Weed Risk Assessment for *Hydrocharis morsus-ranae* L. (Hydrocharitaceae) – European frogbit

Michigan Department of Agriculture and Rural Development

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Version 1

Left: Leaves and stems of *Hydrocharis morsus-ranae*. Right: Dense growth of *Hydrocharis morsus-ranae* among *Phragmites australis* (photos courtesy Cecilia Weibert).

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Introduction The Michigan Department of Agriculture and Rural Development (MDARD) regulates aquatic species through a Prohibited and Restricted species list, under the authority of Michigan's Natural Resources and Environmental Protection Act (NREPA), Act 451 of 1994, Part 413 (MCL 324.41301-41305). Prohibited species are defined as species which "(i) are not native or are genetically engineered, (ii) are not naturalized in this state or, if naturalized, are not widely distributed, and further, fulfill at least one of two requirements: (A) The organism has the potential to harm human health or to severely harm natural, agricultural, or silvicultural resources and (B) Effective management or control techniques for the organism are not available." Restricted species are defined as species which "(i) are not native, and (ii) are naturalized in this state, and one or more of the following apply: (A) The organism has the potential to harm human health or to harm natural, agricultural, or silvicultural resources. (B) Effective management or control techniques for the organism are available." Per a recently signed amendment to NREPA (MCL 324.41302), MDARD will be conducting reviews of all species on the lists to ensure that the lists are as accurate as possible.

We use the United States Department of Agriculture's, Plant Protection and Quarantine (PPQ) Weed Risk Assessment (WRA) process (PPQ, 2015) to evaluate the risk potential of plants. The PPQ WRA process includes three analytical components that together describe the risk profile of a plant species (risk potential, uncertainty, and geographic potential; PPQ, 2015). At the core of the process is the predictive risk model that evaluates the baseline invasive/weed potential of a plant species using information related to its ability to establish, spread, and cause harm in natural, anthropogenic, and production systems (Koop et al., 2012). Because the predictive model is geographically and climatically neutral, it can be used to evaluate the risk of any plant species for the entire United States or for any area within it. We then use a stochastic simulation to evaluate how much the uncertainty associated with the risk analysis affects the outcomes from the predictive model. The simulation essentially evaluates what other risk scores might result if any answers in the predictive model might change. Finally, we use Geographic Information System (GIS) overlays to evaluate those areas of the United States that may be suitable for the establishment of the species. For a detailed description of the PPQ WRA process, please refer to the *PPQ Weed Risk Assessment Guidelines* (PPQ, 2015), which is available upon request.

We emphasize that our WRA process is designed to estimate the baseline—or unmitigated—risk associated with a plant species. We use evidence from anywhere in the world and in any type of system (production, anthropogenic, or natural) for the assessment, which makes our process a very broad evaluation. This is appropriate for the types of actions considered by our agency (e.g., State regulation). Furthermore, risk assessment and risk management are distinctly different phases of pest risk analysis (e.g., IPPC, 2015). Although we may use evidence about existing or proposed control programs in the assessment, the

ease or difficulty of control has no bearing on the risk potential for a species. That information could be considered during the risk management (decision making) process, which is not addressed in this document.

Hydrocharis morsus-ranae L. – European frogbit

Species Information

Family: Hydrocharitaceae

Synonyms: We found no relevant synonyms, however, several are listed by the The Plant List (The Plant List, 2015). *Hydrocharis morsus-ranae* is often mistaken for species of the genus *Limnobium* (Catling & Dore, 1982). In a review of *Hydrochairs morsus-ranae* and *Limnobium spongia*, Catling and Dore (1982) state that the species can be differentiated by " [*Hydrocharis-morsus ranae*] having relatively less well developed aerenchyma on the undersurface of the leaf, relatively longer leaf lobes, leaf veins on either side of the midvein less ascending, free stipules in pairs, roots usually unbranched and stolon buds developing one instead of numerous roots initially."

Common names: European frogbit (Zhu et al., 2014; Skwierawski & Skwierawska, 2013), frogbit (Minshall, 1959; Catling et al., 1988).

Botanical description: *Hydrocharis morsus-ranae* is an aquatic, free-floating herbaceous plant that grows in freshwater systems. It can grow up to 20 cm in length and grows in mats in wave-protected areas (eFloras, 2015; Zhu et al., 2014). For a full botanical description, see the Northern Great Plains Herbaria (2015) or eFloras (Haynes, 2015).

Initiation: In accordance with the Natural Resources and Environmental Protection Act Part 413, the Michigan Department of Agriculture and Rural Development was tasked with evaluating the aquatic species currently on Michigan's Prohibited and Restricted Species List (MCL 324.41302). USDA Plant Epidemiology and Risk Analysis Laboratory's (PERAL) Weed Team worked with MDARD to evaluate and review this species.

Foreign distribution: *Hydrocharis morsus-ranae* is native to most of Europe and parts of Asia (Cook & Lüönd, 1982; NGRP, 2015), as well as northern Africa (i.e. Algeria, Morocco) (NGRP, 2015). This species inhabits a native range in Europe extending from Portugal into the British Isles, France, Sweden, Finland, and Italy (Cook & Lüönd, 1982). *Hydrocharis morsus-ranae* is present in most of northern Europe (GBIF, 2015). It was introduced into Canada in 1932 (Catling & Porebski, 1995). The species was brought into Canada from a botanical garden in Switzerland (Catling et al., 2003), and was cultivated in an arboretum (Dore, 1968). *Hydrocharis morsus-ranae* escaped from the arboretum in 1939 and began spreading, eventually entering the United States (Catling et al., 2003). Dore (1968) cites two Canadian botanical gardens as having the species in cultivation (Ottawa's Central Experiment Farm and Montreal Botanical Garden), but these gardens do not currently cultivate it.

