A Proposed Framework to Assess the Effects of European Frog-bit (*Hydrocharis morsus-ranae*) on Michigan Wetlands

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Cover: Wetland dominated by European frog-bit, taken by B. C. Cahill.

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Abstract

European frog-bit (*Hydrocharis morsus-ranae*; EFB) is an invasive aquatic plant species that has quickly spread within Michigan, yet the detrimental effects to wetland ecosystems remain largely unknown. With funding provided by the Water Resources Division of the Michigan Department of Environment, Great Lakes, and Energy, the Michigan Natural Features Inventory worked with Central Michigan University as they developed an adaptive management plan for EFB. Our work focused on gathering and compiling EFB occurrence data, synthesizing information about the effects of EFB and other aquatic invasive plant species on native species and ecosystem functioning, identifying important knowledge gaps, and developing a research framework to address those information needs. We gathered and aggregated 8,214 records of known EFB status in the U.S. and Canada, with 3,916 unique occurrences being from Michigan. The limited information about the potential effects of EFB and other aquatic invasive plant species on native plants and animals and ecosystem processes was synthesized from available literature. Based on our literature search, we identified several important information needs regarding the impacts of EFB on wetlands: the effects of EFB on other organisms; effects of EFB on ecosystem processes; influence of EFB density and patch size on organisms and processes; interaction of EFB with other invasive species; conditions driving EFB occurrences; ecosystem resiliency to invasion; and effects of EFB to ecosystem services and human values. Five research objectives were proposed to address these knowledge gaps: 1) compare plant and animal communities between wetlands with and without EFB populations; 2) compare measures of ecosystem processes between wetlands with and without EFB populations; 3) examine associations between ecosystem variables and EFB density and patch size; 4) assess if EFB populations interfere with recreational use of wetlands; and 5) evaluate if EFB is affecting ecosystem services. We suggested several elements to strengthen study designs and increase the likelihood of detecting ecological patterns. Study designs should include reference sites representing naturally functioning wetlands as a comparison to wetlands containing EFB populations. The study areas should encompass the range of the invasion gradient (i.e., from well-established, high density to recent, low density sites) and types of wetlands containing EFB (e.g., coastal and inland; marshes, lakes, and ponds). Sampling should be replicated spatially (e.g., across EFB distribution in Michigan) and temporally (e.g., over multiple years) to the greatest extent possible to account for natural variation. We also recommend study designs that evaluate EFB effects to native species, ecological processes, ecosystem services, and human values concurrently. We suggested several measures of organismal communities and ecological functioning and associated sampling methodologies to address the proposed research objectives. Sampling of plant, macroinvertebrate, fish, herptile (frogs, toads, and turtles), and bird (waterfowl, waterbirds, and shorebirds) communities and indicators of ecological processes, such as water chemistry (e.g., DO, pH, nutrients), water movement (e.g., fluctuations, flow), and physical/structural variables (e.g., biomass, soils, light penetration), is recommended to understand potential impacts from EFB. Measuring species and processes across EFB density and size gradients may assist in determining if there are EFB population thresholds at which detrimental impacts to native species and normal ecosystem processes occur. Stakeholder engagement is needed to understand the effects of EFB on human values, such as recreational use. Finally, we recommended developing a conceptual model to describe the interaction of ecosystem processes, ecosystem services, human values, and EFB. New knowledge gained about alterations to processes associated with EFB could then be incorporated into the model to predict resulting changes to ecosystem services and human values.

Introduction

European frog-bit (*Hydrocharis morsus-ranae*; EFB) is an invasive plant species native to Europe and portions of Asia and Africa (Catling et al. 2003). First documented in the U.S. in 1974 and in Michigan in 1996 (Roberts et al. 1981, Reznicek et al. 2011), the species' distribution in the State has been steadily expanding. Despite EFB being present in North America since 1936 (Minshall 1940), much remains unknown about its ecology and the potential detrimental effects on wetland ecosystems. Cahill et al. (2018) highlighted several knowledge gaps regarding EFB, including the need for a better understanding of its distribution in Michigan, impacts to native plant and animal species, ecosystem functioning, and wetland values, and its response to management actions. With growing interest among many conservation partners in better coordinating management, monitoring, research, and information sharing, Central Michigan University (CMU) is leading the European Frog-bit Collaborative, in consultation with an array of partners, to develop an adaptive management plan to guide EFB control efforts in the State.

The Michigan Natural Features Inventory (MNFI) proposed to use funding provided by the Water Resources Division (WRD) of the Michigan Department of Environment, Great Lakes, and Energy (EGLE) to work with CMU in the development of the EFB adaptive management framework by completing tasks complementary to CMU's efforts. We aimed to partner with CMU in identifying existing data sets with information on EFB, such as monitoring conducted by the Great Lakes Coastal Wetland Monitoring Program (GLCWMP), Midwest Invasive Species Information Network (MISIN), and the Great Lakes Invasives Network (GLIN). Spatial information on EFB occurrences and associated metadata would be consolidated into a Geographic Information System (GIS) to facilitate management and research planning. In addition, MNFI planned to search for literature on evaluating the impacts of aquatic invasive plant species on ecosystem services and synthesize the available information to inform future research planning. We also proposed to identify preliminary knowledge gaps to be presented to other partners as part of the adaptive management process.

Based on the preliminary information needs we identified and our collective knowledge of wetland species and systems, MNFI set out to work with CMU and other partners to develop research goals and objectives to better understand the effects of EFB on other biota and ecosystem functioning, which is a critical knowledge gap. We used the spatial information and our knowledge of wetlands to develop a draft research framework, including proposed research objectives, general recommendations, key design elements, and potential sampling methodologies. In this report we describe the following: 1) methods used to gather and compile EFB occurrence data, search for information about the impacts of EFB and other aquatic invasive plants on native species and ecosystem functioning, and identify knowledge gaps; 2) a synthesis of our findings and important knowledge gaps; and 3) a proposed research framework to evaluate the effects of EFB on native species, ecosystem processes, and human values.

Methods

Data Aggregation

The MNFI worked with CMU to query databases for EFB occurrence records. Queries were made to several global, national, and regional databases that could potentially house EFB records. Human observations from natural resource management professionals and citizen scientists and preserved specimens from natural history collections were included as occurrence records. We requested and obtained information on EFB from the GLCWMP. Internal MNFI datasets from wetland studies were also reviewed and EFB detection and non-detection data were transferred to MISIN. Documented EFB records were closely examined to ensure accuracy and remove redundant records. Information on these occurrences were compiled into a single dataset using standardized formatting and appropriate data standards.

Literature Search

We conducted a literature search to ascertain the state of knowledge regarding EFB, which focused on its habits and characteristics in its native range and effects to species and ecosystem functioning in North America. Because information about EFB in North America was limited, we also searched for relevant literature about other aquatic invasive plant species that occupy inundated wetland zones. The search capabilities of the Michigan State University Libraries were used to search on keywords. As simple keywords resulted in well over a thousand hits related to EFB (mainly aspects of reproduction), secondary searches were conducted using combinations of keywords and Boolean operators (e.g., *Hydrocharis* AND invasive, European frog-bit AND unionid). In addition, MNFI's digital library of reports was also searched for relevant studies conducted by MNFI. Google searches were employed primarily to locate government and nongovernmental organization websites that might have relevant information. In the process of reading the literature, relevant studies were also identified in the literature cited sections of the reports and articles identified by the above procedures. Thus, the search was able to identify peer-reviewed articles, books, gray literature, and web resources.

Knowledge Gap Identification

A list of important research needs was developed using information gathered during our targeted literature search, research needs identified in Cahill et al. (2018), examination of the MNFI Natural Heritage Database, and our knowledge of wetland ecosystems and species. To assess the potential for EFB to impact high quality ecosystems and rare/declining species, we intersected documented EFB records with spatial data from MNFI element occurrences using ArcGIS 10.6. Element occurrences considered historical, extirpated, possibly extirpated, or last observed more than 30 years ago were excluded from the analysis. We focused on identifying the knowledge gaps most limiting to our understanding of the influence of EFB on native wetland species and typical ecosystem functioning. A framework for conducting research and monitoring was then developed to address these information needs and thus ultimately improve conservation planning and implementation of control efforts.

