improve the true positive detection of EFB when it is growing undetected in stands of emergent and floating vegetation or in an inaccessible portion of a waterbody. This approach could also reduce the need for labor-intensive field surveys until after EFB was positively detected in an area.

III. Life History and Spread/Dispersal

European frog-bit's sexual reproductive strategy is not fully understood. Some have reported that it has both monoecious (possessing male and female flowers) and dioecious (possessing male or female flowers) genotypes (Catling and Dore 1982; Cook and Lüönd 1982; Scribailo and Posluszny 1984; Martine et al. 2015) while others have reported that it is only monoecious (Lindberg 1873; Dore 1968; Halpern 2017). Lindberg (1873) and Cook and Lüönd (1982) suggested that EFB may appear dioecious due to the difficulty of untangling individual plants from one another.

European frog-bit flowers are imperfect (possessing either male or female reproductive structures) and short-lived, lasting one day once they open (Cook and Lüönd 1982; Catling et al. 2003). Flowering is erratic and may be influenced by small fluctuations in temperature (Cook and Lüönd 1982). The ideal temperature for flowering is unknown. Not all EFB individuals or colonies flower in a given year (Catling et al. 2003). Male and female flowers produce nectar that is visited by a variety of insect pollinators (Scribailo and Posluszny 1984). After the female flower is fertilized and the fruit begins developing, the peduncle recurves so that the fruit ripens in the water (Cook and Lüönd 1982; Scribailo and Posluszny 1984). Once ripe internal pressure causes the sides of the fruit to split, releasing the seeds into the water. The seeds sink to the substrate and remain there until germination begins (Scribailo and Posluszny 1984; Scribailo and Posluszny 1985). Little is known regarding the

germination triggers and viability of EFB seeds in its native or invasive range (Catling et al. 2003).

In addition to sexual reproduction, EFB can reproduce asexually. Clonal daughter plants, called ramets, are produced from terminal buds at the tips of stolons (Sculthorpe 1967). At the end of the growing season, EFB produces modified stolon buds, called turions (Cook and Lüönd 1982). Turions detach from the stolons in the fall and overwinter on the substrate. In the spring and early-summer, turions begin to germinate under the surface of the water and on the surface of the water (Wellons 2018).

Native Range:

In its native range, EFB is a summer annual that can reproduce sexually and asexually (Cook and Lüönd 1982). Seed production is considered rare (Arber 1920; Sculthorpe 1967; Cook and Lüönd 1982; Preston and March 1996). Seeds collected in Europe germinated when water temperature reached 59°F (15°C; Serbanescu-Jitariu 1972 in Catling et al. 2003) but little else is known regarding seed germination.

Asexual reproduction through stolon buds and turions is considered the primary form of reproduction in EFB's native range. When water temperature is between 59°F (15°C) and 77°F (25°C) turion development is initiated by photoperiod. The higher the water temperature the shorter the photoperiod needed to initiate development. When water temperatures are below 50°F (10°C) and above 77°F (25°C) turion initiation becomes independent of photoperiod. Below 50°F (10°C) turion development does not occur and above 77°F (25°C) turion development is immediate (Cook and Lüönd 1982).

Turion freezing temperature and survival are influenced by exposure to frost. In the Czech Republic, dormant turions that were hardened off by natural winter frosts, similar to what they would experience in the fall prior to breaking away from the stolons, had a 76% survival rate. Non-hardened turions that were keep at 36.5°F (2.5°C), representative of the water temperature at the bottom of lakes and rivers during winter in temperate climates, froze at 25.5°F (-3.6°C) and did not germinate (Adamec and Kučerová 2013).

Water temperature and light are the primary drivers of turion germination (Terras 1900 in Halpern 2017; Arber 1920; Sculthorpe 1967). In the United Kingdom, germination rate was greatest at 68°F (20°C) and did not occur below 50°F (10°C; Richards and Blakemore 1975). Two weeks of 59°F (15°C) were needed for the majority of turions to germinate but higher temperatures, approaching 68°F (20°C), were needed for the majority of turions to float. Germination rate was highest with greater light intensity and duration and germination did not occur in the dark.

Invasive Range:

Similar to its native range, EFB in North America can reproduce sexually and asexually. At Rondeau Provincial Park in southern Ontario, EFB started flowering in mid-June, reached peak bloom in mid-July, and was mostly finished by mid-August (Scribailo and Posluszny 1984). Homoptera (Aphidae) and Diptera (*Hydrellia* and *Notiphila* spp.) were most frequently

observed visiting the flowers but hoverflies (Syrphidae) and sweat bees (Halictidae) were considered the primary pollinators.

The reproductive status of EFB populations has been studied at three sites in North America: Lake Champlain, New York (Martine et al. 2015) and Lake Opinicon (Burnham 1998) and Lake Erie (Scribailo and Posluszny 1984), Ontario. In Lake Champlain, EFB was found to be primarily male and almost entirely dioecious. Artificially pollinated plants produced fruits, indicating sexual reproduction is possible in Lake Champlain but it was not observed in the field. In Lake Opinicon, EFB was found to be mostly dioecious, but close to 25% of plants were monoecious. European frog-bit mats of greater density produced less fruit compared to mats of lower density but fruits in the greater density mats contained more seeds. European frog-bit mats of intermediate density (~2000 g/m^2) produced the most seeds (2000 – 3000 per m^2). In a laboratory experiment, 69% of seeds collected from the Lake Opinicon population germinated when exposed to a 15-hour photoperiod and a 79/59°F (26/15°C) temperature regime for 12 months. Although the Lake Opinicon population produced abundant viable seeds, few seedlings were found in the lake. A similar situation was observed in a Lake Erie coastal wetland; 250 seeds per m^2 were produced but only 2 seedlings were found the following growing season.

Although, sexual reproduction occurs in North America, turion production is EFB's primary strategy for persisting overwinter. Turions develop on stolon nodes in the late summer and early fall, detach from the plant in the late fall, and overwinter on the substrate (Catling and Dore 1982; Catling et al. 2003). In Lake Opinicon, turion production increased as EFB biomass increased and reached as high as 1,000 turions per m^2 (Burnham 1998). In coastal wetlands of the Upper St. Lawrence River, turion production differed between vegetation zones, with a median of 208 per m^2 in the emergent vegetation zone, 32 per m^2 in the floating vegetation zone, and in all but one sample that contained 80 turions, zero in the submerged vegetation zone (Halpern 2017).

In southeastern Ontario, turions germinate from late April to early May and by mid-May plants are fully developed (Catling et al. 2003). In the Lower Peninsula of Michigan, turions germinate below the surface of the water from March to April and float to the surface in May. In the Upper Peninsula of Michigan, turions germinate below the surface of the water from April to May and float to the surface in late June to early July (Cahill et al. 2018). The rosette that develops from a single turion can give rise to over 10 ramets, each of which can produce 10 turions of their own (Scribailo and Posluszny 1984). A single turion can grow to cover an area of 1.2 yd^2 (1 m^2) in just one season (Cook and Lüönd 1982; Catling et al. 2003).

Little research has been conducted on turion viability. Burnham (1998) found that turions decayed after being kept at 39.2°F (4°C) for 16 months. Arber (1920) stated that turions can remain viable for up to 2 years but did not describe the methodology of the experiment.

In addition to differences in vegetative reproduction, Halpern (2017) noted differences in EFB density and biomass accumulation between wetland vegetation zones and in a controlled setting with different combinations of light and depth. European frog-bit density

was greatest in the emergent and floating vegetation zone and significantly less in the submerged vegetation zone. Shoot, root, and total biomass were greatest in the floating vegetation zone and similar between the other zones. European frog-bit produced the most biomass in full sun and shallow (11.8 in; 30 cm) to moderate depths (45 cm; 17.7 cm). Halpern (2017) also found differences in EFB morphology and nutrient content between wetland zones: roots were longest in the floating and submerged vegetation zones, root:shoot ratio was highest in the floating vegetation zone, leaves were narrowest in the submerged vegetation zone, and nitrogen content was highest in the emergent vegetation zone.

European frog-bit's free-floating habit allows it to drift on the water's natural flow within and between connected waterbodies. Turions and seeds may also drift on the waters flow. Much of EFB's initial spread in North America is attributed to drifting (Catling and Dore 1982). Some EFB colonies may have intentionally or accidently been introduced by duck hunting clubs to provide refuge and food for waterfowl (Catling and Dore 1982). European frog-bit plants, turions, and seeds may be transported on boats, trailers, and other boating equipment that isn't properly washed and dried following use in an infested waterbody. This mode of dispersal is attributed to much of EFB's recent spread (Catling et al. 2003) but has not been investigated. Wildlife can also contribute to the spread of EFB. Plants can become entangled in the bills and feet of waterbirds and subsequently transported to new waterbodies (Catling and Dore 1982). Seeds and turions may be transported through endozoochory (transport in the digestive tract); however, their viability after passing through the digestive tract is unknown (Systma and Pennington 2015). Although it is difficult to determine, the improper disposal of waste from water gardens and aquariums may also contribute to EFB's spread (Catling and Dore 1982; Catling et al. 2003).

IV. Habitat

Native Range:

European frog-bit is native to Europe and parts of Asia and Africa (Figure 5; Catling et al. 2003). It is critically endangered in Spain and the Czech Republic, endangered in Norway, Switzerland, and parts of France, and vulnerable in the United Kingdom (Lansdown 2014). Habitat loss is regarded as the primary cause of EFB's decline in Switzerland and the United Kingdom (Sager and Clerc 2006; Joint Nature Conservation Committee 2017). Elsewhere in its native range it is widespread and abundant (Lansdown 2014).

