State of Michigan's

Status and Strategy for European Water-Clover (*Marsilea quadrifolia* L.) Management

Scope

Marsilea quadrifolia L. (European water-clover, hereafter EWC) is native to Eurasia and has been documented in Africa, Australia, New Zealand, North America, and South America (Holm et al. 1997). Globally EWC is classified as a species of least concern, but at least 21 European countries and Japan list EWC as a vulnerable, threatened, or endangered species (Strat 2012). It is extinct from the wild in Germany, Poland, Spain, and Switzerland (Schneider-Binder 2014). Other countries in southeast Asia and the Mediterranean consider EWC a weed, especially in rice fields (Holm et al. 1997; Strat 2012). In North America where it is not considered a native species, it has been documented in the United States of America in Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Missouri, New Jersey, New York, North Carolina, Ohio, and Pennsylvania, and in Ontario, Canada (Johnson 1985a; Simpson et al. 2008; USDA 2018). It is on the Michigan "Watch List," a list of priority exotic species that pose an immediate and significant threat to Michigan's natural resources. This document was produced by Central Michigan University (CMU) and reviewed by Michigan Departments of Environmental Quality (MDEQ) and Natural Resources (MDNR) for the purposes of:

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

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

Figure 1. European water-clover (*Marsilea quadrifolia* L.) resembles a "four-leaf clover" with usually floating or emergent leaves. Photograph by A.A. Reznicek, courtesy of Michigan Flora Online (Reznicek et al. 2011)

Biology and Ecology

I. Identification

European water-clover is an aquatic, heterosporous fern (i.e. two types of spores), but it can also be found on wet

Figure 2. Sporocarps in water-clovers are found near the base of a leaf. Sporocarps in European water-clover (*Marsilea quadrifolia* L.) are often branched unlike all but one other species in the genus *Marsilea.* Photograph courtesy of University of Michigan Herbarium (MICH-V-1432876)

ground. Its leaves can float on the surface of the water or emerge above the water. Leaves branch from a rhizome (i.e., horizontal stem) and extend vertically 2 – 6.7 in (5.5 – 17 cm). Each leaf has four similar leaflets, each approximately 0.2 – 0.8 in (0.6 to 2 cm) long, resembling a four-leaf clover (Figure 1). The leaflets are blue-green with a whitish waxy coating (i.e., glaucous). Sporocarps form on a single branched or unbranched stalk found 0.5 in (1.2 cm) above where the leaf branches from the rhizome (Figure 2). Sporocarps are spherical to bean-shaped, approximately 0.2 in (4 to 5 mm) long, and can be pubescent (i.e., hairy) or glabrous (i.e., smooth or non-hairy; Johnson 1986; Holm et al. 1997; eFloras 2018). They contain both microspores (i.e., small, male spores) and megaspores (i.e., larger, female spores).

The four-leaflet arrangement, aquatic habitat, and heterospory distinguish EWC from most other plant species. In Michigan, there are no other species of the genus *Marsilea* that resemble EWC. In areas where other *Marsilea* spp. L. grow, EWC can be differentiated by its nearly glabrous (i.e., non-hairy) leaves and branched sporocarp stalks (Figure 2). Almost all other *Marsilea* spp. have unbranched sporocarp stalks (i.e., one sporocarp per stalk), sometimes with several stalks per leaf. The only other *Marsilea* spp. with branched sporocarp stalks is *M. macropoda*, which is densely pubescent (i.e., hairy) with a larger sporocarp, 0.2 to 0.4 in (6 to 9 mm) long (Johnson 1986; Holm et al. 1997; eFloras 2018).

II. Detection

In the Great Lakes region, EWC is found along the banks of slow-moving streams, ponds, and water gardens. It can grow in dense clumps or interspersed among other vegetation. European water-clover is found in shallow waters and can survive some water-level fluctuation (Stepán and Otahelová 1986; Kiran et al. 2007; Schneider-Binder 2014). Detection efforts are best conducted in summer and fall (Campbell et al. 2010). The four-leaflet arrangement, aquatic habitat, and sporocarps near the base of the leaves distinguish EWC from most all other plant species. Sporocarps are found on stalks near the base of the leaf, but they are not always present. Sporocarps typically develop after the leaves and do not mature until the leaf has withered for the winter (Johnson 1986).

Aerial imagery has been used with botanists or local experts to distinguish emergent and floating aquatic vegetation (e.g., Husson et al. 2013). Given the small size of EWC and its variable density, it may be difficult to detect with anything but imagery with fine spatial resolution (e.g., centimeters). Remote sensing detection would also be limited in its ability to distinguish EWC in mixed stands of other aquatic vegetation or along shores with tree canopies.

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). Little research has been conducted to identify species-distinguishable markers for the detection of this genetic material shed from aquatic plant species (e.g., Scriver et al. 2015; Fujiwara et al. 2016; Matsuhashi et al. 2016; Reef et al. 2017). Identifying such markers for aquatic non-indigenous species such as EWC has the potential to increase the efficacy of field detection and monitoring efforts during the growing season. It could improve the true-positive detection of EWC when it is growing among dense emergent and floating vegetation or in an accessible portion of a waterbody. Although few have reported difficulty in detecting EWC given its shallow water habitat and easily distinguishable features, this sampling approach could reduce the need for labor-intensive field surveys until after EWC was positively detected in an area.

III. Life History and Spread/Dispersal

As a fern, EWC disperses via spores. For the aquatic, heterosporous EWC, spores are contained in a reproductive structure called a sporocarp. Inside the sporocarp, both microspores and megaspores are produced. Sporocarps have two external cell layers and internal gelatinous tissue, which protect the spores from the environment and premature release in animal digestive tracts (Bloom 1955; Bloom 1961; Malone and Proctor 1965; Nagalingum et al. 2006). Thirty-two-year-old sporocarps harvested from herbarium specimens had 99.8% megaspore viability (Bloom 1955), spores from 58-year-old sporocarps have been viable (Kruep 1997), and some other species within the *Marsilea* genus had viable spores in sporocarps that were 100-years-old (Johnson 1985b). The sporocarps of EWC are similar in structure to other *Marsilea* spp. (Nagalingum et al. 2006), so it is likely that EWC sporocarps might have similar long-term viability.

Light is believed to trigger sporocarp germination, which typically occurs after the leaves have matured (Johnson 1986). The sporocarp itself doesn't fully mature until after the leaves have withered.

The microspores inside the sporocarp grew and matured into male gameophytes that shed sperm in water after approximately eight to nine hours after hydration of open sporocarps (Buchholz and Selett 1941; Kruep 1997). Water temperature 77 - 86°F (25 - 30°C) was optimal for sperm survival with sperm death at and above 95°F (35°C); light had no effect on sperm emergence; and sperm lived 2.5 - 4.5 hours (Kruep 1997). The megaspores grew and matured into female gameophytes within 15 hours after hydration of open sporocarps. Once the sperm fertilizes the female gametophyte, a new EWC fern will grow.

Species of *Marsilea* and of the closely related genus *Pilularia* were not believed to hybridize in nature (Buchholz and Selett 1941). The species tested had very different maturation times for the gameophytes and artificially fertilized gametophytes did not produce mature plants.