U.S. distribution and status: The first sighting in the United States was in 1974

in the Oswegatchie River, a tributary of the St. Lawrence, in upstate New York (Catling & Dore, 1982). This introduction spread from the initial escape in Canada via the St. Lawrence River (Catling & Dore, 1982). Since then, it has spread to Michigan, New Jersey, New York, Ohio, Vermont, and Washington as naturalized populations (EDDMapS, 2015; Kartesz, 2015). This species is difficult to find in cultivation; United States-based botanical gardens do not cultivate it (i.e. Fairchild Botanical Garden, Missouri Botanical Garden). This species does not appear to be in trade within the United States, and we found no evidence of its cultivation within the United States. *Hydrocharis morsus-ranae* is currently regulated by nine states: Illinois, Indiana, Maine, Michigan, Minnesota, New Hampshire, Vermont, Washington, and Wisconsin (National Plant Board, 2015; Minnesota Department of Natural Resources, 2015). The Michigan Department of Natural Resources has a control program in place, and is actively attempting eradication, primarily via herbicides (Johnson, 2015). In Vermont, $80,000 was spent over a period of three years controlling *Hydrocharis morsus-ranae* in a Natural Heritage site, primarily through hand-pulling (Lewis Creek Association, 2011).

WRA area[1]: Entire United States, including territories.

1. *Hydrocharis morsus-ranae* analysis

Establishment/Spread Potential

Hydrocharis morsus-ranae is an aquatic plant that forms dense mats, covering the surface of water bodies (Zhu et al., 2014; Catling et al., 2003; Roberts et al., 1981). These mats may also grow and spread quickly, covering linear distances of 5.5 km/year, 11.9 km/year, and 15.6 km/year (Catling & Porebski, 1995). Unintentional spread of plant parts via boats and dumping of bait buckets (Catling et al., 2003; Catling & Dore, 1982; Dore, 1968), and natural dispersal via water currents (Scribailo & Posluszny, 1985b; Scribailo, Carey, & Posluszny, 1984) and water fowl (Catling et al., 2003; Catling & Dore, 1982) provide numerous vectors for the establishment of this species in new areas. *Hydrocharis morsus-ranae* has not escaped or naturalized outside of its native range anywhere other than the somewhat limited range it now inhabits in North America (Ontario's Invading Species Awareness Program, 2015, Dore, 1968), and so studies in regards to methods of dispersal, and sexual reproduction, are lacking in the literature. We had an average amount of uncertainty for this risk element.

Risk score = 15 Uncertainty index = 0.18

Impact Potential

Hydrocharis morsus-ranae has a significant impact on natural systems. This species limits oxygen and light available in the water column (Zhu et al., 2014; Catling et al., 2003; Nault & Mikulyuk, 2009), displacing submergent and

[1] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area"] (IPPC, 2012).

emergent native plant layers (Catling et al., 1998) and replacing floating plant layers (Catling et al., 1998). In anthropogenic systems, thick mats of *H. morsus-ranae* limit water traffic and swimming/fishing (Catling et al., 2003; Dore, 1968). These thick mats may also block irrigation systems (Zhu et al., 2015; Catling & Porebski, 1995). We had a low amount of uncertainty for this risk element.
Risk score = 3.9 Uncertainty index = 0.11

Geographic Potential Based on three climatic variables, we estimate that about 79 percent of the United States is suitable for the establishment of *Hydrocharis morsus-ranae* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *Hydrocharis morsus-ranae* represents the joint distribution of Plant Hardiness Zones 4-11, areas with 10-90 inches of annual precipitation, and the following Köppen-Geiger climate classes: steppe, mediterranean, humid subtropical, marine west coast, humid continental warm summers, humid continental cool summers, subarctic, and tundra.

The area of the United States shown to be climatically suitable (Fig. 1) is likely overestimated since our analysis considered only three climatic variables. Other environmental variables, such as pH, water turbidity, and wave turbulence may further limit the areas in which this species is likely to establish. *Hydrocharis morsus-ranae* is found in wave protected, calm bodies of water (Zhu et al., 2014), often among emergent species such as *Phragmites australis* and *Sparganium erectum* (Cook & Lüönd, 1982). *Hydrocharis morsus-ranae* prefers eutrophic, calcium-poor waters with a peaty soil substrate (Cook & Lüönd, 1982).

Entry Potential We did not assess the entry potential of *Hydrocharis morsus-ranae* because it is already present in the United States (Catling & Dore, 1982).