Results and Discussion

European Frog-bit Occurrences

We searched numerous databases and data sets in North America to compile the most comprehensive set of EFB records available, resulting in over 8,200 records with known EFB status (i.e., present or absent; Table 1). Confirmed EFB occurrences came from eight states (Illinois, Maine, Michigan, New York, Ohio, Pennsylvania, Vermont, and Washington) and two provinces (Ontario and Quebec), with Michigan records representing nearly half (48.2%) of the total occurrences. Details about these records were compiled into a common dataset according to accepted data standards and provided to the Water Resources Division of EGLE as a separate deliverable with this report. Occurrence details and location data were removed from all GLCWMP records; our data-sharing agreement with the GLCWMP does not allow us to distribute the information outside of MNFI and CMU.

For those records having specific location information (98% of records), we aggregated the spatial data into a single ArcGIS shapefile (Figure 1), which was submitted to EGLE as a supplement to this report. As with the digital dataset, the shapefile does not contain detailed information or locations for GLCWMP records because we are not allowed to distribute the data beyond MNFI and CMU. Although EFB has been detected at a few inland sites in Michigan, nearly all (98.5%) of the records for the State are from Great Lakes coastal wetlands or connected waterways. The most extensive EFB populations are from western Lake Erie, the St. Clair Flats, Saginaw Bay, and the St. Mary's River (Figure 1).

Table 1. Data sources queried for European frog-bit (EFB) occurrence records. For each source, the date of last download and number of unique EFB records found is provided.

Data Source	Date of Last Download	No. Records Reviewed
Consortium of Midwest Herbaria (CMH)	05/23/2019	13
Early Detection and Distribution Mapping System (EDDMapS)	04/18/2019	773
Global Biodiversity Information Facility (GBIF)	04/18/2019	1,201
Great Lakes Coastal Wetland Monitoring Program (GLCWMP)	04/29/2019	1,504
Great Lakes Invasives Network (GLIN)	05/09/2019	46
iMapInvasives (NatureServe)	05/08/2019	434
iNaturalist (iNat)	04/30/2019	43
Integrated Biocollections Catalog (iDigBio)	05/23/2019	8
Midwest Invasive Species Information Network (MISIN)	04/30/2019	4,098
United States Geological Survey Nonindigenous Aquatic Species Database (USGS-NAS)	04/18/2019	94
Total No. Occurrence Records Aggregated		**8,214**

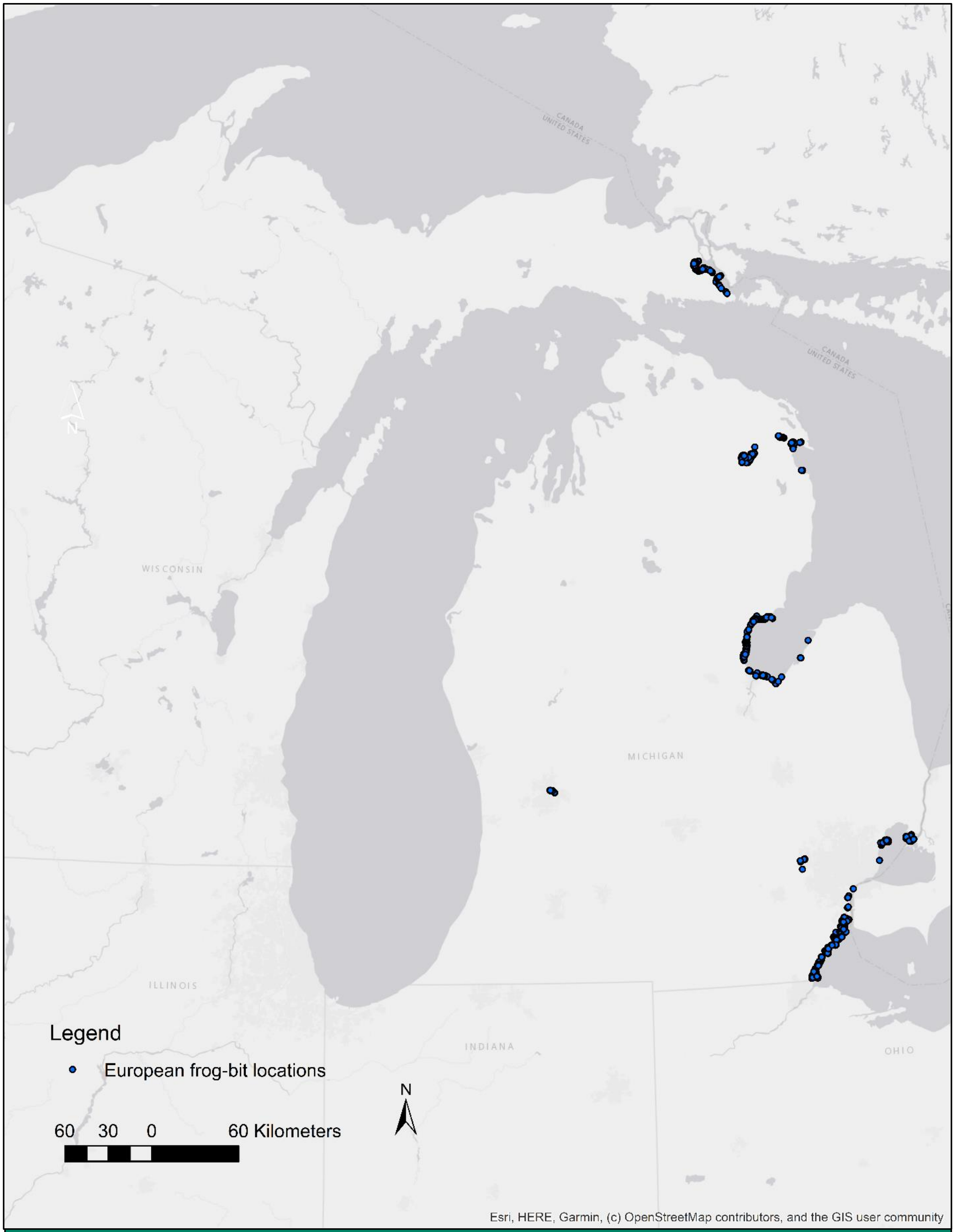

Figure 1. Distribution of confirmed European frog-bit occurrences in Michigan.

Potential Effects of European Frog-bit

Background

Wetlands received legal protection by both the Federal government and the State of Michigan because they are recognized as having characteristics that benefit humans; we refer to these characteristics and benefits as “functions and values”. Some of the inherent ways in which wetlands function results in human benefits (i.e., values or services), such as those listed in Table 2. There are many more functions and values than those listed, and many functions can have more than one associated value. For example, the provision of fisheries and wildlife habitat by wetlands (function) contributes to the maintenance of biodiversity (value), but is also important to fishing, hunting, and bird watching (i.e., recreational value), and those participants contribute to the economy through purchase of fishing and hunting gear, binoculars, etc. (i.e., economic value). The Michigan United Conservation Clubs (2019) reports that hunting and fishing generates $11.2 billion annually in economic activity in Michigan. Thus, fish and wildlife habitat significantly contributes to economic activity, which supports jobs and human well-being in the form of financial security. Any factor that disrupts a wetland function has the potential to negatively impact human well-being; one such factor is invasive plant species.

Table 2. Typical wetland functions and their associated human values.