European water-clover can also reproduce asexually through fragmentation and shedding the apex of its rhizome. The rhizome apex shedding allows EWC populations to spread easily within connected water bodies. It is unknown how long rhizome fragments can survive desiccation, thus it is unknown what role asexual reproduction plays in long-distance dispersal of EWC (Johnson 1986).

There were only two documented differences in life history between EWC studied in its native and North American range: months of the yearly senescence and months sporocarps are likely found. The life history between its native and North American range were not contradictory, but the limited information had subtle differences in the two articles reporting such information: Kiran et al. (2007) in India and Johnson (1986) in North America. While observing macroinvertebrates on a lake in Karnataka, India, from August 2006 to January 2007, Kiran et al. (2007) recorded EWC presence from September to January, but it was possible that EWC was present before or after the study was conducted. In North America, EWC resprouted in April and senesced in November or December (Johnson 1986). Preserved specimen and human observation records support Johnson's statement. The majority of the occurrence records in the United States and Canada occur between April and early November, but these can be biased to when the weather was favorable (i.e., not winter; GBIF 2018; MISIN 2018). There are a few Michigan records collected in December, but these are missing leaves due to either the age of the specimen or senescence (i.e., 1981; CONN00004707). The Midwest Invasive Species Information Network (MISIN) and Global Biodiversity Information Facility (GBIF) also had records of detection of EWC in January and February, but these records were either missing collaborating herbarium images and label information or the observation had the same submission and observation date despite great distances between locations, thus dates may not be reliable.

In its native range Kiran et al. (2007) observed sporocarps from November to January. In North America, Johnson (1986) reported that EWC produced sporocarps from mid-June to mid-October. Of the 15 herbarium specimens collected in Michigan, only those from one collection event at the University of Michigan Matthaei Gardens in September 1972 bore recognizable sporocarps (UNCC_16452; UNCC_16453; UNCC_40755; UNCC_40757; UNCC_40756; UNCC_40758). One additional specimen collected in July had sporocarps, but it was cultivated in a greenhouse at Eastern Michigan University (EMC016443).

IV. Habitat

Native Range:

European water-clover is native to much of Europe and considered a weed in much of the rest of the world (Gupta 2011; Strat 2012; Schneider-Binder 2014). It is considered extinct from the wild in Germany, Poland, Spain, and Switzerland (Schneider-Binder 2014) and at least 21 European countries and Japan list EWC as a vulnerable, threatened or endangered species (Strat 2012). Proposed threats to EWC included herbicide runoff (Aida et al. 2004; Luo and Ikeda 2007; Anwar Bhat 2013; Bruni et al. 2013; Popy et al. 2017), wetland drainage and loss (Schneider-Binder 2014), water eutrophication (Schneider-Binder 2014; Bolpagni and Pino 2016), change in fishpond and farming management (Nemoto and Otsuka 2011; Schneider-Binder 2014; Luo et al. 2017), and rise in muskrat populations (Schneider-Binder 2014). Other countries in southeast Asia and the Mediterranean consider EWC a weed, especially in rice fields (Holm et al. 1997; Strat 2012).

In its native range, EWC was found in slow-moving waters like oxbow lakes, floodplains of major rivers, stream banks, flood channels, ditches, man-made fishponds, clay-pits, gravel pits, pig pastures, rice fields, hemp- and flax-steeping places, and water bodies polluted with heavy metals (Ahmad et al. 2010; Strat 2012; Schneider-Binder 2014). It had also been found to tolerate light to medium saline conditions (Kiran et al. 2007; Schneider-Brinder 2014). European water-clover grew in water bodies near neutral pH (6.9 – 8.0; Stepán and Otahelová 1986; Kiran et al. 2007; Ahmad et al. 2010).

The areas inhabited by EWC had a variety of soil-types and nutrient levels (i.e., oligotrophic to eutrophic), although EWC growth peaks under intermediate nutrient enrichment (Stepán and Otahelová 1986; Schneider-Binder 2014; Bolpagni and Pino 2016). The nutrients nitrate, potassium, and phosphate were relatively low in the water where EWC grew (0.09 – 0.5 ppm, 12 - 31.5 ppm, 0.1 - 0.9 ppm, respectively; Kiran et al. 2007), but some studies showed high nutrient concentrations of potassium, phosphorus, and magnesium in the soils (250 ppm, 55 ppm, 250 ppm, respectively; Stepán and Otahelová 1986). One study that examined water chemistry found upwards of 100x greater nutrient and mineral levels in wastewater where EWC was growing, so those were not listed (Ahmad et al. 2010).

Native populations were usually in sunny and/or recently disturbed areas, and the water was deficient in calcium carbonate (Schneider-Binder 2014). The shade-intolerance lent to the impression that EWC was a pioneer species of aquatic or muddy habitats (Johnson 1986; Schneider-Binder 2014). The lower temperature threshold for EWC growth was -22°F (-30°C; Kaminski 2012 cited in Janiak et al. 2014).

Seasonally fluctuating water levels also played a critical role in the life history of EWC in its native range (Stepán and Otahelová 1986; Kiran et al. 2007; Schneider-Binder 2014). It was found ranging from the shoreline to a maximum water depth of 20 in (0.5 m; Stepán and Otahelová 1986). As the water-level fluctuated throughout the season, so did EWC ability to build biomass. Plants with the greatest biomass were growing in areas that were on exposed soil up to 0.5 in (0.12 m) above the water level at some point in the growing season and were no deeper than 5 in (0.12) below the surface.

In fresh water conditions, EWC was reported to co-occur with water fern (*Azolla filiculoides* Lam.), floating fern (*Salvinia natans* (L.) All.), lesser centaury (*Centaurium pulchellum* (Sw.) Druce), brown flatsedge (*Cyperus fuscus* L.), water-wort (*Elatine triandra* Schkuhr), Canada waterweed (*Elodea canadensis* Michx.), reed mannagrass (*Glyceria maxima* (Hartm.) Holmb.), European frog-bit (*Hydrocharis morsus-ranae* L.), bulbous rush (*Juncus bulbosus* L.), common duckweed (*Lemna minor* L.), greater duckweed (*Lemna polyrhiza* L.), star duckweed (*Lemna trisulca* L.), water mudwort (*Limosella aquatica* L.), false pimpernel (*Lindernia procumbens* (Krock.) Philcox), Eurasian water-milfoil (*Myriophyllum spicatum* L.), alkaline water-nymph (*Najas marina* L.), yellow water-lily (*Nuphar lutea* (L.) Sm.), European water-lily (*Nymphaea alba* L.), yellow floating-heart (*Nymphoides peltata* (S.G. Gmel.) Kuntze), common reed (*Phragmites australis* (Cav.) Trin. ex Steud), grass-leaf pondweed (*Potamogeton gramineus* L.), shining pondweed (*P. lucens* L.), floating pondweed (*P. natans* L.), American pondweed (*P. nodosus* Poir.), clasping-leaf pondweed (*P. perfoliatus* L.), floating crystalwort (*Riccia fluitans* L.), fringed heartwort (*Ricciocarpus natans* (L.) Corda), branched bur-reed (*Sparganium erectum* L.), water soldiers (*Stratiotes aloides* L.), water chestnut (*Trapa natans* L.), narrow-leaf cattail (*Typha angustifolia* L.), broad-leaf cattail (*Typha latifolia* L.), and bladderwort (*Utricularia australis* R. Br.; Strat 2012; Schneider-Binder 2014). Schneider-Binder (2014) claimed that American pondweed was competitive with EWC and threatened EWC populations. Their field observations did not seem to support this claim or a correlative decrease in EWC when American pondweed was present.