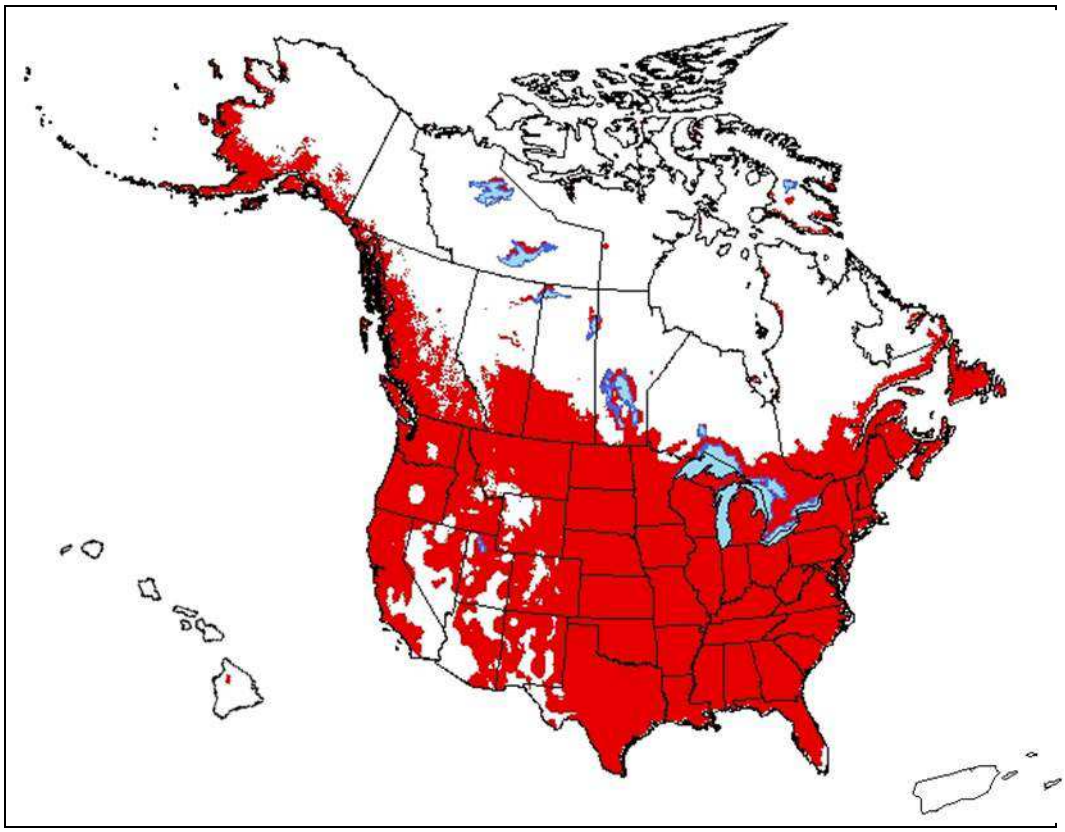

Figure 1. Predicted distribution of *Hydrocharis morsus-ranae* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.

2. Results

Model Probabilities: P(Major Invader) = 85.1%
P(Minor Invader) = 14.3%
P(Non-Invader) = 0.5%

Risk Result = High Risk

Secondary Screening = Not Applicable

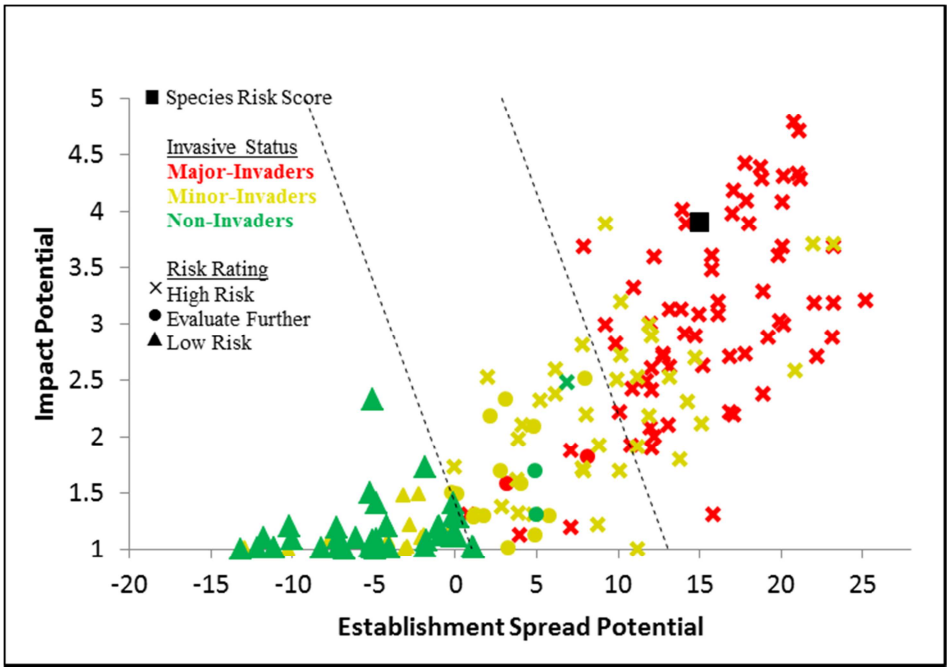

Figure 2. *Hydrocharis morsus-ranae* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