Wetland Function	Human Values
Storm water storage	Flood attenuation
Groundwater recharge/discharge	Water supplies and stream base flow
Provision of fisheries and wildlife habitat	Maintenance of biodiversity
Shoreline stabilization	Maintenance of property values
Contaminant removal	Water quality maintenance
Carbon sequestration	Climate regulation
Provision of natural environments	Aesthetic, recreation, education, cultural

Wetlands are classified primarily using three factors: vegetation, soils, and hydrology. Not surprisingly, hydrology, usually cast in terms of the source and pattern of movement of water into and out of wetlands, is the driving determinant of the vegetation and soil in a wetland. However, interactions among species also influence which plants are present or are dominant in a wetland. Vegetation structure, in the physical sense, can have substantial influence on wetland functioning. As pointed out by Crowder and Painter (1991), macrophytes provide structure along shores that can be used as habitat or food by macroinvertebrates (e.g., insects and snails), which tend to have preferences for specific plant species or structural characteristics. Insects and other invertebrates serve as prey items for fish, amphibians, reptiles, and birds, which can also use the plants as breeding habitat and escape cover. The balance among macrophytes is determined by the specific plant species, habitat preferences of the macroinvertebrates, and the trophic relationships and habitat requirements among the associated fauna. Due to this chain, or web, of causation, introduction of an invasive species can have profound implications for the components and processes of a wetland. It is recognized that invasive species have altered wetlands, affecting the characteristics and ecosystem services of wetlands in a variety of ways (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES] 2018).

We assessed the potential for EFB to threaten ecosystem functioning and biodiversity in Michigan. Escobar et al. (2018) noted that the Great Lakes region is especially vulnerable to the introduction and establishment of aquatic invasive species. Established EFB populations along the coast occur within and near several high-quality examples of the Great Lakes marsh natural community (Cohen et al. 2018, MNFI 2019). Invasive species, such as EFB, common reed (*Phragmites australis*), and narrow-leaved/hybrid cattail (*Typha angustifolia*, *T*. x *glauca*), have the potential to reduce plant diversity and alter ecological functioning. Changes to plant structure and diversity and ecosystem functioning could in turn alter animal use and ecosystem services.

In our analysis of the overlap of EFB records and MNFI element occurrences, 38.4% of the Michigan EFB records fell within polygons mapped for element occurrences of unique natural communities and rare and/or declining plant and animal species (MNFI 2019). An additional 32.8% of EFB records occurred within 250 m of a natural community or species element occurrence. European frog-bit occurred in or near 201 element occurrences, consisting of 19 natural communities (15 Great Lakes marsh, 1 lakeplain wet prairie, 1 lakeplain wet-mesic prairie, 1 lakeplain oak openings, and 1 wet-mesic flatwoods), 36 vascular plant occurrences, 25 invertebrate animal occurrences (23 mussel, 1 dragonfly, and 1 butterfly), 120 vertebrate animal occurrences (100 bird, 16 reptile, 2 amphibian, and 2 fish), and 1 animal assemblage (great blue heron [*Ardea herodias*] rookery). This assessment highlights the potential for EFB to affect an array of rare and declining native species and unique natural communities in Michigan.

Coastal wetlands harboring the largest populations of EFB also support ecologically and economically important species, such as fish (Jude and Pappas 1992), breeding and migrant waterfowl (Bookhout et al. 1989, Prince et al. 1992), and many rare and declining species (e.g., mussels, amphibians, marsh birds, etc.). Trebitz and Hoffman (2015) estimated that fish species that use coastal wetlands make up 50% of the biomass and 60% of the dollar value of the fish landed commercially and approximately 80% of the recreational fish harvest. Furthermore, the authors noted the importance of vegetation structure in predicting the fish species present, with less degraded wetlands supporting more high-value sport and panfish species (Trebitz and Hoffman 2015). Recreational fishing in the Great Lakes contributed $2.2 billion to the economy in 2016 (U.S. Fish and Wildlife Service [USFWS] and U.S. Census Bureau [USCB] 2016). Expenditures in the U.S. for migratory bird hunting, which includes waterfowl, were estimated at $2.3 billion in 2016, with approximately 7% of the population (above the national average) in the east north central U.S. participating in hunting (USFWS and USCB 2016). Wildlife watching contributed $75.9 billion to the national economy in 2016 and an estimated 35% of the population in the east north central region (above the national average) participated in wildlife-watching activities at or away from home (USFWS and USCB 2016). Environment Canada (2019) indicated EFB is a serious threat to several rare species, including Ogden's pondweed (*Potamogeton ogdenii*), Eastern musk turtle (*Sternothermus odoratus*), and least bittern (*Ixobrychus exilis*) in their COSWIC assessments. The presence of EFB in or near ecologically and economically important natural resources emphasize the potential for the invasive species to cause harm to native species, ecological functioning, and derived human values in Michigan and the Great Lakes region.

Ecosystem Functioning and Services

As noted by Cahill et al. (2018), there is little information about the effects of EFB on ecosystem functioning and associated values. Several authors have noted that EFB often forms dense, intertwined mats (Campbell et al. 2010, Martine et al. 2015, Zhu 2018, Johnson and Miner 2018, Jacono and Berent 2019). The formation of dense EFB mats has the potential to alter wetland functioning (e.g., reduced light penetration, dissolved oxygen [DO], and nutrient availability), lower plant and animal diversity, and degrade human values, yet the few past studies available indicated inconsistent patterns. Catling et al. (2003) suggested EFB mats could reduce DO levels, clog navigation and irrigation channels, and limit recreational and commercial uses of water bodies. Catling et al. (1988) and Zhu et al. (2014) found lower plant species richness and abundance under EFB mats compared to areas without EFB. Halpern (2017) observed a negative correlation between EFB surface coverage and aquatic plant species richness and diversity in Lake Ontario wetlands. Conversely, other researchers observed no effects to plant species richness, percent cover, or diversity associated with EFB (Thomas and Daldorph 1991, Houlahan and Findlay 2004, and Trebitz and Taylor 2007). Martine et al. (2015) summarized some findings from an unpublished thesis (Shearman 2011) indicating some submerged native macrophytes (*Ceratophyllum demersum*, *Myriophyllum sibiricum*, *Potamogeton hillii*, *P. zosteriformis*, and *Spirodela polyrhiza*) were more abundant in areas with EFB, which the author suggested may be due to suppression of *Elodea canadensis*, an allelopathic species that may inhibit the growth of native macrophytes.

Dense mats formed by EFB and associated changes to wetland functioning could alter animal use of wetlands. Catling et al. (2003) indicated that the annual decomposition of EFB mats could deplete DO to levels harmful to fish and macroinvertebrates. Zhu et al. (2008) found DO levels as low as low as 1.9 mg/L in some parts of Oneida Lake, New York. In a study of Lake Erie coastal wetlands, Johnson (2018) consistently observed hypoxic conditions within EFB-dominated sites, whereas other wetland types always had DO levels > 2.0 mg/L. Fewer snails, crustaceans, and insect larva were observed on EFB mats compared to stands of native aquatic plants in New York and Ontario (Catling et al. 1988). During a field mesocosm study in Oneida Lake, New York, Zhu et al. (2015) found EFB had no measurable negative effect on surface macroinvertebrates and areas covered by EFB had more diverse insect taxa. Surface samples with EFB had greater mollusk density, amphipod density, taxon richness, and Simpson diversity compared to other sites (Zhu et al. 2015). Benthic samples from areas with EFB had significantly fewer benthic worms, more chironomids, and possibly greater diversity of benthic macroinvertebrates (Zhu et al. 2015). Johnson (2018) observed greater abundance of mobile orders of macroinvertebrates, such as gastropods and odonates, in EFB compared to patches of other invasive plant species and open water. Fish species tolerant of low DO levels (common carp [*Cyprinus carpio*], goldfish [*Carassius auratus*], and central mudminnows [*Umbra limi*]) were more abundant and species intolerant of low DO levels (bluegill [*Lepomis macrochirus*] and pumpkinseed [*L. gibbosus*]) were less abundant in EFB compared to other invasive plant species and open water in Lake Erie (Johnson 2018). Fewer fish species and lower fish abundance was recorded in areas invaded by EFB compared to areas without EFB in coastal wetlands of Munuscong Bay on the St. Mary's River (Daly 2016). We found no published studies investigating amphibian, reptile, or bird use of invasive EFB during our literature search. In thesis research conducted by Johnson (2018), birds were not observed using EFB in Lake Erie coastal wetlands but surveys were based on two visits of short duration in one year. In general, limited information is available on the impacts of non-native invasive plant species, particularly aquatic invasive plants, on native amphibian and reptile species (Martin and Murray 2011, Bucciarelli et al. 2014).