In slight to medium salinity conditions, EWC was found co-occurring with water fern (*Azolla filiculoides* Lam.), feathered mosquitofern (*A. pinnata* subsp. *africana* (Desv.) R.M.K. Saunders & K. Fowler), alligator-weed (*Alternanthera philoxeroides* (Mart.) Griseb.), *Bergia capensis* L., coontail (*Ceratophyllum demersum* L.), Bermuda grass (*Cynodon dactylon* (L.) Pers.), flat sedge (*Cyperus* spp. L.), hydrilla (*Hydrilla verticillata* (L. f.) Royle), swamp morning-glory (*Ipomoea aquatica* Forssk.), sea rush (*Juncus maritimus* Lam.), sea - avender (*Limonium meyeri* Kuntze), pennyroyal (*Mentha pulegium* L.), false pickerelweed (*Monochoria* spp. C. Presl), star lotus (*Nymphaea stellate* Willd.), denseflower knotweed (*Persicaria glabrum* (Willd.) M. Gómez), fleabane (*Pulicaria dysenterica* (L.) Bernh.), saltcedar (*Tamarix ramosissima* Ledeb.), strawberry clover (*Trifolium fragiferum* L.), and cattail (*Typha* spp. L.; Kiran et al. 2007; Schneider-Binder 2014).

Non-indigenous Range:

European water-clover was considered non-indigenous in Africa, southeast Asia, eastern Australia, Madagascar, Pacific islands, New Zealand, North America, and South America (Holm et al. 1997; GBIF 2018). The degree as to which this species is deemed a vulnerable species to be conserved, an inconvenient weed, or a threat to agriculture or native ecosystems differed from country to country. Based on performance, productivity, importance, and relative abundance, EWC was predicted to expand its distribution to disturbed sites and to be a moderate threat to Vietnamese ecosystems (Le and Truong 2016). Some documented reports of North American populations have described limited spread and aggression after decades of establishment (Burk et al. 1976; Henry 1983; Johnson 1985a). For the United States, EWC was classified as a high-risk species on the Notre Dame Risk Analysis Model due to its habitat range (e.g., shallows to land, water fluctuation), sporocarp persistence, dispersal possibilities, and lack of effective controls

(Gordon et al. 2012). In Minnesota it was ranked as only a moderate concern (Madsen 1999). Other North American herbarium records describe populations as vigorous, dense, or abundant (FSU151751; FSU151752; ILL00120234; MICH-V-1432860; MICH-V-1432870; MU-V-000050150; SWSL000149; TENN-V-0011669; TENN-V-0011671; UNCC_16452; UNCC_40757).

In North America, EWC is found in ponds, slow-moving river waters, marshes, wet ditches, and recently disturbed banks (Burk et al. 1976; Henry 1983; Johnson 1986; Serviss and Peck 2008). This species was found in shallow waters up to 4 ft (1.2 m) deep, but most herbarium records report depths less than 12 in (30.5 cm; CM533401; MICH-V-1105932; MICH-V-1105929; MICH-V-1432860; MICH-V-1432869; MICH-V-1432864; MSC0175089; MSC0175092; TENN-V-0011671; Johnson 1986; Cahill et al. 2018). Populations have been found in water bodies with variable pH and alkalinity (i.e., low, neutral, high; Johnson 1986; MICH-V-1432867; ILL00120234; MIN1189166). Little else has been documented about the habitat of EWC in its non-indigenous range that differs from its habitat in its native range.

Associated species documented in North America include buttonbush (*Cephalanthus occidentalis* L.), *Ceratophyllum* spp. L., coontail (*Ceratophyllum demersum* L.), water-hyacinth (*Eichhornia crassipes* (Mart.) Solms), Brazilian elodea (*Egeria densa* Planch.), *Elodea* spp. Michx., water-thyme (*Hydrilla verticillata* (L.f.) Royle), common duckweed (*Lemna minor* L.), water-purslane (*Ludwigia palustris* (L.) Elliott), marsh-dayflower (*Murdannia keisak* (Hassk.) Hand.-Mazz.), Eurasian water-milfoil (*Myriophyllum spicatum* L.), arrow-arum (*Peltandra* spp. Raf.), arrow-arum (*Peltandra virginica* (L.) Raf. ex Schott & Endl.), swamp smartweed (*Persicaria hydropiperoides* (Michx.) Small), arrowhead (*Sagittaria* spp. L.), giant bur-reed (*Sparganium eurycarpum* Engelm.), greater duckweed (*Spirodela polyrhiza* (L.) Schleid.), cattail (*Typha* spp. L.), and Brazilian watermeal (*Wolffia brasiliensis* Wedd.; Serviss and Peck 2008; Simpson et al. 2008; EMC016442; GA001688; GA001689; MICH-V-1105929; MICH-V-1105932; MICH-V-1432879; MSC0175092; MU-V-000109663; MU-V-000041610; MUHW000463).