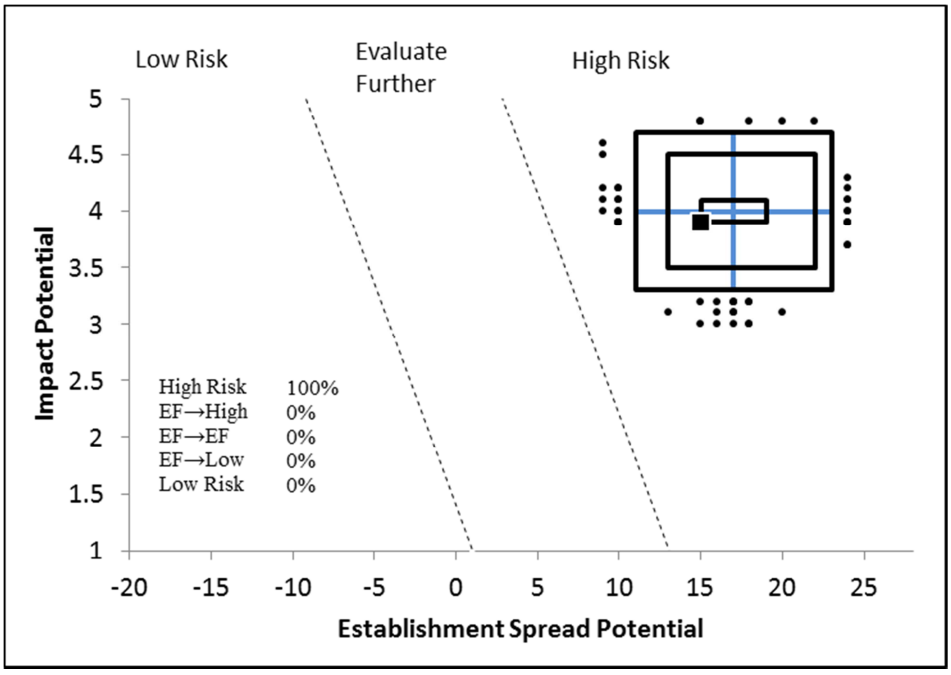

Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for *Hydrocharis morsus-ranae*. The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Hydrocharis morsus-ranae* is High Risk (Figure 2). This categorization of "High Risk" is well supported by the uncertainty analysis (Figure 3). All 5000 iterations of the analysis resulted in a score of "High Risk." This species is one of the species included on Michigan's Department of Natural Resources (DNR) "Watch List," a list of species that may quickly become problematic if they are allowed to establish and naturalize in the state. As part of DNR's Early Detection and Rapid Response program, an eradication program for *Hydrocharis morsus-ranae* is currently in place. The program is designed to identify areas where *H. morsus-ranae* has begun to spread, and uses a range of management strategies, primarily herbicides, to eradicate plants and prevent spread.

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Appendix A. Weed risk assessment for *Hydrocharis morsus-ranae* L. (Hydrocharitaceae). Below is all of the evidence and associated references used to evaluate the risk potential of this taxon. We also include the answer, uncertainty rating, and score for each question. The Excel file, where this assessment was conducted, is available upon request.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ESTABLISHMENT/SPREAD POTENTIAL			
ES-1 [What is the taxon's establishment and spread status outside its native range? (a) Introduced elsewhere =>75 years ago but not escaped; (b) Introduced <75 years ago but not escaped; (c) Never moved beyond its native range; (d) Escaped/Casual; (e) Naturalized; (f) Invasive; (?) Unknown]	f - negl	5	*Hydrocharis morsus-ranae* is native to most of Europe and parts of Asia (Cook & Lüönd, 1982; NGRP, 2015), as well as northern Africa (i.e. Algeria, Morocco) (NGRP, 2015). In 1932, it was introduced into a Canadian arboretum for cultivation from a botanical garden in Switzerland (Catling & Porebski, 1995; Catling et al., 2003). Seven years later, it escaped into neighboring waterways (Roberts et al., 1981). *Hydrocharis morsus-ranae* exists outside of its native range only within North America (Ontario's Invading Species Awareness Program, 2015; Dore, 1968). Catling & Porebski (1995) detail the spread of *H. morsus-ranae* in North America from Ottawa south to New York. From a single hibernaculum turion, an individual plant may grow to cover an area 1 m in diameter in a year (Cook & Lüönd, 1982). Through range expansion, it continues to spread south of Canada (Zhu et al., 2014), and is a source of concern because of its continued invasion of new regions, where its rapid spread is well-documented (Catling et al., 2003). The overall rates of linear spread of *H. morsus-ranae* in three separate areas in Canada were 5.5 km/year, 11.9 km/year, and 15.6 km/year (Catling & Porebski, 1995). Alternate answers for the Monte-Carlo simulation were both "e".
ES-2 (Is the species highly domesticated)	n - low	0	*Hydrocharis morsus-ranae* is cultivated by growers in the Netherlands and is listed for sale by a private seller in the Phillipines, where the plant is native (Netherland Bulb Company, 2015; OLX, 2015), but we found no evidence that it has been bred to reduce weed potential.
ES-3 (Weedy congeners)	y - low	1	The genus *Hydrocharis* contains three species (Cook & Lüönd, 1982). Of these only *Hydrocharis dubia* has been identified as a weed (Randall, 2012). However, we found no information indicating this species is a significant weed. Because this genus is very small, we expanded the scope of this question to include the *Limnobium*, which is very closely related and similar to *Hydrocharis* (Catling et al., 2003; Cook & Lüönd, 1982). *Limnobium* contains two species (Cook & Urmi-König, 1983). One species, *Limnobium laevigatum*, is considered a serious weed in California that spreads rapidly, forms dense mats, and causes problems for boating, fishing, and public infrastructure (Cal-IPC, 2015). This species is regulated as a state noxious weed by the state (California Department of Fodo and Agriculture, 2015).
ES-4 (Shade tolerant at some stage of its life cycle)	n - negl	0	Studies conducted by Zhu et al. (2014) found that shading levels above 50% are effective in controlling *H. morsus-ranae* biomass, while 70% was an ideal cover to actively