Cahill et al. (2018) noted the potential for EFB to alter fungal and bacterial communities, but research in this area has been limited and produced mixed results (see Zhu et al. 2018). Czeczuga et al. (2005) observed fewer zoosporic fungus species on EFB compared to several other aquatic plant species examined in Polish wetlands. Anesio et al. (2000) found that dissolved organic matter leached from EFB impeded bacterial growth after exposure to ultraviolet radiation. Catling et al. (2003) indicated bacteria species had not been documented on EFB.

European frog-bit has the potential to benefit some species or ecosystem services in its invasive range. Some mammals, birds, fish, and invertebrates have been reported to feed on EFB leaves and/or seeds in its native and invasive ranges (Catling and Dore 1982, Catling et al. 2003).

Several turtle species eat the seeds of aquatic macrophytes, such as American waterlily (*Nymphaea odorata*) and yellow pond-lily (*Nuphar advena*; Lagler 1943, Parmenter 1980, Ford and Moll 2004, Padgett et al. 2010), so it is possible that turtles may eat the seeds or turions of EFB. Although dense EFB mats could impede movement of amphibians and reptiles and limit foraging by birds, greater abundance of some fish and invertebrate species could potentially provide increased prey availability for fish, herptiles, and birds. Dense mats of EFB on the water's surface also may provide basking habitat and cover from predators for amphibians and reptiles. European frog-bit could help to maintain/improve water quality due to its ability to uptake high amounts of heavy metals and nutrients (Engin et al. 2015, Polechońska and Samecka-Cymerman 2016).

Given the limited information about the effects of EFB on ecosystems, we also examined past research conducted on other invasive species. Gellardo et al. (2016) conducted a meta-analysis of 733 studies investigating the effects of aquatic invasive species focusing on five functional groups: 1) fish, 2) benthic invertebrates, 3) zooplankton, 4) phytoplankton, and 5) macrophytes. They summarized the overall findings with respect to the effects of invasive macrophytes as follows: overall diversity and abundance was reduced; native macrophyte abundance and diversity were reduced; zooplankton and fish abundance was reduced; benthic invertebrate presence and abundance exhibited negative trends but were not statistically significant; turbidity, organic matter, phytoplankton, and nitrogen increased but the trends were not statistically significant; primary producers were especially impactful on fish and benthic invertebrates; and the effect on abundance but not diversity in some cases may reflect that changes to diversity require long-term effects of invasives to produce local extinctions.

Compared to aquatic invasive plant species, the influence of emergent invasives, such as common reed, narrow-leaved and hybrid cattail, purple loosestrife (*Lythrum salicaria*), and reed canary grass (*Phalaris arundinacea*), on ecosystem functioning has received greater research attention. Reduced plant diversity has been found with the expansion of common reed (Tulbure et al. 2007, Whyte et al. 2008), purple loosestrife (Thompson et al. 1987, IPBES 2018), reed canary grass (Houlahan and Findlay 2004, Perkins and Wilson 2005), and invasive cattail (Lishawa et al. 2010, 2015). Common reed captures detritus and sediment, which affected use of wetlands by fish and benthic invertebrates and raised soil surface levels (Rooth and Stevenson 2000, Rooth et al. 2003). In their study of a western Lake Erie marsh, Duke et al. (2015) indicated that common reed expansion could increase litter decomposition and reduce methane emission through environmental modifications that change microbial activity and/or composition. Several studies have examined impacts of invasive wetland plant species, such as common reed, purple loosestrife, and reed canary grass, on native amphibians and/or reptiles (Maerz et al. 2005, Brown et al. 2006, Rittenhouse 2011, Kapust et al. 2012, Greenberg and Green 2013, Perez et al. 2013, Stephens et al. 2013, Bucciarelli et al. 2014, Mifsud 2014). Most of these studies documented negative impacts on amphibians and reptiles (e.g., lower species richness and abundance, Mifsud 2014; slower development and/or decreased survival of tadpoles, Maerz et al. 2005, Brown et al. 2006, Watling et al. 2011a and 2011b; habitat alteration/loss, Perez et al. 2013). Negative impacts were primarily due to changes in water chemistry, reduced oxygen levels, decreased food quality and quantity, and/or changes in microclimate, hydroperiod, habitat structure, or available cover (Maerz et al. 2005, Brown et al. 2006, Maerz et al. 2010, Rittenhouse 2011, Watling et al. 2011a, 2011b, and 2011c; Kapust et al. 2012, Perez et al. 2013). Other studies described the influence of common reed on invertebrate (Holomuzki and Klarer 2010) and bird (Myer et al. 2010, Lupien et al. 2014, Whyte et al. 2015, Robichaud and Rooney 2017) communities. Narrow-leaved and hybrid cattail was shown to change physicochemical parameters, resulting in cooler waters and homogenized vegetation structure, which in turn reduced macroinvertebrate density and biomass (Lawrence et al. 2016). Whitt et al. (1999) examined bird use of marshes dominated by purple loosestrife and native vegetation on Lake Huron and found that purple loosestrife dominated wetlands had greater total bird densities and lower diversities compared to other types.

Published research on the effects of floating and submersed aquatic invasives to wetland ecosystems was limited but can provide insights into the potential impacts of EFB on wetlands. Boylen et al. (1999) described the expansion of Eurasian watermilfoil (*Myriophyllum spicatum*) and associated decrease in abundance and diversity of native aquatic macrophytes. Macrophyte species richness was lower in New York lakes where *Nitellopsis obtusa*, an invasive macroalgae, was abundant, and *Nitellopsis* biomass exceeded the combined biomass of macrophytes in some lakes (Brainard and Schultz 2017). Urban et al. (2006, 2009) demonstrated the effect of the invasive *Utricularia inflata* on the native *Eriocaulon aquaticum* was principally the result of its shading effect, which in turn produced cascading effects on sediment chemistry. In their review, Villamagna and Murphy (2010) reported that water hyacinth (*Eichhornia crassipes*) can affect water clarity and decrease phytoplankton production, dissolved oxygen, nitrogen, phosphorous, heavy metals, and concentrations of other contaminants. However, they also noted that the relationships are complex, not linear, and the effects on the fish community may be dependent on initial structure. Kuehne et al. (2016) studied sites on a Washington river with varying degrees of infestation by the invasive parrotfeather (*Myriophyllum aquaticum*) and their findings were similar to studies of EFB. The authors found areas with greater amounts of parrotfeather were associated with lower DO, greater diversity of epiphytic invertebrates, dominance by amphipods, lower use by some invertebrates (cladocerans, chironomids and gastropods), greater use by non-native fishes, and an increase in native fishes more tolerant of low DO levels. Researchers found Eurasian watermilfoil supported different macroinvertebrate communities than similarly structured native macrophytes, indicating the replacement of native milfoils may have indirect effects on aquatic food webs (Wilson and Ricciardi 2009). In their study of Hudson River wetlands, Strayer et al. (2003) suggested the replacement of native water celery (*Vallisneria americana*) by water chestnut (*Trapa natans*) has likely increased invertebrate diversity and food for fish but that macroinvertebrates may not be available to fish because of low DO levels in water chestnut beds. Zhu et al. (2015) noted that past research on the effects of water chestnut on macroinvertebrates appear to be location specific and suggested that similar variability may apply to EFB. Kovalenko and Dibble (2011) stated the presence of invasive watermilfoil in Minnesota lakes was associated with changes in trophic diversity but not trophic position of secondary consumers, and in both odonates and fish, trophic diversity appeared to be a more sensitive indicator of environmental disturbance than trophic position. Nichols and Shaw (1986) noted that three nuisance aquatic species, *Myriophyllum spicatum*, *Potamogeton crispus*, and *Elodea canadensis*, all provide habitat for fish and aquatic invertebrates. Hydrilla (*Hydrilla verticillata*) was shown to reduce growth rates of largemouth bass (*Micropterus salmoides*; Brown and Maceina 2002, Sammons et al. 2005) and sportfishing (Slipke et al. 1998). Changes in the abundance of hydrilla have been associated with shifts in the diet of largemouth bass (Sammons and Maceina 2006). Fields et al. (2003) found that hydrilla and parrot's feather were important food items for Texas river cooters (*Pseudemys texana*), although their nutritional values were lower than other plants consumed by the turtles. Non-native milfoil species (*Myriophyllum* spp.) were shown to reduce property values after invasion (Halstead et al. 2003, Horsch and Lewis 2008) and reduced the likelihood that undeveloped lakefront properties become developed (Goodenberger and Klaiber 2016). Research on aquatic invasive plant species occupying niches similar to EFB suggests the effects to wetland ecosystem function from EFB are likely to be complex and the result of interactions with many other native and non-native species.