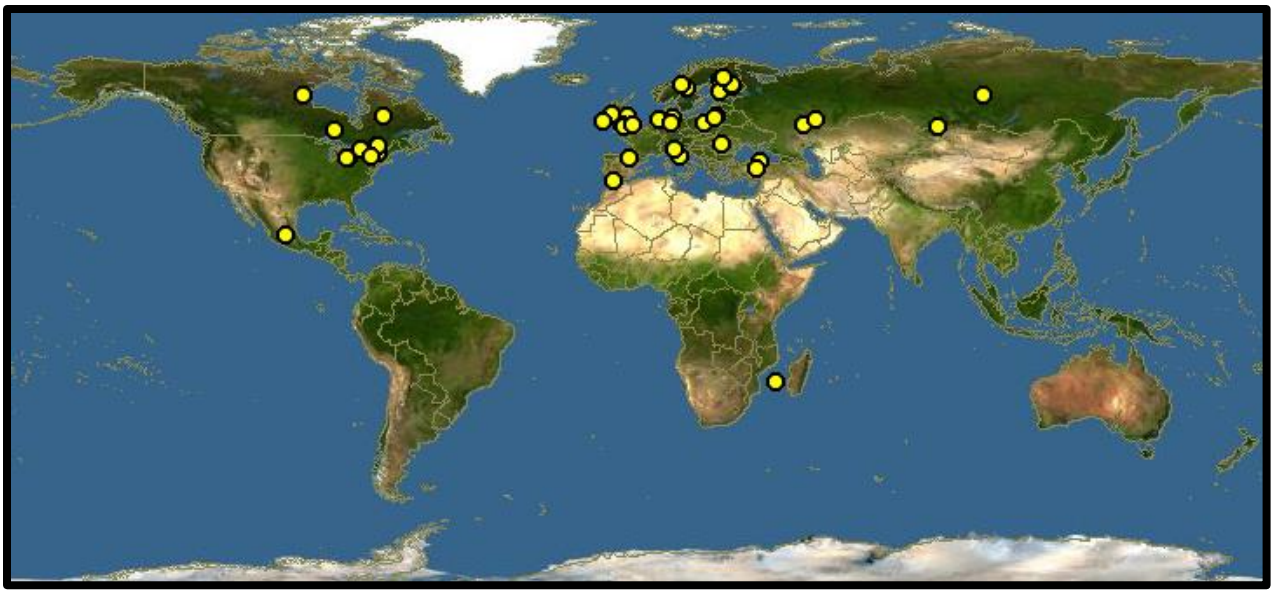

Figure 5. Global distribution of European frog-bit (*Hydrocharis morsus-ranae* L.). Source: http://www.discoverlife.org

European frog-bit tolerates a wide range of climatic conditions across its native range (Catling et al. 2003). It is found in fresh to slightly brackish water with low salinity (≤ 2 ppt; Luther 1951 in Sculthorpe 1967) and favors mesotrophic to oligo-mesotrophic conditions (Cook and Lüönd 1982; Murphy 2002). It has also been found in eutrophic conditions (Suominen 1968; Pitkänen et al. 2013). It occurs in calm to slow moving water with high conductivity (>300 µS cm^{-1}), near neutral pH (7.0 – 8.0), and organic substrate (Husák and Gorbik 1990; Murphy 2002; Sager and Clerc 2006; Steffen et al. 2014). European frog-bit inhabits small waterbodies and inlets, bays, and coves of larger waterbodies and is frequently found in ditches, canals, backwaters, peat diggings, and oxbow lakes (Cook and Lüönd 1982). Associated species include *Typha* spp., tufted sedge (*Carex elata* Mack.), grass-like sedge (*Carex panicea* L.), swamp sawgrass (*Cladium mariscus* (L.) Pohl)*, L. minor*, floating fern (*Salvina natans* (L.) All.), *S. polyrrhiza*, *N. lutea*, arrowhead (*Sagittaria sagittifolia* L.), common bladderwort (*Utricularia vulgaris* L.), *P. natans*, small pondweed (*Potamogeton pusillus* L.), shining pondweed (*Potamogeton lucens* L.), reed manna grass (*Glyceria maxima* (Hartm.) Holmb.), star duckweed (*Lemna trisulca* L.), and *C. demersum* (Husák and Gorbik 1990; Murphy 2002; Sager and Clerc 2006; Steffen et al. 2014).

Invasive Range:

In North America, EFB has been documented in Ontario, Quebec, New York, New Jersey, Vermont, Ohio, Michigan, Maine, Pennsylvania, Illinois, and Washington state (Dore 1968; Roberts et al. 1981; Catling et al. 2003; Gardner 2008; Marsden and Hauser 2009; Lamont et al. 2014; Jacono and Beret 2018; MISIN 2018). Similar to its native range, the EFB established in North America appears tolerant to a wide range of climatic conditions (Catling et al. 2003). It often occurs in nutrient rich water but a range of trophic levels, including oligotrophic conditions, are suitable for establishment (Catling et al. 2003; Zhu et al. 2008). European frog-bit typically occurs in waterbodies with a near neutral pH (6.5 – 7.8; Catling and Dore 1982).

European frog-bit can be found in lakes, rivers, streams, and wetlands as well as artificial waterbodies such as canals, channels, ditches, and ponds (Catling and Dore 1982; Catling et al. 2003). Within wetlands, it can colonize the emergent, floating, and submerged vegetation zones. The floating vegetation zone likely provides the most suitable conditions with sufficient light and nutrient availability (Halpern 2017). In a laboratory experiment conducted by Halpern (2017), EFB biomass production was the greatest in full sun exposure and in 11.8 –17.7 in (30 – 45 cm) of water. In the Cedar Point National Wildlife Refuge, EFB was collected on wet muck at the edge of a diked marsh. Additional colonies were documented along the marsh's shoreline as well as floating within the marsh (Gardner 2008). Species associated with EFB in North America include *L. minor*, northern watermilfoil (*Myriophyllum sibiricum* Kom.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), *P. pusillus*, Vasey's pondweed (*Potamogeton vaseyi* Robb.), *S. polyrhiza*, *U. vulgaris,* broadleaf cattail (*Typha latifolia* L.), narrowleaf cattail (*Typha angustifolia* L.), hybrid cattail (*Typha* x *glauca*), *P. australis*, and native *Phragmites* sp. (Spicer and Catling 1987; Catling et al. 1988; Catling et al. 2003; Central Michigan University Herbarium – CMC).

In wetlands along the Great Lakes coast, particularly those along Lake Ontario, EFB is associated with *Typha* dominated marshes. Of the 100 plant species Halpern (2017) sampled in wetlands of the Upper St. Lawrence River, EFB was the most frequent and ranked second behind *Typha* spp. in abundance. Halpern postulated that the expansion of *Typha x glauca* in Lake Ontario wetlands may be facilitating EFB's establishment. The spread of *Typha* x *glauca* into deeper water may create more suitable habitat for EFB and its decay may provide a readily available nutrient (i.e., nitrates, ammonium, phosphorus) source (Halpern 2017). Floating-leaved plants may also facilitate the expansion and establishment of EFB into more open habitat by providing shelter from wind and wave action (Halpern 2017).

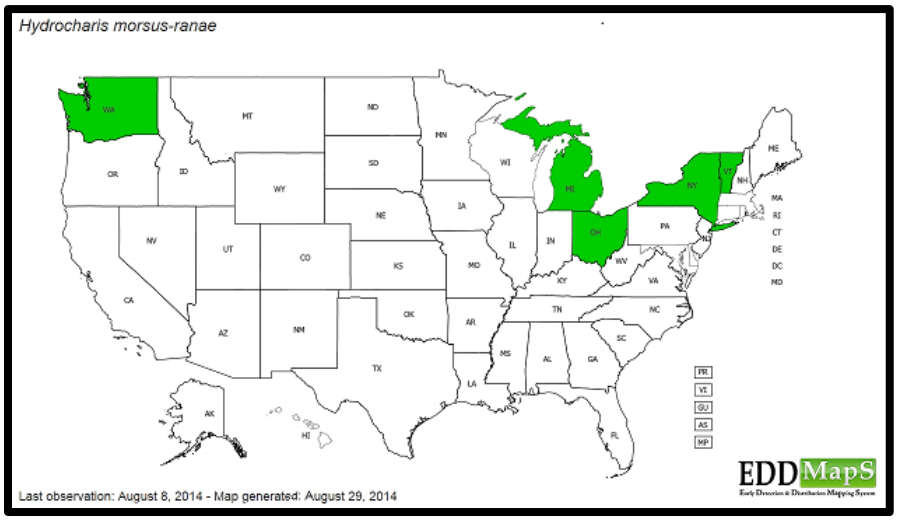

Figure 6. Distribution of European frog-bit (*Hydrocharis morsus-ranae* L.) in the United States. Occurrences are also reported in Illinois, Pennsylvania, New Jersey, and Maine as well as Ontario and Quebec, Canada but are not represented on the map. Map from the Early Detection & Distribution Mapping System (EDDMapS 2018)

V. Effects from EFB

An impact assessment of established nonindigenous species in the Great Lakes basin ranked EFB's potential environmental and socio-economic impacts as moderate and its potential beneficial impacts as low (Sturtevant et al. 2014). A risk assessment conducted by the Michigan Department of Agriculture and Rural Development classified EFB as a high-risk invasive species for its establishment/dispersal, impact, and geographic potential (Weibert 2015). Despite concerns over EFB's invasiveness, few studies have examined its ecological, social, or economic impacts (i.e., Catling et al. 1988; Houlahan and Findlay 2003; Trebitz and Taylor 2007; Zhu et al. 2015; Halpern 2017). The majority of EFB impact reports are based on anecdotal observations. No published studies could be found examining the effects of EFB on food web dynamics, fish habitat, nutrient cycling, or commercial and recreational water use.

a. Negative Effects

European frog-bit can form dense, entangled, floating mats that cover the surface of the water (Figure 4). These mats have the potential to negatively impact the human use of waterbodies by clogging navigation and irrigation channels and inhibiting recreational and commercial activities (Catling et al. 2003). These impacts may result in decreased waterfront property values (Zhu et al. 2018). Research is needed to quantify the social and economic impacts of EFB invasion.