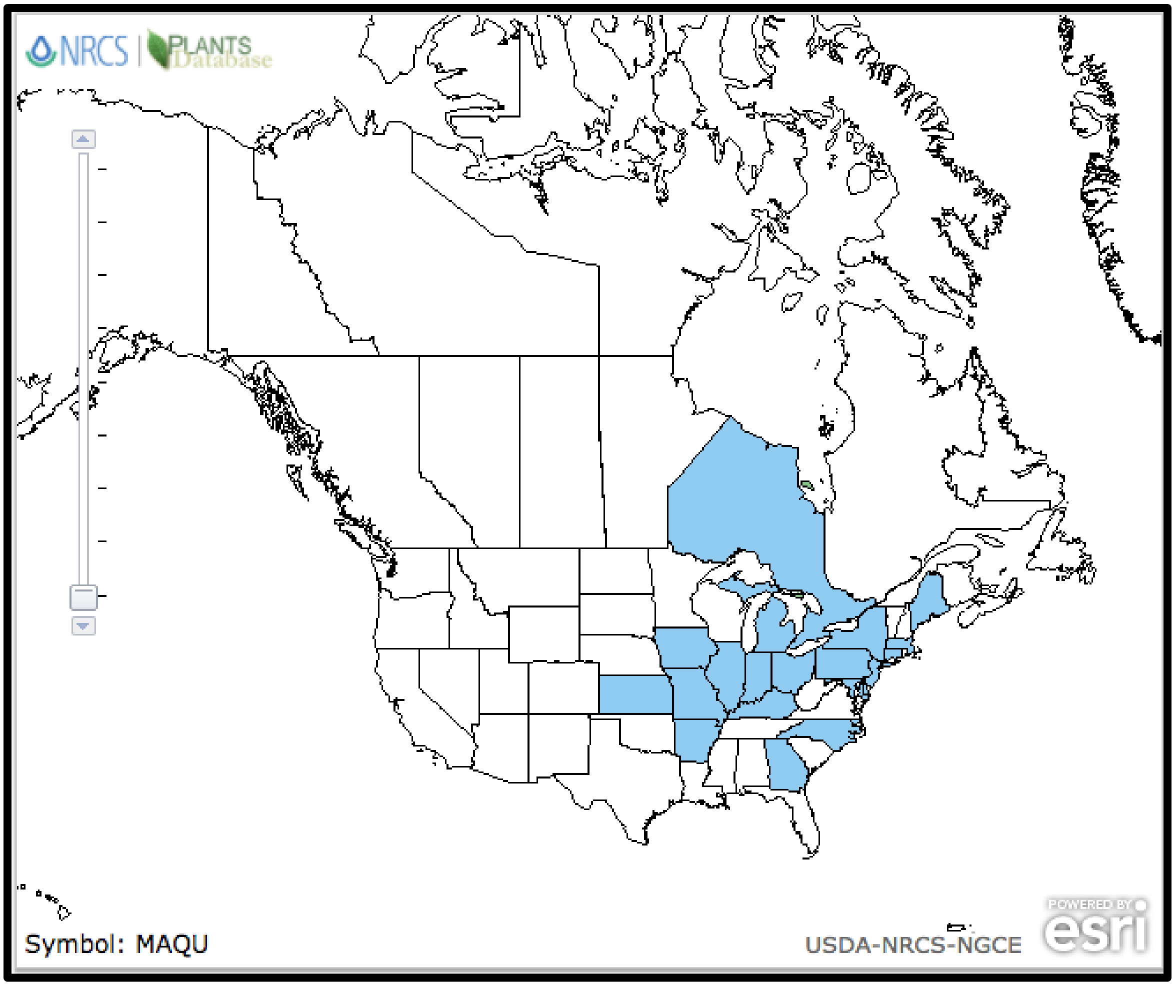

Figure 3. Distribution of European water-clover (*Marsilea quadrifolia* L.) in North America. Populations were reported in Arkansas and Kansas but are not represented on the USDA Plants map (Johnson 1985a; Simpson et al. 2008). Map based on USDA PLANTS Database (2018)

V. Effects from EWC

Few studies have examined the ecological, social, or economic impacts of non-indigenous EWC. Risk analysis for the Great Lakes region, Minnesota, and Vietnam urged moderate to major concern for EWC invasiveness (Madsen 1999; Gordon et al. 2012; Le and Truong 2016). The majority of EWC impact reports are based on anecdotal observations. No published studies could be found examining the effects of EWC on competition with native species, food web dynamics, fish habitat, nutrient cycling, water chemistry, or commercial and recreational water use.

a. Negative Effects

In North America, some herbarium labels and local reports have reported dense or abundant EWC populations in shallow waters or newly disturbed areas, but the effects of these populations on native species or ecosystem functions have not been quantitatively explored (Burk et al. 1976; Henry 1983; Johnson 1985a; FSU151751; FSU151752; ILL00120234; MICH-V-1432860; MICH-V-1432870; MU-V-000050150; SWSL000149;

TENN-V-0011669; TENN-V-0011671; UNCC_16452; UNCC_40757). Studies in rice fields attribute some decrease in grain generation to weeds including EWC (Anwar Bhat et al. 2013; Hossain et al. 2017; Nivetha et al. 2017; Popy et al. 2017; Ramesh et al. 2017). Unfortunately these studies pooled all weeds into the same category and did not examine effects of EWC individually on rice cultivars.

b. Positive Effects

European water-clover has been involved in studies examining sequestering of heavy metals by aquatic plants. In these studies, EWC showed bioaccumulation of some metals, especially cadmium and chromium. In a larger context, EWC accumulated less amounts than other plant species tested (Mishra et al. 2008; Rai 2009; Ahmad et al. 2010; Neha et al. 2017). It is unlikely that it would be the most efficient species to be chosen in a bioremediation effort.

In Asia some areas use EWC as a nutritious food or medicinal resource (Dewanji et al. 1993; Holm et al. 1997; Soni and Lal 2012; Kosaka et al. 2013; Sharma 2017; Vu and Nguyen 2017). Extracts from EWC are currently being studied for treatments of Alzheimer's disease, diabetes, epilepsy, and various cancers (Ashwini et al. 2012; Bhadra et al. 2012; Sahu et al. 2012; Snehunsu et al. 2013; Uma and Pravin 2013; Snehunsu et al. 2015; Sriranjini et al. 2015; Zhang et al. 2015; Chowdhury et al. 2017; Karikalan and Rajangam 2017; Maji et al. 2017).

Schievano et al. (2017) examined the use of EWC in a mix of plant species in floating experimental fuel cells. The cells that contained EWC were not considered suitable for long-term performance.

Current Status and Distribution in Michigan

The known range of EWC in North America is spotty in US states and Canadian provinces east of the Great Plains (Figure 3; USDA 2018). It is generally believed that the first introductions into North America were intentional and that some spreading has occurred from these populations (Burk et al. 1976; Henry 1983; Johnson 1986). The vector and agents of dispersal were not identified definitively. European water-clover has the ability to spread via rhizome fragments and sporocarps, which have been suspected to be dispersed via water currents, animal hitchhiking, or animal defecation.

There are EWC populations recorded in four Michigan counties: three counties in southeast Michigan and one in the northwest Lower Peninsula (Figure 5). The first documentation of EWC in Michigan was in Washtenaw County in southeast Michigan. It was found in the Huron River northwest of Ann Arbor in 1961 (MICH-V-1105931; Reznicek et al. 2011). In 1982, another collection was made from the Huron River at Barton Pond (EMC016442). Since then many occurrences of EWC have been recorded on three river miles of the Huron River in and between Barton Pond and Argo Pond, all in Washtenaw County northwest of Ann Arbor (MICH-V-1105932; GBIF 2018; MISIN 2018). These populations have been described in varying intensities throughout the ponds and across years (MISIN 2018). The Huron River flows to the southeast and empties into Lake Erie. In 2018, a population was documented in a pond in West Park, Ann Arbor (W. Keiper, Michigan Department of Environmental Quality, personal communication).