Important Knowledge Gaps

Little research has been conducted to date on the effects of EFB to native species and ecosystem processes, and results from the few studies completed have been complicated and sometimes conflicting. Studies of EFB have focused on its influence on native macrophytes, invertebrates, and fish, with little information available on its effects to ecosystem functioning and no published investigations of potential impacts to other vertebrate groups (e.g., amphibians, reptiles, birds); however, potential effects on functioning are implied based on changes in biota or physicochemical characteristics. We examined the literature available for other aquatic invasive plants species occupying similar wetland zones (i.e., free-floating, floating-leaved, and submersed species) and found the research to be similarly limited. Several studies documented impacts of invasive species on native plants, invertebrate communities, fish use, and some aspects or indicators of ecosystem processes. Again, little information was available on potential impacts to vertebrate animals other than fish and detailed studies of impacts to ecosystem processes were lacking. Although some research has examined the influence of invasive species on property values, researchers largely have not attempted to translate ecosystem changes to altered human values. Below we list several information needs that we deem important to better understanding the influence of EFB on wetland ecosystems, which would lead to more informed conservation planning and management implementation.

- *Effects of EFB on other organisms*: Research is needed to test the assumption that EFB is altering plant and animal communities in the Great Lakes region. To effectively manage EFB, we need to improve our understanding of the potential negative and positive impacts of EFB to plant and animal communities. Evaluations of EFB effects on plants, invertebrates, fish, herptiles, birds, and mammals (e.g., muskrats [*Ondatra zibethicus*]) are needed. Metrics could include measures of occupancy, abundance, biomass, and diversity (e.g., species richness, diversity indices, floristic quality index).

- *Effects of EFB on ecosystem processes*: Ecosystem functioning is difficult to examine in the field but vital to understanding the impacts of invasive species. Indicators of ecosystem processes should be measured as much as possible and in conjunction with other research and monitoring. Possible factors to investigate include the hydrology (e.g., depth, fluctuations, movement, flow), water conditions (e.g., DO, pH, nutrient levels, temperature), soil conditions, and structural characteristics (e.g., litter, EFB mat depth, light penetration).

- *Influence of EFB density and patch size*: Understanding how EFB density and patch size influence organisms and function could be extremely valuable to future management. There may be thresholds (e.g., percent cover, area of patch) at which EFB becomes detrimental to native species and normal ecosystem processes, so identifying such thresholds could help managers allocate resources effectively, especially if eradication is not possible.

- *Interaction of EFB with other invasive species*: European frog-bit often co-occurs with other invasive species, such as common reed and invasive/hybrid cattail. Study is needed to understand if interactions or management for other invasives facilitates the spread of EFB. At a minimum, the occurrence of other invasive species should be measured while conducting research targeted at EFB to assess if ecosystem metrics appear associated with the co-occurrence of invasive species.

- *Conditions driving EFB occurrences*: The factors most affecting EFB's distribution on the landscape remain unknown, such as variables relating to the hydrology (e.g., water source, depth, flow, wave action), water chemistry (e.g., DO, pH, nutrient levels, temperature), habitat conditions (e.g., native species, other invasives), and landscape context (e.g., human development).

- *Ecosystem resiliency to invasion*: The condition of the wetland when EFB becomes introduced could influence the rate at which EFB expands and the intensity of potential ecological impacts. For example, a wetland with high native plant diversity and low levels of degradation may be more resilient to the establishment of an EFB population than a highly degraded system (e.g., dominance by other invasive species, hydrologic modifications, nutrient loading, etc.). Understanding how these factors interact with EFB invasions could lead to better management planning and resource allocation.

- *Effects of EFB to human services*: Study of EFB effects to species and ecosystem functioning (see above) could help determine EFB's potential or actual effects on human wetland values (e.g., recreation, water quality, biodiversity, fish and wildlife habitat). An improved understanding of the real or perceived effects of EFB to human uses of wetlands (e.g., boating, swimming, fishing, hunting) could be accomplished through stakeholder surveys and valuation studies.

Proposed Research Framework

General Recommendations

We suggest that research to evaluate the impacts of EFB take a multifaceted approach combining investigations of organism diversity, vegetation structure, wetland characteristics, ecosystem processes, and human values. Ruiz-Jaen and Aide (2005) provided recommendations for assessing the success of ecosystem restoration projects that would apply equally well to evaluating the ecological changes associated with invasive species. Their approach aimed to assess various attributes of restored systems identified by the Society for Ecological Restoration International (SER Science and Policy Working Group 2004). Ruiz-Jaen and Aide (2005) suggested studies incorporate a minimum of two variables within each of three ecosystem attributes: diversity (e.g., plants, invertebrates); plant structure (e.g., biomass, density); and ecological processes (e.g., hydrology, nutrient cycling). In addition, at least two reference sites should be sampled to capture the natural variation and provide a comparison to the sites being assessed (Ruiz-Jaen and Aide 2005). We feel these recommendations provide a good approach to assessing the effects of EFB on wetlands and have incorporated them into the objectives and study design elements described below.

Although some work has been done to assess the economic impact (e.g., property values) of invasive aquatic macrophytes, research on effects to ecosystem services are lacking. Pejchar and Mooney (2009) made several observations regarding aquatic invasive species, ecosystem services, and human well-being: 1) the economic impact of aquatic invasives is rarely quantified, resulting in an "invisible tax" on ecosystem services that is not considered in decision making; 2) to capture the full impact of these species on human well-being, we need to consider more than monetary costs and benefits; and 3) the influence of invasive species on culture is likely the most complex and least understood of the ecosystem services, despite strong resonation of these services with stakeholders (e.g., landowners, local communities, cultural practitioners). In our research framework, we propose to examine ecosystem service "chains" (Figure 2), or the flow from nature's inherent processes (e.g., biodiversity and ecosystem functions) through intermediate ecosystem services to final ecosystem goods and services and associated values. Our approach would investigate the influence of EFB on ecosystem service chains by examining multiple "links" of the process, from species interactions and ecological functioning to real or potential changes to ecosystem services, goods, and human values (Table 3). Benefits to humans from nature occur through these chains of factors and processes.