Dense mats of EFB have the potential to reduce light, dissolved gas, and nutrient availability in the water column, thereby negatively impacting native aquatic plants. Studies examining the impact of EFB on aquatic plant communities have yielded inconsistent results. Catling et al. (1988) and Zhu et al. (2014) documented reduced aquatic plant species richness and abundance under EFB mats compared to areas without EFB. Halpern (2017) found that EFB surface coverage and aquatic plant species richness and diversity were negatively correlated in one of six Lake Ontario wetlands studied. When samples from all six wetlands were pooled EFB surface coverage and aquatic plant diversity had a significant, weak, negative correlation (Halpern 2017). Thomas and Daldorph (1991), Houlahan and Findlay (2004), and Trebitz and Taylor (2007) found no effect of EFB on aquatic plant species richness, cover, or diversity. Further studies are needed to elucidate the impact that EFB has on native aquatic plant communities at varying densities and spatial scales.

Dense mats of EFB may also negatively impact fish, wildlife, and invertebrate communities. Catling et al. (1988) observed less snails, crustaceans, and insect larva on EFB mats compared to stands of native aquatic plants in New York and Ontario. In Lake Oneida, New York EFB altered the macroinvertebrate community assemblage (Zhu et al. 2015). A preliminary study in Munuscong Bay documented fewer fish species and lower fish abundance in areas invaded by EFB compared to areas without EFB (Daly 2016). The annual decomposition of EFB mats may deplete dissolved oxygen which can be harmful to fish and macroinvertebrates (Catling et al. 2003). Further research is needed to evaluate the potential for EFB to impact native aquatic fauna.

European frog-bit may alter aquatic fungal and bacterial communities. A study in Poland found fewer fungi species on EFB than several aquatic plant species, including *L. minor* and *C. demersum* (Czeczuga et al. 2004). Dissolved organic matter leached from EFB negatively impacted bacterial growth in a controlled setting (Anesio et al. 2000). Catling et al. (2003) reported that no bacteria species have been documented on EFB.

b. Positive Effects

European frog-bit may benefit some wildlife and invertebrate species by providing food and refuge (Catling et al. 2003; Zhu et al. 2018). In Lake Oneida, New York areas with EFB had greater chironomid abundance and benthic macroinvertebrate diversity compared to areas without EFB (Zhu et al. 2015).

European frog-bit's ability to store pollutants in its tissue makes it a viable candidate for phytoremediation (Zhu et al. 2018). Several studies have demonstrated EFB's uptake of heavy metals (e.g., lead, zinc, nickel, copper) and nutrients (e.g., nitrogen, phosphorus) from polluted environments (Maleva et al. 2004; Shu 2013; Polechońska and Samecka-Cymerman 2016). Caution must be exercised when using EFB for phytoremediation given its capacity for rapid reproduction and dispersal and potential social, economic, and environmental impacts (Zhu et al. 2018).

European frog-bit may also have medicinal uses. Dormant turions contain spermidine, a chemical compound known to have "anti-aging" effects (Villanueva et al. 1985).

Current Status and Distribution in Michigan

European frog-bit was introduced into North America in 1932 when it was intentionally planted in an Arboretum in Ottawa, Ontario. It was first detected outside of the Arboretum in 1939 in the Rideau Canal, which was connected to the original planting site (Minshall 1940). From there EFB spread into the St. Lawrence and Ottawa Rivers (Minshall 1940; Dore 1968). By 1972, it was found in eastern Lake Ontario (Catling and Dore 1982). European frog-bit was first reported in the United States in New York in 1974 (Roberts et al. 1981). It has since been documented in Ontario, Quebec, New York, New Jersey, Vermont, Ohio, Michigan Maine, Pennsylvania, Illinois, and Washington state (Dore 1968; Roberts et al. 1981; Catling et al. 2003; Gardner 2008; Marsden and Hauser 2009; Lamont et al. 2014; Jacono and Beret 2018; MISIN 2018).

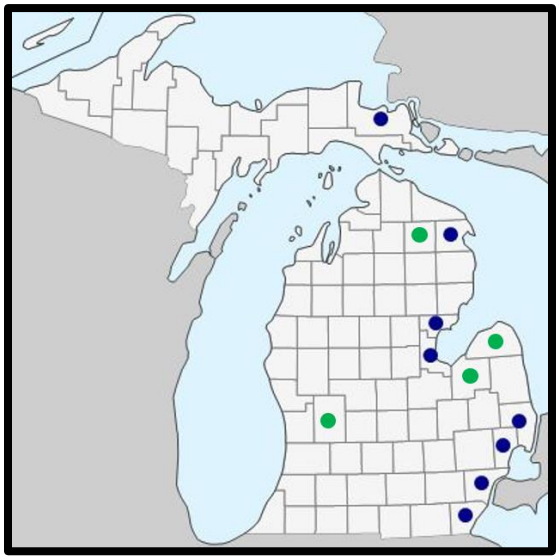

Figure 7. Blue dots indicate counties in Michigan where a specimen of European frog-bit (*Hydrocharis morsus-ranae* L.) has been collected and included in Michigan Flora. Green dots indicate counties where European frog-bit was documented by the Midwest Invasive Species Information Network (MISIN), but not by Michigan Flora. County map developed by Michigan Flora online (Reznicek et al. 2011)

European frog-bit was first documented in Michigan in 1996 at the Ford Yacht Club in Wayne County

(Reznicek et al. 2011). It is now established in 12 counties (Figure 7; MISIN 2018). European frog-bit has been documented in several counties in southeast Michigan. It is established along the Huron-Erie Corridor in Monroe, Wayne, Macomb, and St. Clair counties. In 2018, an inland population was detected in Maybury State Park in Wayne County (MISIN 2018).

European frog-bit has been documented in Arenac, Bay, Tuscola, and Huron counties along the shoreline of the Saginaw Bay. Known occurrences extend from Point Au Gres to the Wildfowl Bay State Wildlife Area and are concentrated at sites along the western shore of Saginaw Bay (e.g., Nayanquing Point State Wildlife Area, Bay City State Park).

In the northeastern Lower Peninsula, EFB has been documented in Alpena and Montmorency counties. Populations in Alpena County occur in the Alpena Wildlife Sanctuary, Lake Winyah, and along the coastline of Lake Huron north and south of the Thunder Bay River mouth and in the Negwegon State Park (MISIN 2018). In Alpena/Montmorency counties, EFB is established in Fletcher Pond.

In west Michigan, an inland population occurs in Kent County. Here, EFB occurs in Reeds Lake, Fisk Lake, a connecting channel between the two lakes, and ponds on the Aquinas College campus.

European frog-bit has been documented in the northeastern Upper Peninsula in Chippewa County. Here, EFB is established along the shoreline of the St. Mary's River, in Munuscong Bay and Raber Bay. European frog-bit has also been documented along the Canadian shoreline of the St. Mary's River (W. Keiper, Michigan Department of Environmental Quality, personal communication).

Management of EFB

I. Prevention

According to the modeling efforts of the Michigan Department of Agriculture and Rural Development, all of Michigan and 79% of the United States is suitable for EFB establishment (Weibert 2015). Since EFB spreads quickly once it is established, it is imperative to take the proper measures toward prevention. European frog-bit is a prohibited species in Michigan under the Natural Resources and Environmental Protection Act 413 of 1994. Under this act it may neither be grown nor sold in the state. Additionally, the transport and sale of EFB are prohibited in Washington, Oregon, Idaho, Minnesota, Wisconsin, Indiana, New York, Vermont, New Hampshire, and Maine (Halpern 2017).

European frog-bit may act as an aquatic hitchhiker (Cahill et al. 2018), so boaters, anglers, and hunters can unintentionally contribute to its spread. The Clean Boats, Clean Waters program, a cooperative program of Michigan Lake and Stream Associations, Inc. and Michigan State University Extension, produced a video that provides instructions for decontaminating equipment to reduce the spread of invasive species, such as EFB, between waterbodies: https://www.youtube.com/watch?v=IWobcoWchsI&feature=youtu.be. The following actions may prevent and limit the dispersal of EFB:

- Build a coalition of local, state, and Great Lakes regional partners to monitor for EFB and other aquatic invasive species
- Build a coalition of states that have classified EFB as a restricted or prohibited species
- Identify and monitor waterbodies that have a high-risk of invasion using known distribution and dispersal knowledge
- Provide boat washing stations for high-traffic public lake accesses
- Develop and sustain a water recreation vehicles and trailers inspection program
- Increase stakeholder awareness of available prevention and control methods
- Actively manage sites where EFB is found

II. Management/Control

A management strategy that incorporates ecological knowledge and several management techniques – called integrated pest management – into an adaptive framework of setting management objectives, monitoring, and plan adaptation over time is often considered the most effective approach to controlling invasive species. It is imperative that treatment of invasive aquatic plants is paired with a scientifically sound monitoring program that is designed to assess the management objectives. Monitoring data should be collected using a standardized protocol, inclusive of pre- and post-treatment assessments in managed and unmanaged reference locations, so statistical inferences on treatment impact can be made.

Consideration of EFB's distribution in wetlands, lakes, canals and other waterbodies is crucial when developing a management plan. European frog-bit can become increasingly difficult to manage once it is established throughout the major wetland vegetation zones (Halpern 2017). Dense mats in the floating vegetation zone are often the target of management actions; however, turions and free-floating plants can reestablish from the emergent and submerged vegetation zones, respectively. Free-floating plants in the submerged vegetation zone are likely to disperse to new areas through wind, waves, and current and should therefore be considered a management priority (Halpern 2017). A coordinated management strategy that targets EFB in the emergent, floating, and submerged vegetation zones simultaneously may be required to reduce EFB's reestablishment and dispersal potential.

Treatment timing is another important consideration when developing a management plan for EFB. To reduce its reestablishment potential, management actions should be conducted prior to seed and turion development. In North America, turion and seed development begin in the fall and late-summer, respectively (Dore 1968; Catling et al. 2003). More detailed studies on the timing of turion and seed development in EFB's invasive range as well as the contribution of seeds and turions to post-management reestablishment could aid in the planning and implementation of management actions.