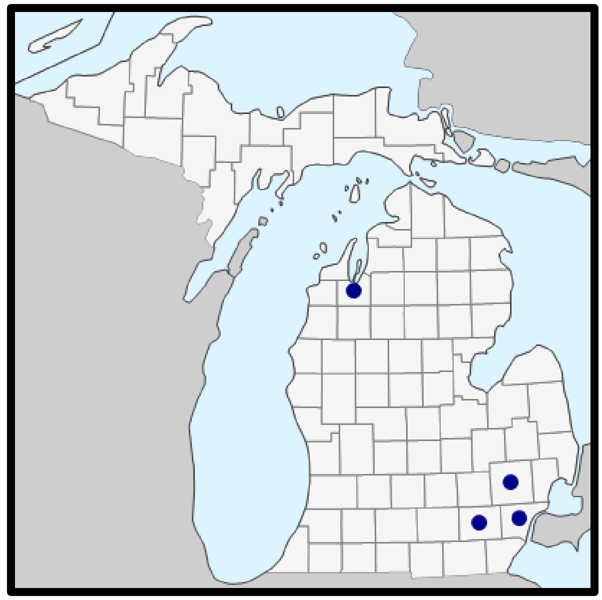

Figure 3. Blue dots indicate counties in Michigan where a specimen of European water-clover (*Marsilea quadrifolia* L.) has been collected and included in Michigan Flora. No documented observations have occurred outside of these four counties within Michigan. County map developed by Michigan Flora online (Reznicek et al. 2011)

Another population was recorded in Washtenaw County: in Willow Pond on Parker Brook near the visitor's center of University of Michigan's Matthaei Botanical Gardens. This population was recorded in 1972 and described as abundant (UNCC_16452), but has not been revisited and documented since. The Parker Brook is a tributary of Fleming Creek that flows into the Huron River southeast of Ann Arbor.

In Oakland County in southeast Michigan, another population was documented along six river miles of the Clinton River from an old MDNR fish hatchery, now Drayton Plains Nature Center, into Cass Lake since 2013 (MICH-V-1484590). With a few exceptions, the EWC along the Clinton River portion was described as sparse or patchy. In the canals where the Clinton River meets Cass Lake, the EWC population was described as dense, sparse, or patchy (MISIN 2018). In May 2017, EWC was reported in Lake Angelus, upstream from Drayton Plains Nature Center, but this occurrence has not been verified (Cahill et al. 2018; MISIN 2018). The Clinton River continues through Cass Lake and flows into Lake St. Clair. Targeted monitoring in 2014 – 2017 detected no other EWC occurrence in the Clinton River and Cass Lake system (Cahill et al. 2018; MISIN 2018).

In 2016, a population was recorded in Wayne County in southeast Michigan. A pond south of Van Horn Road west of where Silver Creek crosses Van Horn Road between Inkster and Arsenal contained a dense population of EWC 0.5 to 1 acre in size. The pond appeared to be self-contained, but was less than 100 ft (30.5 m) from Silver Creek, which flows into the Huron River approximately 1 - 2 miles (1.6 – 3.2 km) upstream from where the Huron River enters Lake Erie. No sporocarps were ever observed on these EWC, despite numerous visits (Cahill et al. 2018). The area was chemically treated in 2016 and 2017 and population has reduced in area (Cahill et al. 2018).

One specimen of EWC was collected from Grand Traverse County in the northwest Lower Peninsula. The EWC population was found in Sabon Pond on Boardman River in 1991 (MICH-V-1105929). No remark was made on the density of the population. An attempt to relocate the population was made in 2016 by the Michigan DNR, but it was not located (Cahill et al. 2016). The Boardman River flows into the Grand Traverse Bay of Lake Michigan.

There are several occurrences reported in the Great Lakes Basin outside of Michigan, but most of them have not been verified as occurring in these locations today: 1) a specimen at Thornden

Park, New York, in the Lake Erie Basin, 2) specimens collected near Cayuga Lake, Ithaca, New York, USA, a part of the Lake Ontario Basin, 3) literature documented occurrences in Lewiston near Niagara, New York, USA, 4) occurrences and specimens along Nanticoke Creek in Nanticoke, Ontario, Canada, within miles of Lake Erie, 5) a preserved specimen at Hurlburt's Pond, Lorne Park, Mississauga, Ontario, Canada, near Lake Ontario, and 6) a preserved specimen at Toronto, Ontario, Canada, near Lake Ontario.

The specimen at Thornden Park, New York, was collected in 1916 (DOV). The only surface water noted on maps and satellite images of Thornden Park is in lily ponds, which are not connected to any surface inlets or outlets. No recent occurrence reports have been made in this area.

The earliest of the Cayuga Lake, New York, specimens was collected in 1917 from Taughannock Falls, Ithaca, New York (v0305196WIS). It was collected again in 1932 and 1937 at the inlet of Cayuga Lake and Stewart Park on the lake, respectively (MIN353129; IAC0000001068; IAC0000001930; GBIF 2018). In 1952, more specimens were collected: at Renwick Sanctuary near the southern edge and inlet of Cayuga Lake and at a place described as Judd Falls pond, which is likely on the campus of Cornell University in East Ithaca (MIN568331; MIN463766; MIN439256). Cayuga Lake flows north through a series of rivers (e.g., Seneca, Oswego) and Cross Lake to Lake Ontario.

A book of Niagara, New York, flora documented that EWC was introduced into a pool near Lewiston, New York, but "did not establish itself" (Zenkert 1934). No recent occurrence reports have been made in this area.

Occurrences were recorded in Nanticoke Creek in Nanticoke, Ontario, Canada since 1951 to as recent as 2012 (CAN328506; CAN347019; MSC0175089; QFA0104595; QFA0230838; UTC00238814; UTC00270991; Miller 1956; GBIF.org 2018; MISIN 2018). All occurrences were described or geo-located as being downstream from the Nanticoke Union Cemetery and before entering Lake Erie.

In 1982, a preserved specimen was collected from the north shore of Hurlburt's Pond, north of the railroad and west of Lornewood Creek in Lorne Park, Mississauga, Ontario, Canada, near Lake Ontario (OAC63296). No recent occurrence reports have been made in this area.

A specimen was collected in 1911 at Toronto, Ontario, and deposited at the herbarium at the University of Toronto (TRTE) according to Miller (1956). The TRTE herbarium is only partially digitized and no further information was found. No recent occurrence reports have been made in this area.

On the US Geological Service Nonindigenous Aquatic Species webpage (http://nas.er.usgs.gov), there was a report of a specimen collected in Franklin County, New York, but the reference for the collection was the US Department of Agriculture's PLANTS Database (http://plants.usda.gov). The PLANTS Database had no occurrence marked in Franklin County. No other specimen record was found in any data aggregator or literature that could be linked to an occurrence in Franklin County.

Reports out of state populations that could disperse into the Great Lakes Basin were in Illinois in Clear Pond of Kickapoo State Park since 1951 (C0047095F; EIV019261; UNA00047520; GBIF2018), Illinois in Riley Creek between North County Road 1100E and where Riley Creek crosses West State Road since 2008 (MISIN 2018), Illinois in the Embrass River on the south boarder of Fox Ridge State Park since 2010 (FLAS236592; MO100756761; GBIF 2018), Ohio southeast of Columbus since 2012 (MISIN 2018), and many occurrences in western Connecticut (GBIF.org 2018; MISIN 2018).

Management of EWC

I. Prevention

European water-clover is considered a "Watch List species" in Michigan at the time of this document. It was not on the Prohibited, Restricted, or Noxious Weed lists under the Natural Resources and Environmental Protection Act 413/329 of 1994. As a species on the Watch List, it was considered to have a limited distribution in Michigan, but professionals and citizens were encouraged to report occurrences. A Watch List species does not always have restrictions or prohibition on the possession, introduction, importation, or selling of the species in Michigan. This is the status it shares in most other North American states and provinces (Cao and Berent 2018). Illinois and Connecticut have enacted stricter restrictions on the sale and purchasing of EWC (Cao and Berent 2018; USDA 2018).