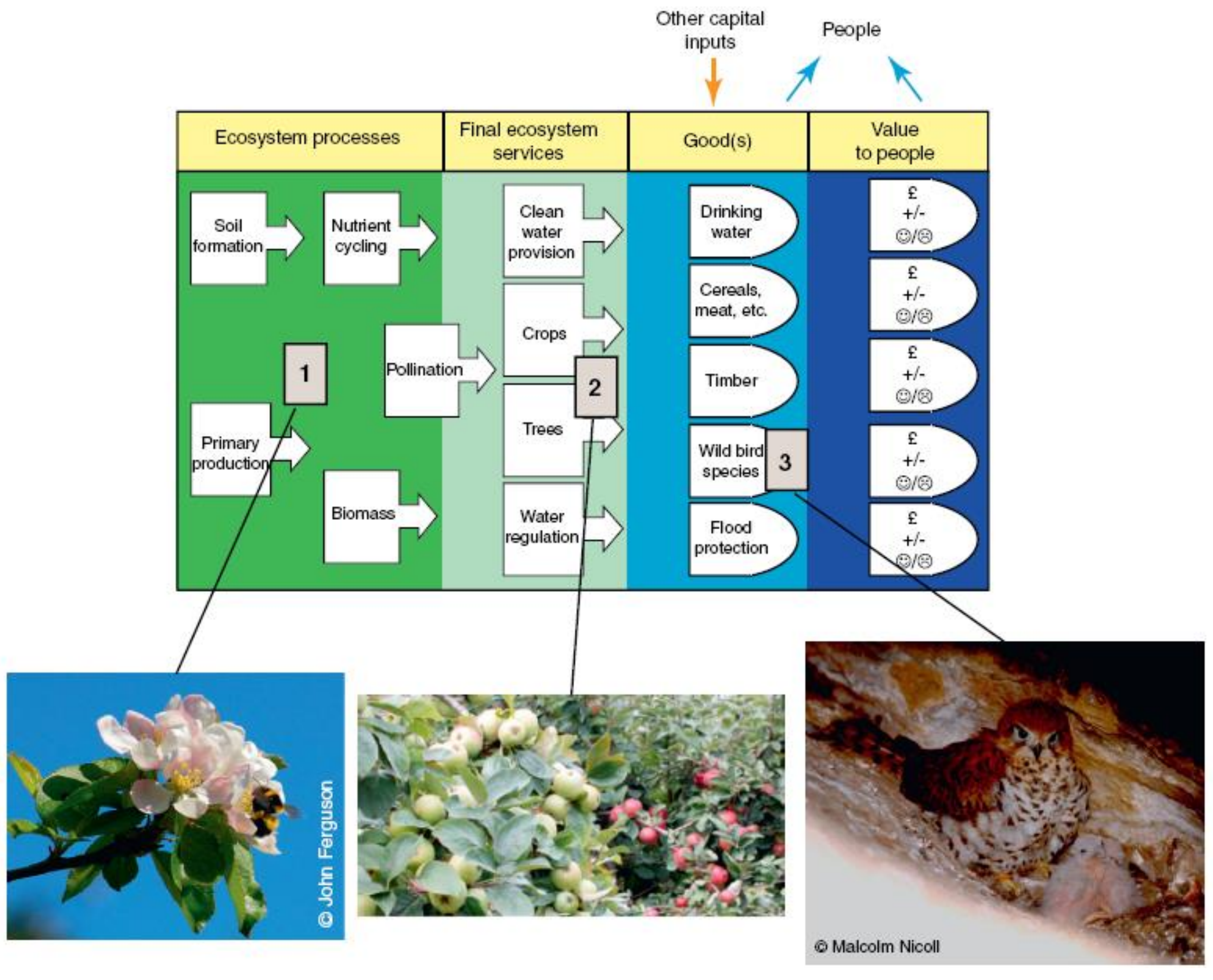

Figure 2. Graphical representation (from Mace et al. 2012) of ecosystem service "chains," or the flow from nature and its inherent processes through intermediate ecosystem services to final ecosystem goods and services and associated values.

Table 3. Examples of ecosystem service chains that could be explored through future research to understand the effects of European frog-bit on ecological processes, ecosystem services, and human values.					
Ecosystem Feature	**Intermediate Service I**	**Intermediate Service II**	**Intermediate Service III**	**Final Service of Good**	**Human Values**
Macrophytes	Substrate for macro-invertebrates	Prey items for fish	Fish	Sport fishing, Food	Recreation, Economic value
Macrophytes	Substrate for macro-invertebrates	Prey items for waterfowl	Waterfowl	Hunting, Food, Wildlife watching	Recreation, Economic value
Macrophytes	Food for waterfowl	---	Waterfowl	Hunting, Wildlife watching	Recreation, Economic value
Open water	Space for boating	---	---	Boating	Recreation, Economic value
Open water	Shallow open water for swimming	---	---	Swimming	Recreation, Economic value

High Priority Objectives

As described above, much remains unknown as to how wetland ecosystems respond to invasion by EFB, and more broadly, aquatic invasive plant species. To address the many knowledge gaps associated with EFB and its influence on the ecosystems in which it occurs, we suggest five research objectives to improve our ability to manage EFB and ameliorate potential impacts. These objectives may change over time as we gain input from other partners and learn more about the species and systems. Although some knowledge gaps could be reduced in a short time (e.g., < 5 years), other questions will probably require long-term study (e.g., 5-10 years or more). These objectives are unlikely to be accomplished by one project or organization. We expect multiple projects and partners will be required to accomplish this ambitious set of objectives.

1. <u>Compare plant and animal communities between wetlands with and without EFB populations</u>: In addition to gaining a better understanding of the potential effects of EFB on native plant species, we recommend studying EFB's influence on ecologically and economically important faunal groups, with a focus on those species/groups most likely to be affected by EFB. At a minimum, we suggest the following animal groups be examined: macroinvertebrates, fish, amphibians (e.g., anurans), reptiles (e.g., turtles), and birds (e.g., waterfowl, waterbirds, and shorebirds). Potential plant variables to be compared include species richness, diversity indices, coefficients of conservatism, and floristic quality index. Animal variables could consist of abundance, occupancy, species richness, and diversity indices.

2. <u>Compare measures of ecosystem processes between wetlands with and without EFB populations</u>: By comparing metrics that serve as indicators of wetland functioning, we will improve our understanding of potential changes to processes caused by EFB infestation. The following variables could be used as indicators of productivity, hydrology, and nutrient cycling: vegetation structure (e.g., mat depth, plant life forms, aquatic macrophyte biomass); soil conditions (e.g., type, organic layer depth); water

depth, temperature, and chemistry (e.g., DO, pH, conductivity, alkalinity); and nutrient levels (nitrogen and phosphorus).

3. Examine associations between ecosystem variables and EFB density and patch size: Data collected to achieve Objectives 1 and 2 could be used to explore if EFB density or patch size is associated with ecosystem metrics, such as plant and animal species/community indices, water chemistry, and nutrient levels. Potential relationships could be investigated using multivariate analyses (e.g., nonmetric multidimensional scaling) and modeling techniques (e.g., occupancy, logistic regression).

4. Assess if EFB populations interfere with recreational use of wetlands: Determining if and how EFB impacts human values is an important information need. The wetlands with growing EFB populations serve as important areas for economically and culturally important activities, such as fishing, hunting, and boating. Much could be learned by developing and implementing targeted surveys (e.g., online, mail) and/or conducting interviews to understand stakeholders' knowledge about EFB and real/perceived impacts.

5. Evaluate if EFB is affecting ecosystem services: Information gathered to address Objectives 1-4 will allow researchers to assess the real or potential impacts to ecosystem services. The wetlands in which EFB occurs provide several key services that should be evaluated: maintenance of biodiversity; provision of fish and wildlife habitat; protection of rare species; maintenance of water quality; and recreation.

Key Design Elements

In developing projects to investigate the proposed research objectives described above, we suggest several elements be incorporated into the study designs. These design elements will help account for the inherent variation encountered in ecosystems, address important knowledge gaps, and strengthen our ability to detect patterns and apply the results. An example study design incorporating many of these elements is provided (Table 4).

- Reference Sites: Reference sites lacking EFB should be a part of study designs for projects aiming to understand the impacts of EFB on organismal communities and ecological processes. Ideally, reference sites would represent naturally functioning systems relative to areas infested by EFB. In practice, we may be left with sites offering the best approximation of a natural reference due to high levels of degradation. For example, high levels of eutrophication and degradation by other invasive species (e.g., common reed, Eurasian watermilfoil, common carp [*Cyprinus carpio*]) could make finding adequate reference sites difficult in places like western Lake Erie and Saginaw Bay. As much as possible, the number of EFB and reference sites should be balanced within and among study areas.

- Gradients of Invasion: In addition to including sites lacking EFB, sampling across gradients of EFB invasion (e.g., low to high density, small to large area, early to long-term establishment) would help to understand the effects of EFB and potentially determine thresholds at which measurable ecological changes occur. These gradients could be incorporated into the design at both large and local spatial scales. At the large spatial scale, we recommend the design include study areas representing the range of EFB occurrence, such as wetland complexes with high (e.g., western Lake Erie, St. Clair Flats), moderate (Saginaw Bay), and low (Munuscong Bay) levels of EFB (or time since population establishment). If feasible, sites within these larger wetland complexes could span the range of EFB density (e.g., low, medium, and high density) and/or patch size (e.g., small, medium, and large areas).

- Wetland Types: Whenever feasible, study designs should include sites representative of the range of wetland types in which EFB occurs. Although most established populations are presently in Great Lakes coastal wetlands, EFB now occurs in several inland sites (Figure 1). Coastal marshes function differently than inland lakes and ponds, so sampling should be done in both coastal and inland sites to understand EFB impacts. Reference sites should be selected to represent the same set of wetland types. For example, if a study was to examine both coastal and inland EFB sites, then the sample design should also include both coastal and inland references sites (Table 4).