Management of other invasive aquatic plants may facilitate the expansion and growth of EFB infestations. European frog-bit has been observed growing in the open water and among the dead stalks of *T. angustifolia* and *Typha x glauca* in Munuscong Bay and *P.*

australis in Saginaw Bay and the Huron-Erie Corridor following management of these emergent species (Cahill et al. 2018). Management techniques or plans that target EFB and these emergent invasive plants simultaneously may provide more effective EFB control (Halpern 2017; Wellons 2018).

Physical and chemical management techniques have been used to control EFB infestations in Michigan (Cahill et al. 2018). See Table 1 for the year that EFB management started and the management techniques used to date in each infested region of Michigan.

Table 1. Summary of management techniques used to control European frog-bit (*Hydrocharis morsus-ranae* L.) in each infested region of Michigan and the year that management started in each region. Techniques with a (+) between them indicate they were part of an integrated management strategy, not that they were implemented at the same time.

Region	Managed Since	Control Technique(s) Used
Saginaw Bay (Arenac, Bay, Tuscola, and Huron Counties)	2010	Manual removal, diquat treatment + manual removal
Northeast Lower Peninsula (Alpena and Montmorency Counties)	2015	Manual removal
West Michigan (Kent County)	2016	Flumioxazin treatment, Flumioxazin treatment + diquat treatment + manual removal, manual removal
Eastern Upper Peninsula (Chippewa County)	2013	Manual removal
Southeast Lower Peninsula (Macomb, Monroe, St. Clair, and Wayne Counties)	Unknown	Unknown

Outside of Michigan, manual removal has been the most commonly used method for EFB control. In Vermont, manual removal, supplemented with metal and bamboo rakes, was used to control EFB in the Charlotte Town Farm Bay and Shelburne Lower LaPlatte (Lewis Creek Association 2011; Lewis Creek Association 2013). Manual removal has also been used on small isolated EFB populations in the Adirondack region of New York (Oles and Flint 2007).

Few studies have been conducted to evaluate the effectiveness of management techniques for EFB control (e.g., Zhu 2014; Zhu et al. 2014; Halpern 2017; Wellons 2018) and many management recommendations are based on qualitative observations and are lacking untreated controls or pre- and post-treatment monitoring. The following is a summary of control methods tested to date and their results.

a. Chemical

Newbold (1975) and (1977) list diquat, paraquat, chlorthiamid, terbutryne, cyanatryn, and dichlobenil as providing effective single season control of EFB. Hauteur and Canetto (1963) reported that amitrole controlled EFB in ditches and canals of France but required retreatment the following year. In Europe, diquat at 1 and 10 ppm and endothall at 5 ppm were effective for controlling EFB in stagnant drainage ditches (Holz 1963; Renard

1963). Only two of the aforementioned herbicide active ingredients, diquat and endothall, are approved for aquatic use by the United States Environmental Protection Agency (EPA). Further research is needed to evaluate the efficacy and optimal use patterns of diquat and endothall for controlling EFB. Care should be taken when using diquat and endothall for EFB management, as they are broad spectrum herbicides that can negatively impact native aquatic plants (Hofstra and Clayton 2001; Bugbee et al. 2015).

A mid-summer treatment of diquat and a late-summer treatment of flumioxazin, separated by three weeks, appeared to provide successful single season control of EFB in Reeds Lake and Fisk Lake, Michigan in 2017 (Cahill et al. 2018). Post-treatment monitoring for effectiveness is ongoing.

Many herbicides are used to control the closely related *L. spongia*, but it is uncertain if EFB is equally susceptible. Herbicides commonly used for *L. spongia* control include diquat, imazapyr, penoxsulam, imazamox, triclopyr, and 2,4-D (Madsen et al. 1998; AERF 2018). A summary of herbicide active ingredients that are approved for aquatic use by the EPA and have shown some effectiveness for EFB or *L. spongia* control is in Table 2.

Table 2. Summary of effective herbicide active ingredients for European frog-bit (*Hydrocharis morsus-ranae* L.; hereafter EFB) control to date that are approved for aquatic use by the United States Environmental Protection Agency. Also included are herbicide active ingredients that are used for American frog-bit (*Limnobium spongia* (Bosc) Rich. ex Steud.) control that could be effective against EFB. For each active ingredient, example trade names, whether it's approved for aquatic use in Michigan (MI), whether EFB is listed on its label, advantages, disadvantages, and the cited literature are listed. Directions on the pesticide label should always be followed and the state Departments of Environmental Quality and Agriculture and Rural Development should be consulted for up to date regulations, restrictions, permitting, licensing, and application information. Table modeled after the MNFI Glossy Buckthorn Factsheet (MNFI 2012).

Herbicide	Approved in MI	Listed on Label	Pros	Cons	References
Endothall (e.g. Aquathol®)	Yes	No	• 5 ppm controlled EFB in stagnant drainage ditches	• Has not been systematically evaluated for EFB control in field or lab trials • May harm non-target species (Broad-spectrum herbicide) • May be toxic to aquatic organisms • Prohibited for use in waterbodies < 600 ft from a potable water intake • May have post-treatment restrictions on water use	(Holz 1963; WDNR 2012; AERF 2018)
Diquat (e.g. Reward®)	Yes	No	• 1 and 10 ppm controlled EFB in stagnant drainage ditches • Possibly effective in an inland lake in MI (anecdotal) • 0.94, 1.87, and 3.75 lb/ac reduced *L. spongia* biomass by 99 – 100% in lab trials • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • May harm non-target species (Broad-spectrum herbicide) • Toxic to aquatic invertebrates • Ineffective in turbid water or conditions with a lot of wave action • Post-treatment restrictions on drinking and irrigation water	(Holz 1963; Renard 1963; WDNR 2012; AERF 2018)
Triclopyr (e.g. Renovate®)	Yes	No	• 0.76, 1.51, 3.02 lb/ac reduced *L. spongia* biomass by 78 – 95% in lab trials • Less harm to non-target species (Selective herbicide) • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • Post-treatment restrictions on potable and irrigation water	(Madsen et al. 1998; WDNR 2012; AERF 2018)
2,4-D (e.g. Navigate®)	Yes	No	• 0.96, 1.93, and 3.85 lb/ac reduced *L. spongia* biomass by 53 – 80% in lab trials • Less harm to non-target species (Selective herbicide)	• Has not been systematically evaluated for EFB control in field or lab trials • May be toxic to fish and invertebrates • May have post-treatment restrictions on swimming and irrigation water	(Madsen et al. 1998; WDNR 2012; AERF 2018)

Herbicide	Approved in MI	Listed on Label	Pros	Cons	References
Imazamox (e.g. Clearcast®)	Yes	No	• Used for *L. spongia* control • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • May harm non-target species (Broad-spectrum herbicide) • Restricted concentration when near potable water intakes • Post-treatment restrictions on potable and irrigation water	(WDNR 2012; AERF 2018; UF/IFAS 2018)
Imazapyr (e.g. Habitat®)	Yes	No	• Used for *L. spongia* control • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • May harm non-target species (Broad-spectrum herbicide) • Post-treatment restrictions on potable water	(WDNR 2012; AERF 2018; Texas A&M AgriLife Extension 2018)
Penoxsulam (e.g. Galleon® SC)	Yes	No	• Used for *L. spongia* control • Less harm to non-target species (Selective herbicide) • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • Post-treatment restrictions on irrigation water	(WDNR 2012; AERF 2018
Flumioxazin (e.g. Clipper®)	Yes	No	• Possibly effective in an inland lake in MI (anecdotal) • Used for *L. spongia* control • Lists *L. spongia* on label	• Has not been systematically evaluated for EFB control in field or lab trials • May harm non-target species (Broad-spectrum herbicide) • Toxic to fish and aquatic invertebrates • Post-treatment restrictions on irrigation water	(WDNR 2012; AERF 2018)

b. Physical or Mechanical Control

Manual removal has been effective for the control of small isolated EFB populations (Martine et al. 2015). Manual removal is time and labor-intensive and often requires repeated visits to maintain control (Bailey and Calhoun 2008; Kelting and Laxson 2010; Zhu et al. 2015; Cahill et al. 2018). Catling et al. (2003) recommended that manual removal should occur in the spring and early-summer once germinating turions are floating on the surface of the water but before dense mats form. In Michigan, EFB plants are found floating on the surface of the water beginning in May in the Lower Peninsula and late-June to early-July in the Upper Peninsula (Cahill et al. 2018; Wellons 2018). If done too late in the year manual removal can proliferate the spread of stolon buds and turions (Catling et al. 2003). Manual removal of EFB has been shown to have no impact on surface or benthic macroinvertebrates (Zhu et al. 2015) but its effect on native aquatic plants is unknown.

Over a five-year period in the Town Farm Bay in Vermont, 55.9 tons (50,711.63 kg) of EFB were removed at a cost of $79,000 and 6,208 hours, reducing EFB cover to less than 6%. Annual spring maintenance visits were required to maintain control of the Town Farm Bay population (Lewis Creek Association 2013). At the Alpena Wildlife Sanctuary in Michigan, 10,000 lbs (4,536 kg) of EFB were removed in both 2015 and 2016 and 3,000 lbs (1,361 kg) were removed in 2017. Removal effort was similar across 2015 – 2017, suggesting that the manual removal was effective. Similarly, in Munuscong Bay and Raber Bay, Michigan, over 10,000 lbs (4,536 kg) of EFB has been removed since 2013 with similar effort but reduced biomass returns each year. Scientifically-sound studies are needed to verify the efficacy of manual removal for EFB control.

Shading can be a time and cost-efficient method for EFB control in areas where recreational and commercial activity is limited (Zhu et al. 2014). The effectiveness of shading with floating cloth for EFB control has been demonstrated in greenhouse and field trials (Zhu et al. 2014). In greenhouse trials, 100% shading completely removed EFB and shading between 50% – 80% significantly reduced EFB biomass. One hundred percent shading completely removed EFB in field trials and 70% shading significantly reduced EFB biomass. Shading does have the potential to negatively impact native aquatic plant and macroinvertebrate communities (Zhu et al. 2014; Zhu et al. 2015).