Question ID	Answer - Uncertainty	Score	Notes (and references)
			reduce populations. Shading reduces root growth by 90% (Minshall, 1959).
ES-5 (Plant a vine or scrambling plant, or forms tightly appressed basal rosettes)	n - negl	1	This species is neither a vine nor a terrestrial herb with a basal rosette (Cook & Lüönd, 1982; Catling et al., 2003).
ES-6 (Forms dense thickets, patches, or populations)	y - negl	2	*Hydrocharis morsus-ranae*'s dense growth covers a large surface area (Zhu et al., 2014). Stolons become interwoven and form dense networks of large masses (Catling et al., 2003), and large mats grow over water (Roberts et al., 1981).
ES-7 (Aquatic)	y - negl	1	*Hydrocharis morsus-ranae* is a free-floating aquatic (Catling et al., 2003; Scribailo & Posluszny, 1985b) and is often found in calm, shallow areas of freshwater ecosystems (Zhu et al., 2015).
ES-8 (Grass)	n - negl	0	This species is a member of the family Hydrocharitaceae (Scribailo & Posluszny, 1985a) and is therefore not a grass.
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	We found no evidence that this species fixes nitrogen, nor is it in a plant family known to have N-fixing capabilities (Martin and Dowd, 1990). Further, this is not a woody plant.
ES-10 (Does it produce viable seeds or spores)	y - negl	1	This species produces viable seeds that germinate in the field (Catling et al., 2003; Scribailo & Posluszny, 1985b).
ES-11 (Self-compatible or apomictic)	? - max	0	The majority of plants are dioecious (Catling & Dore, 1982; Scribailo, Carey, & Posluszny, 1984), with male and female flowers occurring on different plants. However, Scribailo and Posluszny (1984) found that 5-10% of naturally occurring plants are monoecious, where different sex flowers do not occur on the same rosette but appear on different ramets of the same individual. We found no other information on this species' breeding system. Without knowledge of any mechanisms that prevent self-pollination within the monoecious individuals, we answered unknown.
ES-12 (Requires specialist pollinators)	n - low	0	Flies and bees (*Toxomerus marginatus* and *Dialictus* sp.) were the observed pollinators in field studies conducted by Scribailo and Posluszny (1984).
ES-13 [What is the taxon's minimum generation time? (a) less than a year with multiple generations per year; (b) 1 year, usually annuals; (c) 2 or 3 years; (d) more than 3 years; or (?) unknown]	b - low	1	*Hydrocharis morsus-ranae* is an annual species (Cook & Lüönd, 1982). This species reproduces both vegetatively and sexually (Cook & Lüönd, 1982). Vegetatively, this species produces turions, modified stolon buds designed for overwintering (Catling et al., 2003). Turions are produced in the autumn, detach and sink before winter, and then germinate in the spring (Catling et al., 2003). Dormancy in the winter requires several weeks of chilling, and when water temperatures begin to warm in the spring, turions float to the surface and begin to germinate (ISSG, 2015; Catling et al., 1988). Sexually, fruits mature underwater and detach from the fruit in late autumn (Cook & Lüönd, 1982). Seeds undergo a similar chilling dormancy period, whereby germination takes place after water temperature reaches 15^{o}C (Cook & Lüönd, 1982). Alternate answers for the Monte-Carlo simulation are both "a," as plant cuttings and stolons transported to other areas

Question ID	Answer - Uncertainty	Score	Notes (and references)
			may regenerate into new individuals (Minshall, 1959; Dore, 1968; Catling et al., 2003; Catling & Dore, 1982).
ES-14 (Prolific reproduction)	n - low	-1	In its native range in Poland, plants produce 3000-4000 seeds/m^2 (Toma, 2013). In Rondeau Park, Ontario, plants produce about 250 seeds/m^2, however, this may be because male plants outnumber female plants by about 8.5:1 (Scribailo & Posluszny, 1984). Because neither estimate met our threshold, we answered no. Vegetative reproduction of this species is achieved via turions (winter buds), but no data was found regarding the level of production of these structures.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - negl	1	*Hydrocharis morsus-ranae* is prone to spread from one body of water to another via anchors, ropes, and boat motors, due to the interwoven mats formed by dense *H. morsus-ranae* growth (Catling et al., 2003; Catling & Dore, 1982; Dore, 1968). The dumping of bait buckets containing water from infested locations also contributes to unintentional spread (Catling et al., 2003; Dore, 1968).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	? - max	0	Aquatic plants are often mislabeled due to similarity in growth forms (Thum et al., 2012)*Hydrocharis morsus-ranae* may be a hitchhiker plant with other species ordered through water garden catalogs (Nault & Mikulyuk, 2009). However, without definite evidence that this species follows a trade pathway, we answered unknown with maximum uncertainty.
ES-17 (Number of natural dispersal vectors)	2	0	Fruit and seed traits for questions ES-17a through ES-17e: Fruit is a globose berry containing up to 74 seeds, the average being 26-42 (Catling et al., 2003; Toma, 2013). Fruits dihesce underwater and split to release the seed (Cook & Lüönd, 1982). Seeds are transversely elliptic in shape and are approximately 1 mm in length when fully mature. Testas are covered in knoblike tubercles, giving seeds a spiny appearance (Scribailo & Posluszny, 1985b). Seeds are covered with a gelatinous mass when they emerge from the fruit (Cook & Lüönd, 1982; Catling & Dore, 1982). Vegetative turions (winter buds) 5-7 mm in length, ellipsoidal, are produced on stolons and detach and sink underwater (Cook & Lüönd, 1982).
ES-17a (Wind dispersal)	n - negl		Seeds are released underwater, and have no adaptations for wind dispersal (Scribailo & Posluszny, 1985b).
ES-17b (Water dispersal)	y - negl		After pollination, flowers are pulled underwater, and fruits are released under water (Scribailo & Posluszny, 1985b; Cook & Lüönd, 1982). The fruits sink to the bottom of the body of water once mature and split open, releasing the seeds in a gelatinous mass (Cook & Lüönd, 1982). These seeds may float to the surface and travel via surface currents (Cook & Lüönd, 1982). Plants produce overwintering turions which are dispersed by water currents (Scribailo, Carey, & Posluszny, 1984).
ES-17c (Bird dispersal)	y - low		Great blue herons have been observed flying with interlocking plants of *Hydrocharis morsus-ranae* attached to their feet (Catling et al., 2003). Plant parts may be carried by waterfowl over long distances by lodging in