- Spatial and Temporal Replication: Given the inherent high variation across locations in ecosystem measures, such as species abundance/diversity and water chemistry, we recommend sampling the maximum number of sites possible within each wetland category (e.g., EFB, reference, coastal, inland) given funding and logistical constraints. Ecological variables can also vary considerably over time, especially in wetland ecosystems that regularly experience short- and long-term water level fluctuations. Sampling the same sites over multiple years would help account for this variation, and we suggest studies of 2-3 years should be viewed as the minimum length of time for ecological research.

- Species, Processes, and Services: We suggest a powerful study design that combines measures of plant and animal species, ecosystem processes, and ecosystem services/values. This approach would help to not only understand potential ecological changes associated with EFB invasion but also subsequent impacts (negative or positive) to ecosystems services and human values. An understanding of both is needed to make informed decisions about management strategies and resource allocation. Following the recommendations of Ruiz-Jaen and Aide (2005) would ensure that elements of ecosystem diversity, structure, and processes are incorporated into the study design. Investigations into the effects to ecosystem services (e.g., predictive models) and/or human values (e.g., valuation analyses, stakeholder surveys) could then be layered onto the design developed to understand species and processes.

Table 4. Example study design to assess the effects of European frog-bit (EFB) on wetlands in Michigan that considers spatial distribution, level of infestation, and landscape context.

Study Area	Landscape Context	Level of EFB Infestation	Wetland Type	No. Sites
Western Lake Erie	Coastal	High	EFB	3
			Reference	3
Saginaw Bay, Lake Huron	Coastal	Moderate	EFB	3
			Reference	3
Munuscong Bay, St. Mary's River	Coastal	Low	EFB	3
			Reference	3
Fletcher Pond, Alpena County	Inland	High	EFB	3
			Reference	3
Small Ponds, Oakland County	Inland	Low	EFB	3
			Reference	3

Potential Sampling Methodologies
Below we provide guidance on sampling methodologies to employ when addressing the high priority research objectives (Table 5). Several sampling methods can be used to address multiple objectives. We suggest using standard methods employed in past/ongoing research and monitoring efforts in the Great Lakes region whenever available because comparisons with other locations or time periods (e.g., before EFB introduction) may be possible. For some variables, multiple suitable methods are available, whereas for other sampling, new protocols may need to be developed.

1. <u>Compare plant and animal communities between wetlands with and without EFB populations</u>: *Vegetation*) No standardized protocol is readily available to assess the influence of EFB on plant diversity, so it is likely a new method or protocol used for similar research will need to be implemented. Aspects of several protocols/studies could inform the development of the methodology, such as the protocol being developed to evaluate EFB management in Michigan (CMU 2019), vegetation sampling done as part of the GLCWMP (Uzarski et al. 2017), or studies related to management of Great Lakes coastal wetlands (e.g., Herrick and Wolf 2005, Monfils et al. 2014). As estimating plant diversity is part of this objective, we suggest an area-proportional method in which level of sampling is scaled to the size of the site (e.g., EFB or wetland patch). Hackett et al. (2016) demonstrated the use of an area-proportional design in their study of plant diversity in prairie fens.

   Macroinvertebrates) We recommend sampling macroinvertebrates using the dip net sweeping technique used by the GLCWMP (Uzarski et al. 2017) within aquatic bed zones with and without EFB. Sampling should occur during mid-June to early September, with southern Michigan being sampled first and moving northward with phenological changes (Uzarski et al. 2017).

   Fish) If possible, fish could be sampled using fyke nets according to Uzarski et al. (2017). However, dense aquatic vegetation (e.g., EFB mats, submersed aquatic vegetation) could make the use of fyke nets infeasible. Sampling with small minnow traps and/or an electroshocker (see Johnson 2018) may be feasible alternatives to overcome logistical challenges of dense aquatic vegetation.

   Herptiles) To assess and determine the impacts of EFB on amphibian and reptile diversity and relative abundance, we recommend focusing surveys primarily on frog, toad, and turtle species. These groups are most likely to co-occur with and be impacted by EFB. There are 8-10 species of frogs and toads and about 7 species of turtles within the range of EFB in Michigan. We recommend nighttime auditory surveys (Luhring 2013, Uzarski et al. 2017), basking surveys (Buhlmann 2013), and aquatic funnel trapping (Willson 2013) to sample these herptile groups. Auditory point counts would survey calling male frogs and toads at both EFB and reference wetlands to assess species richness and relative abundance, with three surveys being conducted during March – July. Point count locations should be at least 500 m apart (Uzarski et al. 2017). Three basking surveys should be done during the same time periods as auditory surveys (March – July) at each demarcated EFB area and reference site (i.e., aquatic bed wetland lacking EFB). Basking surveys involve scanning with binoculars/spotting scopes to identify and count basking reptiles and amphibians. Aquatic funnel trapping would be conducted to assess turtle diversity and relative abundance at reference and EFB occupied sites but may also capture snakes and amphibians. The number of traps in a wetland will vary based on wetland area, shape, accessibility, and habitat heterogeneity.

Table 5. Potential sampling methodologies to address proposed European frog-bit research objectives. Methods applicable to a given objective are indicated with an "X." When available, sources and citations for recommended protocols are provided.

Variable Group	Method Type	Source(s)	Citation	Objective 1 (plants and animals)	Objective 2 (ecosystem processes)	Objective 3 (associations w/ EFB)	Objective 4 (recreational value)	Objective 5 (ecosystem services)
Anurans	Frog and toad call surveys	Partners in Amphibian Reptile Conservation	Luhring 2013	X		X		X
		Marsh Monitoring Program/GLCWMP	Uzarski et al. 2017	X		X		X
Birds	Visual surveys of aquatic bed zones	Integrated Waterbird Management and Monitoring Initiative	Loges et al. 2017	X		X		X
	Visual surveys of aquatic bed zones	MNFI	Monfils et al. 2014	X		X		X
	Point counts	Marsh Monitoring Program/GLCWMP	Uzarski et al. 2017	X		X		X
		North American Marsh Bird Monitoring Protocols	Conway 2011	X		X		X
Fish	Fyke nets	GLCWMP	Uzarski et al. 2017	X		X		X
	Minnow traps	---	---	X		X		X
Macroinvertebrates	Dip net sweeps	GLCWMP	Uzarski et al. 2017	X		X		X
Vegetation	Plant diversity transect-quadrat sampling	EFB Standard Treatment Impact Monitoring Protocol	CMU 2019	X		X		X
		GLCWMP	Uzarski et al. 2017	X		X		X
		Prairie Fen Research Collaborative	Hackett et al. 2016	X		X		X
Recreation value	Stakeholder survey	---	---				X	X

Table 5. Continued.

Variable Group	Method Type	Source(s)	Citation	Objective 1 (plants and animals)	Objective 2 (ecosystem processes)	Objective 3 (associations w/ EFB)	Objective 4 (recreational value)	Objective 5 (ecosystem services)
Soils	Organic soil depth	GLCWMP	Uzarski et al. 2017		X	X		X
Turtles	Aquatic funnel trapping	Partners in Amphibian Reptile Conservation	Willson 2013	X		X		X
	Basking surveys	Partners in Amphibian Reptile Conservation	Buhlmann 2013	X		X		X
Water	Chemical and physical variables	GLCWMP	Uzarski et al. 2017		X	X		X
	Nutrients (N and P) levels	GLCWMP	Uzarski et al. 2017		X	X		X
	Water flow	---	---		X	X		X

Birds) Because EFB often occurs near the edge of and interspersed with emergent vegetation, we recommend two survey techniques be used: point counts for emergent zones, and visual surveys for aquatic bed zones. Point counts target breeding marsh birds (i.e., bitterns, rails, grebes) and employ electronic call broadcasts to elicit responses from secretive species. Two methods have been used in the Great Lakes region, Marsh Monitoring Program/GLCWMP method (Uzarski et al. 2017) and the North American Marsh Bird Monitoring Protocol (Conway 2011, Michigan Bird Conservation Initiative 2015). Although the two methods are similar, they have differing sample designs, survey frequencies, and point count durations. Researchers should weigh the costs and benefits of each and select the one best suited to the study objective. Timed-area surveys, or visual surveys of defined areas of aquatic bed wetlands, would be an important method to assess direct use of areas with and without EFB. The Integrated Waterbird Management and Monitoring Initiative uses visual surveys to document nonbreeding bird use of wetlands (Loges et al. 2017). Similar visual surveys were used to examine breeding and nonbreeding bird use of coastal wetlands in Michigan (Monfils et al. 2014, 2015). We recommend conducting visual surveys during both breeding and nonbreeding periods (i.e., spring and fall migration).