Water level drawdowns may effectively control EFB, but they are only possible in waterbodies with artificially controlled water levels. To be effective, drawdowns would likely need to occur overwinter or in the spring/early summer (Catling et al. 2003). European frog-bit's ability to survive on mud flats for months at a time (W. Keiper, Michigan Department of Environmental Quality, personal communication) may lessen the efficacy of spring and early summer drawdowns. The length of time needed for drawdowns to be effective is unknown. Drawdowns can have many negative effects on aquatic ecosystems, particularly to native aquatic plants and macroinvertebrates (Madsen 2000; Harman et al. 2005).

Mechanical harvesting has been employed to control EFB in the Rideau Canal in southeastern Ontario (Spicer and Catling 1987). Research is needed to evaluate its efficacy as well as its potential to disperse free-floating individuals, turions, and stolon buds, further spreading EFB. Non-target impacts to native plant, fish, and invertebrate species is also a concern when using mechanical harvesting to control an invasive plant population (Engel 1990; Madsen 2000).

Researchers at Loyola University Chicago (Lishawa et al.) are currently evaluating the effectiveness of combined EFB management and hybrid *Typha* management techniques (Wellons 2018). Thus far, the above water harvest of hybrid *Typha*, below water harvest of hybrid *Typha*, above water harvest of hybrid *Typha* combined with EFB manual removal, and below water harvest of hybrid *Typha* combined with EFB manual removal have been evaluated. None of the aforementioned treatments significantly reduced EFB cover one-year post-treatment. Monitoring of these treatments will continue through 2020 (Wellons 2018). The Loyola University Chicago researchers (Lishawa et al.) are currently evaluating the effectiveness of chemical treatments alone and in combination with above and below water harvest of hybrid *Typha* for EFB control (Cahill et al. 2018).

c. Biological

Many organisms are known to consume EFB, including water-birds, rodents, insects, snails, and fish (Catling and Dore 1982; Sviridenko et al. 1988; Vaananen and Nummi 2003; Catling et al. 2003). Few studies have evaluated these species potential as biological control agents (i.e., Sanders et al. 1991; Zhu 2014; Halpern 2017).

Zhu (2014) conducted field and laboratory experiments to test the utility of snails as biological control agents for EFB. Zhu sampled EFB at sites across the Great Lakes region, collected snails that were on the EFB samples, and quantified the amount of EFB leaf damage at each site. A significant weak correlation between the number of snails and the amount of leaf damage at each site was detected; however, when a single outlier was removed the correlation was no longer significant. In the laboratory experiment, Zhu tested the impact of the tadpole physa (*Physa gyrina* Say) on parameters associated with EFB growth (e.g., number of roots, stems, and leaves; biomass). There were no significant differences between EFB plants with and without snails. Zhu (2014) concluded that snails are unlikely to serve as biological control agents and that further studies are needed to find species that could, particularly in EFB's native range.

In a laboratory setting, Halpern (2017) investigated the use of the waterlily leafcutter moth (*Elophila obliteralis* (Walker)), a semi-aquatic moth native to eastern North America, for control of EFB. Halpern introduced *E. obliteralis* larva to young EFB plants at varying densities and measured their impact on vegetative reproduction and productivity. When five larvae were introduced per EFB plant, clonal production was significantly reduced compared to untreated controls. Total biomass was significantly reduced when one, three, and five larvae were introduced per plant. Artificially enhancing the abundance of *E. obliteralis* early in the growing season may serve as a

viable biological control option for EFB management (Halpern 2017); however, this has not been verified in the field.

Grass carp (*Ctenopharyngodon idella* Val.) are also known to consume EFB; however, it is not a preferred food source (Sanders et al.1991). The utility of *C. idella* as a biological control agent is not feasible as they can have detrimental impacts to ecosystems and are a prohibited species in Michigan.

No bacteria, viruses, or plant parasites have been recorded on EFB. The plant does, however, host a variety of rusts, smuts, and molds, but their effects on EFB have not been studied (Catling et al. 2003).

d. Indirect Management

No indirect management techniques have been investigated for the control of EFB at the time of this report. European frog-bit establishment may be prevented by altering flow regimes. In waterbodies that have artificially controlled flow regimes, such as some canals and ditches, the flow of water could be increased to make conditions unsuitable compared to the calm, slow moving waterbodies that EFB typically prefers. However, this could result in the further spread of EFB plants and propagules.

Research Needs

I. Biology and Ecology

In eastern North America, EFB has been documented in Ontario, Quebec, New York, New Jersey, Vermont, Ohio, Michigan, Maine, Pennsylvania, and Illinois (Dore 1968; Roberts et al. 1981; Catling et al. 2003; Gardner 2008; Marsden and Hauser 2009; Lamont et al. 2014; Jacono and Beret 2018; MISIN 2018). Plant specimens from each population should be collected and deposited in herbaria for future genetic analysis. Genetic analysis of the relationships between established EFB populations may provide insight into EFB's introduction and dispersal pathways and aid in the development of more efficient education, prevention, and monitoring programs.

European frog-bit is established in 12 counties in Michigan: Alpena, Arenac, Bay, Chippewa, Huron, Kent, Macomb, Monroe, Montmorency, St. Clair, Tuscola, and Wayne counties (Figure 7; MISIN 2018). Occurrences outside of its known distribution in Michigan are likely, particularly along the coastline of Lake Huron between the known occurrences in St. Clair and Huron counties and Arenac and Alpena counties. Surveys in these areas would elucidate the full extent of EFB's distribution in Michigan and potentially shed light on its pathways of dispersal. Further understanding of EFB's distribution could alter state-wide management objectives and approaches for EFB management in Michigan. There is also a need for comprehensive surveys in areas surrounding each known infestation. Further understanding of EFB's local distribution could impact the management objectives for (i.e., exclusion, containment, adaptation) each infestation; therefore, altering the local management approach employed.

To date, modeling of suitable EFB habitat has been coarse, examining a regional scale in North America. Using a trio of climate variables (plant hardiness zones, precipitation, Köppen-Geiger climate classes), Weibert (2015) predicted that 79% of the United States and all of Michigan could be suitable for EFB. Understanding local characteristics (e.g., depth, pH, turbidity, flow velocity) that characterize EFB occurrence in its invasive range will improve predictions of EFB spread and guide monitoring efforts. An ongoing project led by Loyola University Chicago (Lishawa et al.) is expanding a habitat suitability model created for Munuscong Bay to the rest of Michigan (Cahill et al. 2018).

Turions and seeds are produced in the late-summer to early fall in Ontario, Canada (Dore 1968; Catling et al. 2003). The phenology of turion and seed development in Michigan is expected to be similar to that of Ontario but it has yet to be investigated. Understanding EFB's seasonal growth pattern in Michigan could help guide the timing of management efforts so that the spread of propagules is reduced.

Temperature and photoperiod are known to influence turion and seed germination (Serbanescu-Jitariu 1972 in Catling et al. 2003; Richards and Blakemore 1975; Cook and Lüönd 1982). Turions are reported to remain viable for 16 months to 2 years (Arber 1920; Burnham 1998). Little else is known regarding the triggers of turion and seed germination or their long-term viability. The potential for regrowth through seeds and turions is important to understand when controlling populations of EFB.

In its native and invasive range, EFB populations can be entirely composed of one sex, making seed production rare (Cook and Lüönd 1982; Catling et al. 2003). Although, populations in Ontario have been documented producing abundant seeds (Catling and Dore 1982; Burnham 1998). Comprehension and delineation of seed producing populations in the Great Lakes region could provide insight into the role of seed production in EFB reproduction and dispersal. If seed production is widespread, EFB persistence (i.e., seeds in the seed bank) and dispersal (i.e., transport of seeds by wildlife) may be greater than previously thought.

Much of EFB's spread between connected waterbodies in North America is believed to be a result of plants and propagules drifting on the waters natural flow and its overland dispersal is believed to be a result of hitch-hiking on boats and boating equipment (Catling et al. 2003). Understanding how far and for how long turions and seeds can float before sinking could help predict the natural spread of EFB and guide prevention and monitoring efforts. Similarly, understanding the tolerance of EFB plants, turions, and seeds to desiccation are crucial for predicting over-land dispersal and developing effective watercraft decontamination procedures.

Dense mats of EFB have been shown to impact native aquatic flora and fauna (Catling et al. 1988; Zhu et al. 2015; Dray 2016) but its impacts at varying densities and scales is lacking. Anecdotal reports suggest EFB has detrimental social and economic impacts but data demonstrating these impacts is lacking. Understanding EFB's ecological, social, and economic impacts at varying levels of infestation can help managers prioritize sites for management and contribute to the cost-benefit analysis of managing an invasive population.

II. Detection

Genetic markers have been developed for detecting EFB genetic material shed into the environment (Scriver et al. 2015) but they have not been evaluated in the field. This approach may not be prudent for EFB detection, given its near-shore habitat and easily distinguishable features. Sampling for genetic material shed into the environment by EFB could improve the efficiency of early detection, especially when it is growing in stands of emergent and floating vegetation or in an inaccessible portion of a waterbody.

Remote sensing technology, at a 2.4 m resolution, has been used to detect and distinguish EFB infestations (Proctor et al. 2012). Using this resolution, populations that don't form dense mats may go undetected because a EFB plant is smaller than the resolution of the imagery. Imagery gathered by unmanned aerial systems would likely be required to gather imagery at a resolution fine enough to detect individual EFB plants or small EFB mats. European frog-bit is also difficult to detect using remote sensing when it is growing interspersed among emergent vegetation. An ongoing project led by Loyola University Chicago (Lishawa et al.) is currently evaluating the use of aerial imagery gathered after emergent vegetation has senesced for EFB detection (Cahill et al. 2018).