Question ID	Answer - Uncertainty	Score	Notes (and references)
			beaks or feet, and seeds, which are surrounded by a gelatinous mass, may adhere to birds (Catling & Dore, 1982). Seeds of *Limnobium spongia*, a very closely related species, have been identified in the stomachs of ducks, but it is unknown if the seeds of *H. morsus-ranae* would be able to similarly pass through the digestive tract. (Catling et al., 2003).
ES-17d (Animal external dispersal)	? - max		The gelatinous coating of the seeds of *H. morsus-ranae* (Catling & Dore, 1982) may allow the seeds to attach to other water dwelling creatures (i.e. beavers), but there is no direct evidence of this form of dispersal. Therefore, we answered unknown.
ES-17e (Animal internal dispersal)	? - max		The berry-like fruit of *H. morsus-ranae* is borne underwater (Scribailo & Posluszny, 1985b), and there is no evidence of this mode of dispersal. We answered unknown because it is possible that some animal may consume the fruit and disperse viable seeds.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - low	-1	In a study of seeds banks formed by native, established plant taxa, Combroux & Bornette (2004) found no evidence of a seed bank formed for *Hydrocharis morsus-ranae*.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	y - mod	1	Anecdotal evidence suggests that *Hydrocharis morsus-ranae* stolon fragments that are transported between water bodies can regenerate (Catling et al., 2003; Catling & Dore, 1982; Dore, 1968), however, regeneration rates have not been studied. These fragments generally occur as cuttings on boat anchors, motors, propellors, etc. (Catling et al., 2003). Thus, we answered yes but with moderate uncertainty.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - negl	0	We found no evidence this species is resistant to herbicides. Furthermore, it is not listed by Heap (2013) as a weed that is resistant to herbicides. *Hydrocharis morsus-ranae* is susceptible to diquat, paraquat, chlorthiamid, terbutryne, and cyanatryn (Catling et al., 2003).
ES-21 (Number of cold hardiness zones suitable for its survival)	8	0	
ES-22 (Number of climate types suitable for its survival)	8	2	
ES-23 (Number of precipitation bands suitable for its survival)	8	1	
IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	n - low	0	We found no evidence that this species is allelopathic.
Imp-G2 (Parasitic)	n - negl	0	We found no evidence that this species is parasitic. Furthermore, *Hydrocharis morsus-ranae* does not belong to a family known to contain parasitic plants (Heide-Jorgensen, 2008; Scribailo & Posluszny, 1985a).
Impacts to Natural Systems			
Imp-N1 (Changes ecosystem processes and parameters that affect other species)	y - negl	0.4	*Hydrocharis morsus-ranae* is a free-floating aquatic plant (Catling et al., 2003; Scribailo & Posluszny, 1985b). Leaves of free-floating species exchange oxygen directly with the atmosphere, rather than the surrounding water

Question ID	Answer - Uncertainty	Score	Notes (and references)
			(Pierobon et al., 2010). When the vegetation dies, it sinks and decays, utilizing oxygen from the water column (Catling et al., 2003). Thus, *H. morsus-ranae* lowers oxygen levels in waters where it is found (Catling et al., 2003). In a study that measured the impact of *H. morsus-ranae* on oxygen levels in water, dissolved oxygen content in a lake with a floating mat of *H. morsus-ranae* at a density of 142.6 g/m2 was 0.23 mg/L, while the content under an area with *H. morsus-ranae* density of 5.7 g/m2 was 1.66 mg/L (Zhu et al., 2014). The dense mats formed by *H. morsus-ranae* are also effective at blocking light from reaching far into the water column (Zhu et al., 2015; Catling et al., 2003; Nault & Mikulyuk, 2009).
Imp-N2 (Changes habitat structure)	y - negl	0.2	Catling et al. (1988) found that at sites in New York and Ontario, *Hydrocharis morsus-ranae* displaced the submerged plant layer (e.g. *Potamogeton pusillus*, *Myriophyllum heterophyllum*), replaced the free-floating plant layer (e.g. *Utricularia vulgaris*, *Lemna minor*, *Nuphar variegate*), and displaced the emergent plant layer (e.g. *Potamogeton nodosus*, *Spirodela polyrhiza*).
Imp-N3 (Changes species diversity)	y - negl	0.2	*Hydrocharis morsus-ranae* reduces biodiversity by competing with and displacing native vegetation, and is capable of changing the fauna and flora of an ecosystem (Nault & Mikulyuk, 2009). *Hydrocharis morsus-ranae* is dominant where it occurs (Toma, 2013; Catling & Porebski, 1995). Native flora supports a greater diversity of native aquatic animals than do the floating mats of *H. morsus-ranae* (Catling et al., 2003). Without direct evidence regarding the mechanisms by which *H. morsus-ranae* changes species diversity, we answered yes, but with moderate uncertainty.
Imp-N4 (Is it likely to affect federal Threatened and Endangered species?)	y - low	0.1	*Hydrocharis morsus-ranae* forms dense, interwoven, free-floating mats on the surface of freshwater systems (Roberts et al., 1981; Dore, 1968; Catling et al., 1988),. *Hydrocharis morsus-ranae* also outcompetes native vegetation (Nault & Mikulyuk, 2009; Toma, 2013; Catling & Porebski, 1995) and either removes vegetation layers or replaces them, altering the habitat and food source available for other species (Catling et al., 1988). These habitat alterations are likely to affect T&E species, thus we answered this question yes, with low uncertainty.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions?)	y - negl	0.1	Its predicted distribution in the United States includes globally outstanding ecoregions as defined by Ricketts et al. (1999). *Hydrocharis morsus-ranae* is already present as a noxious weed in a county in Washington (BONAP, 2014) which occurs in a globally outstanding ecoregion (Ricketts et. al, 1999), and may move to other nearby counties in globally outstanding ecoregions via the dispersal methods discussed in ES-17. This species alters nutrient regimes within areas it becomes established in, and outcompetes native species to reduce the submerged vegetation layers to near zero, and replace the free-floating vegetation present in an area (Nault & Mikulyuk, 2009; Toma, 2013; Catling & Porebski, 1995; Catling et al.,