Water garden and aquarium enthusiasts should purchase native or non-invasive species whenever possible. Unwanted plants should be disposed of properly and never released into the environment. The following actions may prevent and limit the dispersal of EWC:

- Classify EWC as a "restricted" or "prohibited species" in Michigan under the Natural Resources and Environmental Protection Act 413
- Build a coalition of local, state, and Great Lakes regional partners to monitor for EWC and other aquatic invasive species
- Identify and monitor waterbodies that have a high-risk of invasion using known distribution and dispersal knowledge
- Encourage water garden and aquarium enthusiasts to use native or non-invasive species
- Educate water garden and aquarium enthusiasts on the proper disposal of unwanted plants and waste
- Increase stakeholder awareness of available prevention and control methods
- Actively manage sites where EWC is found

II. Management/Control

A management strategy that incorporates ecological knowledge and several control 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.

Few studies have been conducted to evaluate control methods for EWC (e.g., Aida et al. 2004; Luo and Ikeda 2007; Anwar Bhat et al. 2013; Bruni et al. 2013; Nivetha et al. 2017; Popy et al. 2017) and most have had the purpose of conserving EWC or been focused on a unique weed management system for rice fields. Many EWC management recommendations are based on qualitative observations and are lacking untreated controls or pre- and post-treatment monitoring. Management of EWC is not a priority through much of its North American range. When EWC is managed, manual removal and chemical treatments are the most commonly used control methods. In Michigan, a population in a retention pond has been managed with chemical treatments (Cahill et al. 2018). The following is a summary of control methods tested to date and their results. Given the longevity of the sporocarps, treatment prior to sporocarp development is optimal, but may not be practical.

a. Chemical

There were two types of experiments that tested the impact of chemical treatments on EWC at the time of this report: 1) effectiveness of certain herbicides at controlling common rice field weeds, like EWC, without damaging rice production (Anwar Bhat et al. 2013; Popy et al. 2017), and 2) effects of herbicide runoff on growth of the vulnerable or endangered EWC in Eurasia (Aida et al. 2004; Luo and Ikeda 2007; Bruni et al. 2013; Nivetha et al. 2017). Although the intent of these studies may be different than that of this strategy, some of the information was usable and could guide future efficacy studies. Since these studies are conducted in EWC's native range or in rice fields, which are not common in Michigan, several of the herbicides that have been tested are not approved for aquatic use in Michigan (MDEQ 2015). Many of the herbicides that were approved for application in rice fields cannot be applied to any other aquatic system due to their toxicity to fish and macroinvertebrates (e.g., Crop Care Australasia Pty Ltd 2008; Dow ArgoSciences 2016). A summary of herbicide active ingredients that have shown some effectiveness at controlling EWC are in Table 1.

In Michigan, some chemical testing was conducted in a retention pond where EWC growth was dense. In 2016, glyphosate was used on emergent plants and penoxsulam was used on submerged plants. The chemical treatments were observed to have little effect and the population was considered dense again in 2017. In 2017, glyphosate was again used on emergent plants and imazamox on submerged plants. Regrowth has not yet been evaluated (Cahill et al. 2018).

Effective in the field – All field experiments described were conducted on pooled rice field weeds (Anwar Bhat et al. 2014; Nivetha et al. 2017; Popy et al. 2017). For these experiments, the relative density of EWC was less than 5% of the total vegetation in the field plot. The pooled nature of these experiments does not allow for a decisive answer on whether the herbicide was effective against EWC. It is possible that the herbicides were effective on all weeds except EWC. Regrowth the following season was not examined.

Penoxsulam was effective in controlling rice field weeds including EWC in field experiments (Anwar Bhat et al. 2013; Popy et al. 2017) and against EWC specifically in laboratory experiments (Bruni et al. 2013). Penoxsulam alone at 0.0179 – 0.0201 lbs of active ingredient (a.i.) acre^{-1} (20.0 – 22.5 g a.i. ha^{-1}) was 71.3 – 75.2% effective against rice field weeds, but it is important to note that hand pulling performed at almost the same level of effectiveness (69.0 – 70.3%; Anwar Bhat et al. 2013). Penoxsulam at greater dosage 0.0223 lbs a.i. acre^{-1} (25.0 g a.i. ha^{-1}) was approximately as effective as hand-pulling (67.0 – 71.0%).

Penoxsulam was also paired with pre-emergent pretilachlor or pendimthalin. While these treatments showed 64.7 – 73.9% and 63.5 – 71.4% control of rice paddy weeds, respectively, those treatments were not significantly different from the treatment of pre-emergent herbicide combined with one session of hand-pulling (68.1 – 77.0%, 65.6 – 73.2%, respectively; Popy et al. 2017). The effectiveness of penoxsulam may be no greater than that of hand-pulling.

Other herbicide treatments with the pre-emergent showed less, but significant control of rice paddy weeds: pretilachlor followed by 2,4-D dimethyl amine, pendimethalin followed by 2,4-D dimethyl amine. Note: liquid 2,4-D dimethyl amine in liquid form is prohibited from application in aquatic habitats in Michigan due to toxicity to non-target organisms (MDEQ 2006).

Treatments of bensulfuron methyl with pretilachlor at 0.054 + 0.54 lbs a.i. acre^{-1} (60 + 600 g a.i. ha^{-1}) followed by hand weeding several weeks after treatment was slightly more efficient (88.1 – 95.1%) than two hand weeding sessions at combating rice field weeds (85.6 – 92.3 %; Nivetha et al. 2017). Treatments of bensulfuron methyl with pretilachlor at the same dosage above followed by an application of 2,4-D sodium salt at 0.071 lbs a.i. acre^{-1} (80 g a.i. ha-1) several weeks after the first treatment were approximately as efficient as two hand weeding sessions (83.2 – 90.7%).

“Mustard crop residue” inhibited EWC growth from 83.98 – 91.73% at concentrations of 2.0 t ha^{-1} and provided 50% inhibition between 1.0 – 2.0 t ha^{-1} in rice fields (Hossain et al. 2017). Mustard crop residue was not described further than “crop or its parts left in field for decomposition after it has been thrashed or harvested,” so there may be repeatability issues. The low number of replicates (i.e., 3) was concerning to the repeatability and strength of the conclusions of the experiment as well.

Effective in the laboratory – The studies described in the laboratory section were all conducted for the purpose of determining EWC sensitivity to widely used herbicides, because it is a vulnerable species in those countries (Aida et al. 2004; Bruni et al 2013). The EWC was most sensitive to profoxydim (Aura) at 0.1 ppm, Aura with adjuvant Dash at 0.02 ppm, and Aura and Dash at 0.2 ppm (Bruni et al. 2013), exhibiting means of 82%, 85%, and 95% control, respectively (Bruni et al. 2013). Cyhalofop-butyl (Clincher) at 0.005 ppm provided 80% EWC control. Penoxsulam (Viper) provided 95% EWC control at its effective concentration of 20 ppm, but it was 50% or less effective at greater dilutions.