III. Management

There is little known regarding the efficacy of chemical treatments for EFB control. Newbold (1975) and (1977) reported that EFB is susceptible to diquat, paraquat, chlorthiamid, terbutryne, cyanatryn, and dichlobenil. Holz (1963), Renard (1963), and Hauteur and Canetto (1963) reported that amitrole, diquat, and endothall provided effective EFB control in drainage ditches and canals of Europe. No research has been published on the efficacy of chemical treatments for EFB management that is inclusive of untreated controls and pre- and post-treatment monitoring. Research evaluating the effectiveness of herbicide treatments used on the closely related American frog-bit (e.g., triclopyr, diquat, imazamox) for EFB control could be useful for management. An ongoing project led by Loyola University Chicago (Lishawa et al.), is evaluating the efficacy of chemical treatments for control of mixed stands of *T. angustifolia, Typha* x *glauca,* and EFB in Munuscong Bay, Michigan (Cahill et al. 2018).

Understanding how ramet, turion, and seed production are impacted by chemical treatment could lead to more effective management strategies. If ramets, turions, or seeds are not impacted by treatment or if production of these reproductive structures is enhanced following treatment, repeated applications will likely be required to maintain control.

On a small scale, manual removal is considered an effective technique for EFB control (Martine et al. 2015) but often requires repeated visits (Zhu et al. 2015). Mechanical harvesting has also been employed for EFB management (Spicer and Catling 1987). Combinations of *Typha* harvesting techniques and EFB management techniques are currently being evaluated by Loyola University Chicago (Lishawa et al.) in Munuscong Bay, Michigan (Wellons 2018). Thus far, no treatments have been found to significantly reduce EFB but post-treatment monitoring is ongoing (Cahill et al. 2018; Wellons 2018). The

efficacy of physical and mechanical management techniques for EFB control as well as their potential to disperse seeds, turions, and stolon buds requires further investigation. Research into more efficient methods and devices for physical or mechanical management of EFB could be beneficial.

Halpern (2017) demonstrated the utility of *E. obliteralis* larva for control of young EFB plants in lab trials. Studies that examine the efficacy of *E. obliteralis* in the field as well as its non-target impacts are needed. Other species, such as snails (Zhu 2014), have been evaluated but their impact to EFB was not severe enough to be useful for management. A variety of rusts, smuts, and molds are also found on EFB (Catling et al. 2003). The impact that these species have on the productivity and reproductive output of EFB might be worthy of investigation. Further research exploring potential biological control agents, particularly in EFB's native range, could provide a long-term control option.

Future Directions for Michigan and EFB Management

European frog-bit is a free-floating aquatic plant native to Europe, Asia, and Africa (Catling et al. 2003). In North America, it has been documented in Ontario, Quebec, New York, New Jersey, Vermont, Ohio, Michigan, Maine, Pennsylvania, Illinois, and Washington state (Dore 1968; Roberts et al. 1981; Catling et al. 2003; Gardner 2008; Marsden and Hauser 2009; Lamont et al. 2014; Jacono and Beret 2018; MISIN 2018). European frog-bit's rapid reproductive and dispersal ability as well as its potential for ecological, social, and economic impacts make it a great concern to natural resource managers in the Great Lakes region.

Prevention – Prevention of new colony establishment is likely the most cost-effective approach to EFB management. Potential pathways of EFB dispersal include waterway currents, fish and wildlife, and transportation of plants and propagules by recreational waterbody users. The development of outreach and education programs designed to raise stakeholder (e.g., lake associations, anglers, waterfowl hunters) awareness of prevention and control methods may reduce the human-mediated spread of EFB. Likewise, a sustainable boat washing and inspection program, particularly at high-risk waterbodies, could aid in containing its spread. Active management to eradicate or suppress established EFB populations could reduce the likelihood of dispersal through non-human mediated vectors.

Monitoring – Early detection of a EFB introduction makes eradication a more realistic option. Adding EFB to existing monitoring programs will assist in early detection and increase the potential of eradication. A cohesive monitoring and reporting system involving local municipalities, non-profit organizations, lake associations, recreation clubs and organizations, and waterfront property owners, would increase the number of known EFB locations and enable early detection and rapid response to new colonies. Connecting waterfront property owners and boaters with resources such as MISIN could improve early detection efforts. Working with herbaria for confirmation, documentation, and vouchering will provide verifiable long-term data that can be used to examine changes in macrophyte communities.

European frog-bit monitoring would benefit from a direct and targeted monitoring strategy. To develop a targeted monitoring strategy, EFB occurrences and associated environmental

variables could be modelled to identify suitable waterbodies for establishment. Human use patterns, such as whether a waterbody has a public boat access, could also be included in the distribution models. Suitable waterbodies that have a high-risk of EFB introduction could then be prioritized for monitoring.

Networking data – Statewide monitoring methods would benefit from creating or participating in systems that centralize and provide open access to diversity data (e.g., MISIN, Weed Map – Cooperative Weed Management Area, Michigan Clean Water Corps (MiCorps) Data Exchange Network – Great Lakes Commission, Nonindigenous Aquatic Species Database – USGS (NAS – USGS), Biodiversity Information Serving Our Nation (BISON), Global Biodiversity Information Facility (GBIF), Integrated Digitized Biocollections (iDigBio)). These databases house biological specimen or observation data including species location, verification, photographs, density, and even links to genetic data. Preliminary efforts within the state of Michigan have agencies contributing to regional databases (e.g., MISIN, Cooperative Weed Management Area, Nonindigenous Aquatic Species Database), but participation is not consistent and data standards are not established across programs. Currently state databases are not always networked within an agency, across the state, throughout the region or relative to national efforts.

Participation in a national or global information network will standardize data collecting practices, record comparable data using designated data standards across projects, ease data acquisition, avoid data redundancies, and promote projects with a larger scope of study than the original project for which the data sets were initially collected. Information networks that are continually linked to other resources and updated, can be used to develop effective and efficient monitoring and management plans. When information networks are not linked or periodically synchronized, a person collecting information must independently identify, locate, and consolidate data from separate and often difficult-to-access sources. The result is that information is missed and data collection becomes redundant and inefficient.

Networking with and contributing to state, regional, national, and international databases will advance research in areas that could improve the way invasive aquatic species are managed. Researchers can easily access the data and use it to model suitable habitat, model distribution, research population genetics across many spatial scales, predict new introductions, study changes due to climate change, or locate areas most beneficial for new projects or collections. The public could also use these data to know which species they may encounter when visiting specific waterbodies.

Rapid response – The ability to rapidly respond to reports in new or high-value locations submitted by the public or through a regular monitoring strategy is essential to battling invasive species. Invasive species are easier to treat prior to establishment and when an infestation is small. If the procedure to manage an infestation takes several years to achieve action, the infestation may have grown beyond realistic management. The Michigan Departments of Environmental Quality, Natural Resources, and Agriculture and Rural Development have developed a response plan that outlines the steps to take when a new aquatic invasive species occurrence is reported and serves as a guide for determining when and what type of response

is needed (MDEQ et al. 2014). The workflow begins at reporting the occurrence to the appropriate personnel, who determine the threat level of the species and verifies the species identification. Next a risk assessment is completed to determine if a species is a candidate for a response. If a response is deemed appropriate, options are assessed, and the response is planned and implemented. Finally, a report is made and adaptive management of the population is initiated. Although it is called a rapid response, it may not end rapidly.

Management – When managing EFB, it is important to delimit the extent of the infestation, contain already established populations, and protect high-value sites. An integrated pest management plan combined with an adaptive management framework is likely the most effective approach for controlling EFB.

Educating residents on the identification, legal restrictions, and potential negative impacts of EFB could aid in the detection of infested sites, assist in preventing new occurrences, and alert managers prior to the establishment of dense floating mats.

Measuring effective control: The effectiveness of a management action for EFB control can be quantitatively assessed by documenting any regrowth, reduction in EFB biomass or cover, or reductions in turion and seed production. Pairing a management plan with a monitoring program, inclusive of pre- and post-treatment assessments in treated and reference areas, is crucial for determining the efficacy of any management action.

The goal of aquatic invasive species management strategies is to preserve or restore ecologically stable aquatic communities. Minimal chemical, biological, and physical controls should be required to maintain these communities. Any management plan should involve the integration of prevention and control methods that consider factors impacting the long-term ecological stability of an aquatic community.

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Table 3. Objectives, strategic actions, leads, and expected outcomes of European frog-bit (*Hydrocharis morsus-ranae* L.; hereafter EFB) management.