Question ID	Answer - Uncertainty	Score	Notes (and references)
			1988). Dense mats of *H. morsus-ranae* deplete oxygen levels and light attenuation in the water column beneath them, altering the natural system (Zhu et al., 2014; Catling et al., 2003; Nault & Mikulyuk, 2009).
Imp-N6 [What is the taxon's weed status in natural systems? (a) Taxon not a weed; (b) taxon a weed but no evidence of control; (c) taxon a weed and evidence of control efforts]	c - negl	0.6	This species invades relatively small bodies of water or sheltered bays and inlets of larger lakes (Cook & Lüönd, 1982), where the water is slow moving or still and wave/wind protected (Zhu et al., 2014). *Hydrocharis morsus-ranae* is one of five alien plants reported to have a major impact on natural ecosystems in Canada (Catling et al., 2003; Catling & Porebski, 1995). Mechanical and chemical methods are the most frequent control strategies utilized (Nault & Mikulyuk, 2009). The herbicides diquat, paraquat, chlorthiamid, terbutryne, and cyanatryn are effectively used to manage populations of *H. morsus-ranae* in natural areas in Canada (Catling et al., 2003). Alternate answers for the Monte Carlo simulation are both "b."
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Negatively impacts personal property, human safety, or public infrastructure)	? - max	0.1	*Hydrocharis morsus-ranae* blocks inlets of the Rideau Canal (Dore, 1968), a UNESCO World Heritage site that connects the cities of Ottawa, Ontario, to Kingston, Ontario. Extensive growth of the species was also found in an inlet that had been partly excavated for a marina at the Carleton Golf and Yacht club in Ottawa (Dore, 1968). Without further direct evidence of the extent of the impact of the growth, and the age of the source, we have answered unknown.
Imp-A2 (Changes or limits recreational use of an area)	y - low	0.1	In Ontario and New York, thick mats limit water traffic and swimming/fishing (Catling et al., 2003; Dore, 1968). *Hydrocharis morsus-ranae* can form dense mats that interfere with recreational activities such as boating, fishing, swimming, water skiing, canoeing, and kayaking (Nault & Mikulyuk, 2009).
Imp-A3 (Affects desirable and ornamental plants, and vegetation)	n - mod	0	We found no evidence that this species affects desirable and ornamental plants and vegetation.
Imp-A4 [What is the taxon's weed status in anthropogenic systems? (a) Taxon not a weed; (b) Taxon a weed but no evidence of control; (c) Taxon a weed and evidence of control efforts]	c - low	0.4	*Hydrocharis morsus-ranae* inhabits slow-flowing ponds, streams and lakes (Zhu et al., 2014; Cook & Lüönd, 1982). It invades many water sources used for recreation and public infrastructure (Dore, 1968; Catling et al., 1988; Nault & Mikulyuk, 2009). Mechanical and chemical control strategies are the most commonly utilized management techniques; weed control teams in British channels use weed-cutting boats, weed rakes, dredgers, and herbicides to treat areas infested with *H. morsus-ranae* (Catling et al., 2003). Alternate answers for the Monte Carlo simulation are both "b."
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product	n - mod	0	We found no evidence that this species affects crop or