2. <u>Compare measures of ecosystem processes between wetlands with and without EFB populations</u>: *Water Chemistry*) To assess potential EFB effects to ecosystem processes, we suggested measuring the following parameters in the field using a water quality sonde according to Uzarski et al. (2017): temperature, dissolved oxygen (especially at peak of EFB senescence), chlorophyll, oxidation-reduction potential, total dissolved solids, turbidity, pH, and specific conductance. Measurements should be taken at the same locations as organismal surveys (i.e., plants, inverts, fish, herps, and birds). Water samples should be collected at the same sites for nutrient (nitrogen and phosphorus) and alkalinity analysis according to Uzarski et al. (2017).

   Water Movement) EFB does not occur in running water in its native range, so factors such as water level fluctuation, flow, and fetch could influence where EFB occurs, its density, and ecosystem impacts. Water dynamics could be explored by examining Great Lakes water level fluctuations, landscape position, and potentially in situ measurements of water levels and flow (e.g., pressure/acoustic sensors).

   Physical and Structural Variables) We recommend the following physical and structural variables be measured while conducting vegetation sampling: water depth, organic soil depth (Uzarski et al. 2017), EFB mat depth, light penetration (photosynthetically active radiation), and macrophyte wet weight biomass (EFB and other species; Bickel and Perrett 2016).

3. <u>Examine associations between ecosystem variables and EFB density and patch size</u>: The same methods described for Objectives 1 and 2 will be used to accomplish this objective. Species richness and diversity measures are positively associated with patch size and survey effort, so proportional sampling designs are warranted when assessing the influence of patch size on ecosystem impacts.

4. <u>Assess if EFB populations interfere with recreational use of wetlands</u>: Research to understand the impacts of EFB on recreational values of wetlands will consist of online/written surveys of stakeholders to gain information about their knowledge of EFB and perceived/real effects to their use of wetland resources (e.g., Bremner and Park 2007, Andreu et al. 2009, Kapler et al. 2012). This is an area where development of new

methodologies would be necessary but could be based on approaches used in other applications, such as roving creel surveys, stakeholder interviews, and stakeholder mail surveys (e.g., Malvestuto et al 1978, Kozfkay and Dillon 2010). It may be possible to use the findings from such a study to evaluate potential impacts to recreational use of frog-bit impacted areas, especially inland waters. Evaluation can be based on previous studies relating local recreation models to ecosystem services (e.g., Kovacs 2012) and scenario analysis as has been done for hydrilla and Great Lakes recreational fishing (Lauber et al. 2016).

5. <u>Evaluate if EFB is affecting ecosystem services</u>: To accomplish Objectives 1-4, analyses will be required to assess potential changes to plant and animal communities, indicators of ecosystem processes, and human values. We suggest using the results of these analyses to perform a process similar to an ecological risk assessment to evaluate how ecological changes caused by EFB could result in altered services. The first step in this process would be to develop a conceptual model describing ecosystem service chains (i.e., relationships among ecosystem processes, services, and human values), connections with EFB, and associated potential effects to services and values. Connections between EFB and native species/processes could then be further described with knowledge gained from Objectives 1-4 (e.g., changes to native plant cover/diversity, macroinvertebrate abundance/diversity, invertebrate predators, etc.) and remaining areas of uncertainty identified. After describing the connections among EFB, species, and processes, potential changes to services and values (e.g., recreation) could be better assessed.

Summary

In this project, the MNFI set out to assist CMU in developing its adaptive management plan by gathering and compiling EFB occurrence data, synthesizing information about the effects of EFB and other aquatic invasive plant species on native species and ecosystem functioning, identifying important knowledge gaps, and developing a research framework to address those information needs. We worked with CMU to obtain 8,214 records of confirmed EFB status (i.e., present or absent) in the U.S. and Canada, of which 3,916 (48.2%) were from Michigan. These occurrences were aggregated into a common data set and an ArcMap shapefile was created for those records having spatial information.

We searched the literature for information about the potential effects of EFB and other aquatic invasive plant species on native plant and animals and ecosystem processes. Some studies indicated EFB and other aquatic invasive plant species alter plant, invertebrate, and fish populations (Catling et al. 1988; Zhu et al. 2014, 2015; Halpern 2017), although the patterns were not consistent among projects. There is little information about the potential impacts to vertebrates other than fish (e.g., amphibians, reptiles, birds, mammals). Several studies indicated mats of EFB or other aquatic invasives created low oxygen conditions (Catling et al. 2003; Urban et al. 2006, 2009; Zhu et al. 2008; Johnson 2018), but investigations of other changes to ecosystem processes are lacking. Based on our literature search, we identified several information needs hindering our understanding of the impacts of EFB on wetland ecosystems: effects of EFB on other organisms, effects of EFB on ecosystem processes, influence of EFB density and patch size, interaction of EFB with other invasive species, conditions driving EFB occurrences, ecosystem resiliency to invasion, and effects of EFB to human services. Five high-priority research objectives were developed to address these knowledge gaps: 1) compare plant and animal communities between wetlands with and without EFB populations; 2) compare measures of ecosystem processes between wetlands with and without EFB populations; 3) examine associations between ecosystem variables and EFB density and patch size; 4) assess if EFB populations interfere with recreational use of wetlands; and 5) evaluate if EFB is affecting ecosystem services.

We suggested researchers consider incorporating several elements into their study designs to increase the likelihood of detecting patterns and ecosystem impacts. To assess the effects of EFB on native species and ecosystem functioning, study designs should include reference sites representing the best available examples of naturally functioning wetlands as a comparison to wetlands infested with EFB. Study sites should represent the range of the invasion gradient (i.e., from well-established, high density to recent, low density sites) and types of wetlands containing EFB (e.g., coastal and inland; marshes, ponds, and lakes). Environmental factors, species, and ecological processes can vary considerably over space and time, so sampling should be replicated spatially (e.g., across EFB distribution in Michigan) and temporally (e.g., over multiple years) to the greatest degree practical to account for natural variation. Finally, we suggest a strong study design would evaluate EFB effects to native species, ecological processes, ecosystem services, and human values, so that informed decisions about management and resource allocation can be made.

Several measures of plant and animal groups and ecological processes and associated sampling methodologies were recommended to address the proposed research objectives, with a focus on the use of standardized protocols when available. To evaluate potential effects of EFB on organisms at multiple trophic levels, we suggest sampling the plant, macroinvertebrate, fish, herptile (frogs, toads, and turtles), and bird (waterfowl, waterbirds, and shorebirds) communities. Sampling several indicators of ecological processes, such as water chemistry (e.g., DO, pH, nutrients), water movement (e.g., fluctuations, flow), and physical/structural variables (e.g., biomass, soils, light penetration), will facilitate understanding potential impacts to functioning. Measuring these communities and processes across EFB density and size gradients could allow the identification of EFB population thresholds (e.g., percent cover, area of patch) at which detrimental impacts to native species and normal ecosystem processes occur. Engagement with stakeholders through surveys and interviews would be necessary to understand the effects of EFB on human values, such as recreational use of wetlands. We recommend an analysis akin to ecological risk assessment to evaluate the effects of EFP on ecosystem services. This analysis would consist of a conceptual model of ecosystem processes, ecosystem services, and human values, and their connections with EFB. Knowledge gained from EFB research would be incorporated into the model by describing documented or predicted alterations to processes associated with EFB to allow assessment of changes to ecosystem services and human values.

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