Guidance and Outreach for EFB Management			
Objective	**Strategic Action**	**Who is leading effort in Michigan?**	**Expected Outcome**
Increase public awareness of prevention methods	• Coordinate and collaborate with local and regional stakeholders managing water bodies with an infestation or high likelihood of introduction • Educate public on identification, prevention, and early-detection	• Michigan State University Extension • Michigan Lake and Stream Associations, INC. • CISMA's	• Increase public awareness of EFB • Increase the frequency and use of boat washing stations • Protect high-value sites • Contain established populations
Provide technical guidance to those interested in EFB management	• Develop a framework to prioritize management of EFB infestations • Educate stakeholders on available control methods	• CISMA's	• Increase management efforts
EFB Monitoring and Data Management			
Develop a mechanism for detecting, monitoring, and reporting AIS species	• Develop a system of identifying water bodies with high likelihood of introduction • Survey waterbodies with high likelihood of introduction	• Cooperative Lakes Monitoring Program (CLMP) • MDEQ – Water Resources Division (WRD) • MISIN • MiCorps	• Develop a more thorough and up-to-date statewide distribution of EFB • Evaluate dispersal pathways and vectors
Develop standard operating procedures for monitoring treatment efficacy	• Develop guidelines for pre/post-treatment monitoring to determine treatment efficacy	• CMU (Monfils et al.)	• Develop best management practices for EFB control
Contribute regularly to regional, national, and global diversity information networks	• Consolidate Michigan biological and abiotic data • Standardize resources • Standardize data collection • Network existing data • Regularly synchronize data	• MISIN • MiCorps Data Exchange Network • iDigBio • NAS - USGS • BISON • GBIF	• Develop adaptive monitoring strategy that responds to up-to-date distribution • Promote AIS research of regional, national, and global extents • Prevent data redundancies
Educate public on identification and reporting of AIS in Michigan	• Target users of water bodies that are infested or have a high-likelihood of introduction	• MISIN • MiCorps • CISMA's • Management agencies	• Increase public awareness of AIS • Identify water bodies that need professional confirmation of AIS
Research Needs for EFB Management			
<u>Chemical:</u> Evaluate the effectiveness of current chemical treatments	• Study the effectiveness of chemical treatments for reducing/eliminating EFB	• Loyola University Chicago (Lishawa et al.) • MDEQ – WRD	• Determine whether or not chemical treatment is a cost-effective management approach

			• Effective treatment of EFB resulting in containment, suppression, or eradication
Biological: Establish biological control methods	• Identify and study the effectiveness of any potential biological control species		• Increase long-term control success
Mechanical: Evaluate effectiveness of current mechanical controls	• Study the effectiveness of hand-pulling and mechanical harvesting for reducing/eliminating EFB	• Loyola University Chicago (Lishawa et al.)	• Determine whether or not physical/mechanical removal is a cost-effective management approach • Effective treatment of EFB resulting in containment, suppression, or eradication
Physical: Evaluate effectiveness of current physical controls	• Study the effectiveness of shading and water level draw-down for reducing/eliminating EFB		• Determine whether or not physical controls are a cost-effective management approach • Effective treatment of EFB resulting in containment, suppression, or eradication

Literature Cited

Adamec L, Kučerová A (2013) Overwintering temperatures affect freezing temperatures of turions of aquatic plants. Flora 208:497–501.

(AERF) Aquatic Ecosystem Restoration Foundation (2018) Aquatic Herbicides/Agents. Web. http://www.aquatics.org/herbicides.html. Accessed 23 Jan 2018

Anesio AM, Theil-Nielsen J, Graneli W (2000) Bacterial growth on photochemically transformed leachates from aquatic and terrestrial primary producers. Microb Ecol 40:200–208.

Arber A (1920) Water plants: A study of aquatic angiosperms. Cambridge University Press, Cambridge, United Kingdom.

Bailey JE, Calhoun JK (2008) Comparison of three physical management techniques for controlling variable-leaf milfoil in Maine lakes. J Aquat Plant Manage 46:163–167.

Bakker J, Wangensteen OS, Chapman DD, et al. (2017) Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. Sci Rep 7:16886. doi: 10.1038/s41598-017-17150-2

Bugbee GJ, Gibbons JA, June-wells M (2015) Efficacy of single and consecutive early-season diquat treatments on curlyleaf pondweed and associated aquatic macrophytes: A case study. J Aquat Plant Manage 53:171–177.

Burnham JC (1998) The contribution of seeds and turions towards population growth and persistence of *Hydrocharis morsus-ranae* L. Master's Thesis. University of Guelph, Guelph, Ontario.

Cahill BC, Hackett RA, Monfils AK (2018) Proceedings of the Aquatic Invasive Plant Species Stakeholders Workshop. T. Alwin et al., Eds. March 8 – 9, 2018, Central Michigan University, Mount Pleasant, Michigan, USA. Project # 2016-0114, Michigan Department of Environmental Quality, Lansing, Michigan.

Catling PM, Dore WG (1982) Status and identification of *Hydrocharis morsus-ranae* and *Limnbobium spongia* (Hydrocharitaceae) in northeastern North America. Rhodora 84: 523–545.

Catling PM, Spicer KW, Lefkovitc LP (1988) Effects of the introduced floating vascular aquatic, *Hydrocharis morsus-ranae* (Hydrocharitaceae), on some North American aquatic macrophytes. Nat Can 115:131–137.

Catling PM, Mitrow G, Haber E, et al. (2003) The biology of Canadian weeds. 124. *Hydrocharis morsus-ranae* L. Can J Plant Sci 83:1001–1016.

Cook CDK, Lüönd R (1982) A revision of the genus *Hydrocharis* (Hydrocharitaceae). Aquat Bot 14:177–204.

Czeczuga B, Godlewska A, Kiziewicz B, et al. (2005) Effect of aquatic plants on the abundance of aquatic zoosporic fungus species. Pol J Environ Stud 14:149–158.

Daly D (2016) Effects of European frog-bit on water quality and the fish assemblage in St. Marys River coastal wetlands. Senior Thesis Project. School of Biological Sciences, Lake Superior State University, Sault Ste. Marie, Michigan. (Poster Presentation)

Dore WG (1968) Progress of the European frogbit in Canada. Can Field-Nat 82:76–84.

EDDMapS (2018) Early Detection & Distribution Mapping System. The University of Georgia – Center for Invasive Species and Ecosystem Health. Web. http://www.eddmaps.org. Accessed 26 Mar 2018

Engel S (1990) Ecological impacts of harvesting macrophytes in Halverson Lake, Wisconsin. J Aquat Plant Manage 28:41–45.

Ganie AH, Bashir A, Khuroo AA, et al. (2016) A new record of an invasive aquatic plant *Hydrocharis morsus-ranae* (*Hydrocharitaceae*), reaching to the Kashmir Himalaya. J Jpn Bot 91:100–104.

Gardner RL (2008) Noteworthy collection – Ohio*, Hydrocharis morsus-ranae* L. (Hydrocharitaceae). Common Frog-bit. Mich Bot 47:77–78.

Gingera TD, Bajno R, Docker MF, Reist JD (2017) Environmental DNA as a detection tool for zebra mussels *Dreissena polymorpha* (Pallas, 1771) at the forefront of an invasion event in Lake Winnipeg, Manitoba, Canada. Manag Biol Invasions 8:287–300. doi: https://doi.org/10.3391/mbi.2017.8.3.03

Hackett RA, Hilts DJ, Monfils AK (2014) Status and Strategy for European Frog-bit (*Hydrocharis morsus-ranae* L.) Management. Michigan Department of Environmental Quality, Lansing, Michigan.

Halpern AD (2017) *Hydrocharis morsus-ranae* L. in the Upper St. Lawrence River in New York: Its success within heterogenous wetland habitat and potential management approaches. Doctoral Dissertation. State University of New York, New York, New York (State).

Harman WN, Hingula LP, Macnamara CE (2005) Does long-term management in lakes affect biotic richness and diversity? J Aquat Plant Manage 43:57–64.

Hauteur J, Canetto R (1963) Destruction of aquatic plants with Aminotriazole. In: Compte rendu Conference du Comite Francais de Lutte contre les Mauvaises Herbes (COLUMA). pp 7.

Hofstra DE, Clayton JS (2001) Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: l. The use of endothall, triclopyr and dichlobenil. J Aquat Plant Manage 39:20–24.

Holz W (1963) Chemical weed control in ditches: Trials on the control of submerged plants. 5. Dtsch. Arbeitsbesprechung uber Fragen der Unkrautbiologie u. -bekampfung, Hohenheim, pp 4.

Houlahan JE, Findlay CS (2003) Effect of invasive plant species on temperate wetland plant diversity. Conser Bio 18:1132–1138.

Husák S, Gorbik VP (1990) Macrophyte biomass in the Kiev Reservoir (Ukraine). Folia Geobot Phytotaxon 25:265–273.

Jacono CC, Berent L (2018) *Hydrocharis morsus-ranae* L. United States Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, Florida, USA. Web. https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1110. Accessed 8 Sept 2018

Joint Nature Conservation Committee (2014) Unlucky 13 – Examples of New Additions on the Red List. Web. http://jncc.defra.gov.uk/page-3378. Accessed 29 Dec 2017

Kelting DL, Laxson CL (2010) Cost and effectiveness of hand harvesting to control the Eurasian watermilfoil population in Upper Saranac Lake, New York. J Aquat Plant Manage 48:1–5.

Lamont EE, Werier D, Glenn SD (2014) Noteworthy plants reported from the Torrey Range – 2011 and 2012. J Torrey Bot Soc 141:95–108.

Lansdown RV (2014) *Hydrocharis morsus-ranae.* The IUCN Red List of Threatened Species 2014. Web. *(The link provided was broken and has been removed)*. Accessed 23 Oct 2017

Lewis Creek Association (2011) European Frogbit Spread Prevention Pilot Program – Year 3 Final Report. Web. http://www.lcbp.org/publications/european-frogbit-spread-prevention-pilot-project-year-three/. Accessed 29 Dec 2017

Lewis Creek Association (2013) European Frogbit Removal Program, Shelburne and Charlotte 2013 Summary Report. Web. https://www.scribd.com/document/183279058/2013-European-Frogbit-Removal-Program-Shelburne-amp-Charlotte-Summary-Report. Accessed 29 Dec 2017

Lindberg SO (1873) Is *Hydrocharis* really dioecious? Trans Proc Bot Soc Edinburgh 11:389.

Luther H (1951) Verbreitung und Ökologie der höhoren Wasserpflanzen im Brackwasser der Ekenäs-Gegend in Südfinnland. Acta Bot Fenn 49:1–231; 50:1–370.

Madsen JD (2000) Advantages and Disadvantages of Aquatic Plant Management Techniques. ERDC/EL Mp-00-1. United States Army Corps of Engineers, Environmental Laboratory, Vicksburg, Mississippi.

Madsen JD, Owens CS, Getsinger KD (1998) Evaluation of four herbicides for management of American frogbit (*Limnobium spongia*). J Aquat Plant Manage 36:148–150.

Maleva MG, Nekrasova GF, Bezel VS (2004) The response of hydrophytes to environmental pollution with heavy metals. Russian J Ecol 35:230–235.

Marsden JE, Hauser M (2009) Exotic species in Lake Champlain. J Great Lakes Res 35:250–265.