Question ID	Answer - Uncertainty	Score	Notes (and references)
yield)			commodity yield.
Imp-P2 (Lowers commodity value)	n - mod	0	We found no evidence that this species lowers commodity value.
Imp-P3 (Is it likely to impact trade?)	? - max		Aquatic plants in the water garden trade commonly travel as hitchhikers of each other (Maki & Galatowitsch, 2004); however, there is no direct evidence that this species has been identified as following a pathway of trade. The South American country of Namibia requires phytosanitary certificates for the entire family of Hydrocharitaceae (APHIS, 2015), and Illinois, Indiana, Maine, Michigan, New Hampshire, Vermont, Washington, and Wisconsin regulate this species (National Plant Board, 2015). Without further evidence that this species affects trade, we answered "unknown," with maximum uncertainty.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	y - low	0.1	*Hydrocharis morsus-ranae* limits water flow in irrigation canals (Catling et al., 2003). Dense mats of *H. morsus-ranae* growth can block irrigation canals (Zhu et al., 2014; CDFA, 2015).
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - mod	0	We found no evidence that this species is toxic to animals.
Imp-P6 [What is the taxon's weed status in production systems? (a) Taxon not a weed; (b) Taxon a weed but no evidence of control; (c) Taxon a weed and evidence of control efforts]	a - low	0.6	*Hydrocharis morsus-ranae* inhabits still, slow-moving waters such as canals (Catling et al., 2003), irrigation ditches, and water-intake pipes (Zhu et al., 2015; Catling & Porebski, 1995), however, aquatic species will inhabit any body of water that fits their growth habit. There is no evidence that this species is considered a weed in production systems. Very little information specifically regarding production systems is available for *H. morsus-ranae*. Alternate answers for the Monte Carlo simulation were both "b."
GEOGRAPHIC POTENTIAL			Unless otherwise indicated, the following evidence represents geographically referenced points obtained from the Global Biodiversity Information Facility (GBIF, 2015).
Plant hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z2 (Zone 2)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z3 (Zone 3)	n - high	N/A	There are 3 points in Canada near the edge of zone 4. Because of potential mapping error, we answered no because we didn't find any evidence this species occurs in other areas of this zone.
Geo-Z4 (Zone 4)	y - negl	N/A	Finland and the United States. Two points in Germany.
Geo-Z5 (Zone 5)	y - negl	N/A	Finland and the United States. Two points in Sweden.
Geo-Z6 (Zone 6)	y - negl	N/A	Germany and Sweden.
Geo-Z7 (Zone 7)	y - negl	N/A	Germany and Sweden.
Geo-Z8 (Zone 8)	y - negl	N/A	France, Germany, and the United Kingdom.
Geo-Z9 (Zone 9)	y - negl	N/A	France, Ireland, and the United Kingdom.
Geo-Z10 (Zone 10)	y - low	N/A	A few points in France and the United Kingdom. One point in Ireland.
Geo-Z11 (Zone 11)	y - high	N/A	A few clustered points in Spain.
Geo-Z12 (Zone 12)	n - mod	N/A	We found no evidence that it occurs in this hardiness zone.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Geo-Z13 (Zone 13)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	n - low	N/A	We found no evidence that it occurs in this climate class.
Geo-C2 (Tropical savanna)	n - low	N/A	We found no evidence that it occurs in this climate class.
Geo-C3 (Steppe)	y - high	N/A	See notes under Geo-C4.
Geo-C4 (Desert)	n - high	N/A	One point in Spain in a very small area characterized as desert and which is embedded in a larger area of steppe habitat. Because of potential mapping error, we answered this question as no, but answered yes for steppe. Note there is no reason an aquatic plant couldn't occur in these dry areas as long as there is a permanent source of water available, whether it is natural or artificial.
Geo-C5 (Mediterranean)	y - negl	N/A	Some points in France and a few in Spain. Present in one county in Washington, United States (Kartesz, 2015).
Geo-C6 (Humid subtropical)	y - high	N/A	One point in Italy.
Geo-C7 (Marine west coast)	y - negl	N/A	France and the United Kingdom.
Geo-C8 (Humid cont. warm sum.)	y - low	N/A	A few counties in southeastern Michigan, United States (Kartesz, 2015).
Geo-C9 (Humid cont. cool sum.)	y - negl	N/A	Germany, Sweden, and the United States.
Geo-C10 (Subarctic)	y - negl	N/A	Finland. One point in Sweden and Norway, and a few points in the French Alps.
Geo-C11 (Tundra)	y - high	N/A	Two points in mountainous regions in France. This species may be adapted for growth in cold areas due to the "chilling" process which the species's reproductive structures (i.e. turions and seeds) undergo as a necessary dormancy period prior to germination (ISSG, 2015; Catling et al., 1988; Cook & Lüönd, 1982).
Geo-C12 (Icecap)	n - low	N/A	We found no evidence that it occurs in this climate class.
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - low	N/A	We found no evidence that it occurs in this precipitation band.
Geo-R2 (10-20 inches; 25-51 cm)	y - low	N/A	A few points in Spain.
Geo-R3 (20-30 inches; 51-76 cm)	y - negl	N/A	France, Germany, the United Kingdom, and the United States.
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	The United Kingdom and the United States.
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	Denmark, Ireland, and the United Kingdom.
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	Germany and Ireland.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	A few points in France, Ireland, Germany, and the United Kingdom.
Geo-R8 (70-80 inches; 178-203 cm)	y - low	N/A	A couple of points in France and Germany.
Geo-R9 (80-90 inches; 203-229 cm)	y - mod	N/A	Two points in Germany.
Geo-R10 (90-100 inches; 229-254 cm)	n - high	N/A	We found no evidence that it occurs in this precipitation band.
Geo-R11 (100+ inches; 254+	n - high	N/A	We found no evidence that it occurs in this precipitation

Question ID	Answer - Uncertainty	Score	Notes (and references)
cm)			band.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	The first sighting in the United States was in 1974 in the Oswegatchie River, a tributary of the St. Lawrence, in Northern New York (Catling & Dore, 1982).
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	