Bensulfuron-methyl also inhibited growth of EWC (Luo and Ikeda 2007; Aida et al. 2014). A concentration of 0.00067 lbs a.i. $acre^{-1}$ (0.75 g a.i. ha^{-1}) resulted in 40% control and concentrations 0.022 lbs a.i. $acre^{-1}$ (25 g a.i. ha^{-1}) and greater controlled resulted in 90% control (Aida et al. 2014).

Simetryn has also shown success to inhibit growth of EWC, but the results were not communicated in an easily comparable units or concentrations to other experiments (Luo and Ikeda 2007).

Ineffective trials – In rice field experiments, butachlor at 1.3 lbs a.i. $acre^{-1}$ (1500 g a.i. ha^{-1}) was 60.5 – 63.4% effective at controlling rice field weeds, but this was less effective than hand-pulling in the same field experiment (69.0 – 70.3%; Anwar Bhat et al. 2013).

In laboratory experiments, mefenacet and theiobencarb were shown to be effective at 6 and 50 µmol L^{-1} concentrations, but they were not as effective as the other two herbicides tested in the trials (i.e., bensulfuron-methyl, Simetryn; Luo and Ikeda 2007). Glyphosate at 10 ppm, iso-oxazolidinone (Command) at 0.5 ppm, sulfonylurea class (Gulliver) at 15 ppm, and dinitroaniline class (Most MC) at 20 ppm, were least effective at controlling EWC of those tested by Bruni et al. (2013) with mean survival rates of 38%, 35%, 47%, and 76%, respectively. The dosages tested were the suggested dose for applications to rice fields in the European Union.

Table 1. Summary of effective herbicide active ingredients for European water-clover (*Marsilea quadrifolia* L.; hereafter EWC) control to date. 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 Michigan	Listed on Label	Pros	Cons	References
Bensulfuron-methyl (Londax™)	No	No	• Effective in laboratory (90%) when applied at 0.022 lbs a.i. acre^{-1}, 7 days after EWC transplant • Less harm to non-target plant species (Selective herbicide)	• Has not been tested in field • Regrowth not examined • Permitted for aquatic use in only rice fields • Moderate toxicity to fish, algae • Resistance can be built • Application in water less than 70°F delays activity	(University of Hertforshire ND; MDEQ 2006; RiceCo LLC 2011; MDEQ 2015; Aida et al. 2004; Luo and Ikeda 2007)
Bensulfuron-methyl + pretilachlor (e.g. Londax™ Power)	No	No	• Effective in rice field (88.1-95.1%) when applied at 0.054 + 0.54 lbs a.i. acre^{-1}, 3 days after rice transplant • Slightly more efficient than hand weeding	• Regrowth not examined • Experimented in rice field • Results pooled with other weeds • Moderate toxicity to fish, aquatic invertebrates, algae	(MDEQ 2006; Du Pont 2015; MDEQ 2015; Nivetha et al. 2017)
Cyhalofop-butyl (e.g. Clincher®)	No	No	• Effective in laboratory (80%) when applied at 0.005 ppm, 4 days after EWC transplant	• Has not been tested in field • Regrowth not examined • Toxic to fish and macroinvertebrates • Permitted for aquatic use in only rice fields • May contaminate groundwater • May harm non-target plant species (Broad-spectrum, contact herbicide)	(MDEQ 2006; Bruni et al. 2013; MDEQ 2015; Dow AgroSciences 2016)
Imazamox + glyphosate (e.g. Clearcast® + AquaPro®)	Yes + Yes	Yes + No	• Possibly effective in pond in Michigan (anecdotal)	• Regrowth not yet monitored • Restricted concentration when near potable water intakes • Prohibited for use in water bodies < ½ mile from a potable water intake • May harm non-target species (Broad-spectrum, systemic herbicide)	(SePro 2016a; SePro 2016b; Cahill et al. 2018)
Mustard crop residue	No	NA	• Effective in rice field (83.98-91.73%) at 2.0 t ha^{-1}	• Residue not thoroughly described • Low number of replicates • Non-target effects not measured	(MDEQ 2006; MDEQ 2015; Hossain et al. 2017)

Herbicide	Approved in Michigan	Listed on Label	Pros	Cons	References
Pendimethalin (e.g. Drexel Aquapen®)	No	No	• Effective in rice field (59.5-73.2%) when applied at 0.071-0.76 lbs a.i. acre^{-1}, 2 days after rice transplant • Less harm to non-target plant species (Selective herbicide)	• Results pooled with other weeds • Used as pre-emergent with a post-control treatment (e.g., penoxsolum, 2,4-D amine, hand-pulling) • Regrowth not examined • Toxic to fish • Permitted for aquatic use in only rice fields • Does not control established weeds • Not listed on either the aquatic approved or not approved list for Michigan	(MDEQ 2006; Drexel Chemical Company 2010; MDEQ 2015; BASF 2017; Popy et al. 2017)
Penoxsulam (e.g. Viper® India)	Yes	No	• Effective in rice field (71.3-75.2%) when applied at 0.0179-0.0201 lbs a.i. acre^{-1}, 3-12 days after rice transplant • Effective in laboratory (95%) when applied at 20 ppm • Less harm to non-target plant species (Selective herbicide)	• Not effective in pond with glyphosate due to next season regrowth (anecdotal) • Results pooled with other weeds • Performed at almost same effectiveness as hand-pulling • Regrowth not examined • Post-treatment irrigation water restrictions	(Anwar Bhat et al. 2013; Bruni et al. 2013; MDEQ 2015; Popy et al. 2017; Cahill et al. 2018)
Pretilachlor	No	No	• Effective in rice field (88.1-95.1%) when applied at 0.071-0.54 lbs. a.i. acre^{-1}, 2 days after rice transplant • Less harm to non-target plant species (Selective herbicide)	• Results pooled with other weeds • Used as pre-emergent with a post-control treatment (e.g., penoxsolum, 2,4-D amine, hand-pulling) • Regrowth not examined • Moderate toxicity to fish, aquatic invertebrates, algae • Permitted for aquatic use in only rice fields	(University of Hertforshire ND; MDEQ 2006; MDEQ 2015; Popy et al. 2017)
Profoxydim (e.g. Aura®)	No	No	• Effective in laboratory (82-95%) when applied at 0.02-0.2 ppm, 4 days after EWC transplant • More effective with Dash™ HC adjuvant • Less harm to non-target plant species (Selective herbicide)	• Has not been tested in field • Regrowth not examined • Permitted for aquatic use in only rice fields	(BASF ND; MDEQ 2006; Crop Care Australasia Pty Ltd 2008; Bruni et al. 2013; MDEQ 2015)

Herbicide	Approved in Michigan	Listed on Label	Pros	Cons	References
Simetryn	No	No	• Effective in laboratory (significantly different from controls) when applied at 0.0004 – 40 μmol L^{-1}, when inoculated for 10 days • Less harm to non-target plant species (Selective herbicide)	• Has not been tested in field • Measured fresh weight (not dry) • Regrowth not examined • High toxicity to beneficial algae • Moderate toxicity to fish, aquatic invertebrates	(University of Hertforshire ND; Kasai and Hanazato 1995; MDEQ 2006; Luo and Ikeda 2007; MDEQ 2015)

b. Physical or Mechanical Control

Hand pulling weeds was as effective or more so than most chemical treatments in rice fields (Anwar Bhat et al. 2013; Popy et al. 2017). No other research has been conducted on any physical or mechanical control techniques. Given the preferred habitat of EWC, shading, dredging, or benthic barriers may reduce EWC populations.

c. Biological

No studies or observations were noted in the literature of any possible biological controls of EWC at the time of this report.

d. Indirect Management

No indirect management techniques have been investigated for the control of EWC at the time of this report. Given the shallow water habitat preferred by EWC, the prevention of soil erosion and sediment deposition during disturbances may reduce the likelihood of new or growing infestations, but research is needed.