Martine CT, Langdon SF, Shearman TM, et al. (2015) European frogbit in the Champlain/Adirondack region: Recent inferences. Rhodora 117:499–504.

(MDEQ) Michigan Department of Environmental Quality, Michigan Department of Natural Resources, Michigan Department of Agriculture and Rural Development (2014) Response Plan for Aquatic Invasive Species in Michigan. Michigan Department of Environmental Quality, Michigan Department of Natural Resources, Michigan Department of Agriculture and Rural Development, Lansing, Michigan. Web. http://www.michigan.gov/documents/deq/wrd-ais-response-plan_455659_7.pdf. Accessed 22 Jan 2018

Minnesota Pollution Control Agency (2009) Low Dissolved Oxygen in Water: Causes, Impacts on Aquatic Life – An Overview. Minnesota Pollution Control Agency, St. Paul, Minnesota. https://www.pca.state.mn.us/sites/default/files/wq-iw3-24.pdf. Accessed 22 Oct 2017

Minshall WH (1940) Frog-bit *Hydrocharis morsus-ranae* L. at Ottawa. Can Field-Nat 54:44–45.

(MISIN) Midwest Invasive Species Information Network (2018) European frog-bit (*Hydrocharis morsus-ranae*). Web. http://www.misin.msu.edu. Accessed 23 Mar 2018

(MNFI) Michigan Natural Features Inventory (2012) Glossy Buckthorn (*Frangula alnus*). In: Michigan Natural Features Inventory & Michigan Department of Natural Resources. Web. *The link provided was broken. This online document was revised 3/18/2019.* Accessed 23 Jan 2014

Murphy KJ (2002) Plant communities and plant diversity in softwater lakes of northern Europe. Aquat Bot 73:287–324.

Newbold C (1975) Herbicides in aquatic systems. Biol Cons 7:97–118.

Newbold C (1977) Aquatic herbicides: Possible future developments. Linnean Society Symposium Series 5:119–131.

Oles H, Flint S (2007) Adirondack Park Invasive Plant Program 2007 Annual Report. Adirondack Park Invasive Plant Program, Keene Valley, New York. Web.

http://adkinvasives.com/wp-content/uploads/2014/01/2007-APIPP-Annual-Report.pdf. Accessed 29 Dec 2017

O'Neill Jr CR (2007) European Frog-Bit (*Hydrocharis morsus-ranae*) - Floating Invader of Great Lakes Basin Waters. New York Sea Grant, Brockport, New York. NYSG Invasive Species Factsheet Series:07–1.

Pitkänen H, Peuraniemi M, Westerbom M, et al. (2013) Long-term changes in distribution and frequency of aquatic vascular plants and charophytes in an estuary in the Baltic Sea. Ann Bot Fenn 50:1–54.

Polechońska L, Samecka-Cymerman A (2016) Bioaccumulation of macro-and trace elements by European frogbit (*Hydrocharis morsus-ranae* L.) in relation to environmental pollution. Environ Sci Pollut Res 23:3469–3480.

Preston CD, March MD (1996) *Hydrocharis morsus-ranae* L. (Hydrocharitaceae) fruited in Britain in 1995. Watsonia 21:206–208.

Proctor C, Robinson V, He Y (2012) Multispectral detection of European frog-bit in the South Nation River using Quickbird imagery. Can J Remote Sensing 38:476–486.

Renard C (1963) The use of diquat and paraquat to control aquatic plants. Compte rendu Conference du Comite Francais de Lutte contre les Mauvaises Herbes (COLUMA). pp 9.

Reznicek AA, Voss EG, Walters BS (2011) MICHIGAN FLORA ONLINE. University of Michigan. Web. *(The link provided was broken and has been removed)*. Accessed 22 October 2017

Richards AJ, Blakemore J (1975) Factors affecting the germination of turions in *Hydrocharis morsus-ranae* L. Watsonia 10:273–275.

Roberts ML, Stuckey RL, Mitchell RS (1981) *Hydrocharis morsus-ranae* (Hydrocharitaceae): New to the United States. Rhodora 83:147–148.

Sager L, Clerc C (2006) Factors influencing the distribution of *Hydrocharis morsus-ranae* L. and *Rumex hydrolapathum* Huds. in a mowed low-lying marshland, Réserve de Cheyres, lac de Neuchâtel, Switzerland. Hydrobiologia 570:223–229. doi: 10.1007/s10750-006-0184-2

Sanders L, Hoover JJ, Killgore KJ (1991) Triploid Grass Carp as a Biological Control of Aquatic Vegetation. Information Exchange Bulletin A–91–2. United States Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi.

Scribailo RW, Posluszny U (1984) The reproductive biology of *Hydrocharis morsus-ranae*. 1. Floral biology. Can J Bot 62:2779–2787.

Scribailo RW, Posluszny U (1985) The reproductive biology of *Hydrocharis morsus-ranae*. 2. Seed and seedling morphology. Can J Bot 63:492–496.

Scriver M, Marinich A, Wilson C, Freeland J (2015) Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants. Aquat Bot 122:27–31.

Sculthorpe CD (1967) The biology of aquatic vascular plants. Edward Arnold Publishers, London, England.

Serbanescu-Jitariu G (1972) Contributions à la connaissance du fruit et de la germination de quelques representants de la famille des Hydrocharitaceae. Bull Soc Hist Nat Afr Nord (Alger) 63:19–28.

Shu L (2013) Research of different aquatic plants on purify the domestic sewage. Guangdong Agricult Sci 6:161–163.

Spicer KW, Catling PM (1987) Vascular aquatic weeds of the Rideau Canal, southeastern Ontario. Agriculture Canada Technical Bulletin 1990–3E:1–50.

Steffen K, Leuschner C, Müller U, et al. (2014) Relationships between macrophyte vegetation and physical and chemical conditions in northwest German running waters. Aquat Bot 113:46–55.

Sturtevant R, Larson J, Berent L, et al. (2014) An Impact Assessment of Great Lakes Aquatic Nonindigenous Species. NOAA Technical Memorandum GLERL-161. National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan.

Suominen J (1968) Changes in the aquatic macroflora of the polluted Lake Rautavesi, SW Finland. Ann Bot Fenn 5:65–81.

Sviridenko VG, Lapittskaya SK, Avseenko SV, Tolkachev VI (1988) Chemical characterization of aquatic plants and helophytes eaten by the European beaver. Rastit Resur 23:621–625.

Sytsma MD, Pennington T (2015) Vectors for spread of invasive freshwater vascular plants with a North American analysis. In: Canning-Clode J (eds), Biological invasions in changing ecosystems. De Gruyter Publishers, Berlin, Germany. pp 55–74.

Terras JA (1900) Notes on the germination of the winter buds of *Hydrocharis morsus-ranae*. Trans Proc Bot Soc Edinburgh. Session LXIV:318–329.

Texas A&M AgriLife Extension (2018) AQUA-PLANT: A Pond Managers Diagnostics Tool – Frog's-Bit. Web. https://aquaplant.tamu.edu/management-options/frogs-bit/. Accessed 18 Feb 2018

Thomas JD, Daldorph PWG (1991) Evaluation of bioengineering approaches aimed at controlling pulmonated snails: The effects of light attenuation and mechanical removal of macrophytes. J Appl Ecol 28:532–546.

Trebitz AS, Taylor DL (2007) Exotic and invasive aquatic plants in Great Lakes coastal wetlands: Distribution and relation to watershed land use and plant richness and cover. J Great Lakes Res 33:705–721. doi: *The link provided was broken. This online document was revised 3/18/2019.*

(UF/IFAS) University of Florida – Institute for Food and Agriculture Science (2018) Imazamox Considerations. Web. http://plants.ifas.ufl.edu/manage/developing-management-plans/chemical-control-considerations/imazamox-considerations/. Accessed 18 Feb 2018

Vaananen VM, Nummi P (2003) Diet of sympatric dabbling ducks in eutrophic wetlands. Suomen Riista 49:7–16.

Villanueva VR, Simolat LK, Mardon M (1985) Polyamines in turions and young plants of *Hydrocharis morsus-ranae* and *Utricularia intermedia*. Phytochemistry 24:171–172.

(WDNR) Wisconsin Department of Natural Resources (2012) Aquatic Plant Management Fact Sheets. Web. https://dnr.wi.gov/lakes/plants /factsheets/. Accessed 23 Jan 2018

Weibert C (2015) Weed Risk Assessment for *Hydrocharis morsus-ranae* L. (Hydrocharitaceae) - European frogbit. Version 1. Michigan Department of Agriculture and Rural Development, Lansing, Michigan.

Wellons K (2018) Ecophenology and control of European frog-bit in a hybrid cattail marsh of the St. Marys River, Michigan. Master's Thesis. Oregon State University, Corvallis, Oregon.

Wittwer C, Stoll S, Strand D, et al. (2018) eDNA-based crayfish plague monitoring is superior to conventional trap-based assessments in year-round detection probability. Hydrobiologia 807:87–97. doi: https://doi.org/10.1007/s10750-017-3408-8

Zhu B (2014) Investigating snails as potential biological control agents for invasive European frogbit (*Hydrocharis morsus-ranae*). J Aquat Plant Manage 52:102–105.

Zhu B, Ellis MS, Fancher KL, Rudstam LG (2014) Shading as a control method for invasive European frogbit (*Hydrocharis morsus-ranae* L.). Plos One 9:e98488.

Zhu B, Eppers ME, Rudstam LG (2008) Predicting invasion of European frogbit in the Finger Lakes of New York. J Aquat Plant Manage 46:186–189.

Zhu B, Kopco J, Rudstam LG (2015) Effects of invasive European frogbit and its two physical control methods on macroinvertebrates. Freshw Sci 34:497–507. doi: https://doi.org/10.1086/680987

Zhu B, Ottaviani CC, Naddafi R, et al. (2018) Invasive European frogbit (*Hydrocharis morsus-ranae* L.) in North America: An updated review 2003–16. Plant Ecol 11:17–25.