Research Needs

I. Biology and Ecology

To date, only water depth, calcium concentration, percent organic matter, and nutrient concentrations/eutrophication have been studied in relation to EWC populations (Gopal 1968; Gopal 1969; Stepán and Otahelová 1986; Bolpagni and Pino 2016). A greater understanding of local characteristics (e.g., pH, turbidity, sediment, dissolved oxygen) could inform habitat suitability modeling and guide monitoring efforts. Monitoring efforts would benefit from a set of range-wide standardized procedures; a multi-state sample design and pre- and post-treatment monitoring effort would allow for large-scale studies that could inform best practices for EWC control. Understanding the vectors and conditions that accompany the dispersal and establishment of new EWC colonies may contribute to its prevention and improve predictive modeling. An understanding of conditions that support dense versus sparse colonies may also provide insight into EWC's invasive properties.

Many of the first EWC populations in North America were thought to be started by deliberate introduction (Johnson 1986). More recent introductions were attributed to water flow, waterfowl ingestion, waterfowl adhesion, and unintentional human introduction (Burk et al. 1976; Henry 1983; Johnson 1985a). Only waterfowl ingestion was examined experimentally (Malone and Proctor 1965), but it is unknown if waterfowl eat sporocarps in the wild. A better understanding of the vectors of dispersal would increase the ability to prevent new infestations. European water-clover can disperse using spores or rhizome fragments, but it is unknown if one is more prevalent than the other or by what means EWC is colonizing new areas. Understanding if and how long EWC can survive desiccation would lend itself to the probability of unintentional human introduction as hitchhikers on boats or other means of transfer. Population genetics analyses could lend itself to understanding EWC dispersal pathways and vectors.

There has been no research at this time into competition of EWC with other aquatic macrophyte species with the exception of rice fields. There has been no research into impacts on macroinvertebrates, fish, or other organisms. Likewise, there is a need to evaluate the impact of EWC when it's growing at varying densities. Understanding EWC's ecological, social, and economic impacts can help managers prioritize sites for management and contribute to the cost-benefit analysis of managing an invasive population.

II. Detection

Aerial imagery has been used to distinguish emergent and floating aquatic vegetation (e.g., Husson et al. 2013) but no studies have evaluated its use for EWC detection. European water-clover detection using aerial imagery may be difficult due to its small size and varying densities. Imagery with fine spatial resolution (e.g., centimeters) would likely be needed to detect EWC.

Given the near shore, sunny habitat EWC occupies and its easily identifiable features, it may not be efficient to research into the use of environmental DNA identification for detection. To prepare for such methods, genetic markers would need to be identified that distinguish EWC from co-existing and closely related species. Laboratory and field sampling would need to be performed to find the detection rates. This approach may detect EWC growing in stands of emergent and floating vegetation or in an inaccessible portion of a water body.

III. Management

Penoxsulam is the only herbicide tested for its effectiveness in controlling EWC that is approved for aquatic use in Michigan (Anwar Bhat et al. 2013; Bruni et al. 2013; MDEQ 2015; Popy et al. 2017). It was shown to be effective against EWC in the laboratory, so it is likely control of EWC contributed to the reduced weed density detected in rice fields (Anwar Bhat et al. 2013; Bruni et al. 2013; Popy et al. 2017). It should be tested in the field in a more natural situation than is seen in rice fields. Hand-pulling was documented to be almost as effective as penoxsulam treatments (Anwar Bhat et al. 2013; Popy et al. 2017).

A cost-benefit analysis and analysis of regrowth needs to be conducted between hand-pulling and chemical treatments. Untreated control comparisons and quantitative pre- and post-treatment monitoring are required to properly measure the efficacy of any treatment.

No research had been conducted into biological control agents. Pathogens or insects could be researched in its native range for effects on growth or spread of EWC.

The recent spread of EWC could be a result of improved conditions and increased habitat creation near existing populations. A temporal study of habitat suitability over time and conditions that trigger germination of sporocarps and rhizome fragments could lend to a broader understanding of the issue.

Future Directions for Michigan and EWC Management

European water-clover is a rooted aquatic plant with floating or emergent leaves that is native to Eurasia. In North America, it has been documented in several states and provinces east of the Great Plains including Michigan, Illinois, Ohio, New York, and Ontario in the Great Lakes region (Johnson 1986; USDA 2018; GBIF 2018; MISIN 2018). European water-clover's early productivity, its ability to form dense colonies in shallow waters, and the longevity of its sporocarps make it a concern to natural resource managers in the Great Lakes Basin (Malone and Proctor 1965; Johnson 1985b; Madsen 1999; Le and Truong 2016).

Prevention – Prevention of new colony establishment is likely the most cost-effective approach to EWC management. Potential pathways of EWC dispersal are deliberate and accidental introduction, hitchhiking on boating equipment, natural waterway currents, and wildlife.

Monitoring – Early detection of an EWC introduction makes eradication a more realistic option. Adding EWC 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 EWC 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. Suitable water bodies that have a high-risk of EWC 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 aquatic invasive 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 be exposed to when visiting specific water bodies.

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 if the 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 EWC, 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 EWC.

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

Measuring effective control: Following the treatment of EWC, treatment efficacy can be quantitatively assessed through documenting any regrowth, reduction in EWC biomass, percent cover, height, or sporocarp production. 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 affecting the long-term ecological stability of an aquatic community.

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Table 2. Objectives, strategic actions, leads, and expected outcomes of European frog-bit (*Marsilea quadrifolia* L.; hereafter EWC) management

Guidance and Outreach for EWC 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.	• Increase public awareness of EWC • Increase the frequency and use of boat washing stations • Protect high-value sites • Contain established populations
Provide technical guidance to those interested in EWC management	• Develop a framework to prioritize management of EWC infestations • Educate stakeholders on available control methods	• MDEQ – Water Resources Division (WRD)	• Increase management efforts
EWC 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 – WRD • MISIN • MiCorps	• Develop a more thorough and up-to-date statewide distribution of EWC • 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 EWC 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 EWC Management			
Chemical: Evaluate the effectiveness of current chemical treatments	• Study the effectiveness of chemical treatments for reducing/eliminating EWC	• MDEQ – WRD	• Determine whether or not chemical treatment is a cost-effective management approach

			• Effective treatment of EWC 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 EWC		• Determine whether or not physical/mechanical removal is a cost-effective management approach • Effective treatment of EWC 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 EWC		• Determine whether or not physical controls are a cost-effective management approach • Effective treatment of EWC resulting in containment, suppression, or eradication

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