United States Department of Agriculture

United States Department of Agriculture

Animal and Plant Health Inspection Service

December 15, 2017

Version 1

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

Left: Stoloniferous ramet of *H. morsus-ranae* plant. Right: dense mat of *H. morsus-ranae* growing on the surface of a pond. [source: Cecilia Weibert, Great Lakes Commission, Ann Arbor, MI].

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1. Introduction

Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). We use the PPQ weed risk assessment (WRA) process (PPQ, 2015) to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

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., Federal regulation). Furthermore, risk assessment and risk management are distinctly different phases of pest risk analysis (e.g., IPPC, 2016). 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.

2. Plant Information and Background

SPECIES: *Hydrocharis morsus-ranae* L. (NGRP, 2017)

FAMILY: Hydrocharitaceae

SYNONYMS: None

COMMON NAMES: European frog-bit, hydrocharide grenouillette (French) (Darbyshire et al., 2000), frog's-bit (Gleason and Cronquist, 1991), frogbit (Stace, 2010)

BOTANICAL DESCRIPTION: *Hydrocharis morsus-ranae* is a free-floating, stoloniferous, perennial aquatic plant, 0.1-1.5 m across, with individual rosette diameters of 1-30 cm (Catling et al., 2003). Leaves are small, heart-shaped, and floating, 1.2-6 cm long and 1.3-6.3 cm wide (Nault and Mikulyuk, 2009). The majority of *H. morsus-ranae* plants are dioecious (Catling et al., 2003); both male and female flowers contain a trimerous whorl of white petals, enclosed in a whorl of greenish sepals (Scribailo and Posluszny, 1984). Between 5 and 10 percent of plants may be monoecious, with male and female flowers appearing on different ramets within a genet (Scribailo and Posluszny, 1984). During the autumn, plants produce turions: buds that sink to the bottom of water bodies in the winter and resurface in the spring, producing new clonal plants (Catling et al., 2003). For more in-depth botanical descriptions, see Catling et al. (2003) or Haynes (2000).

INITIATION: On August 13, 2015, the PPQ Cross-Functional Weed Group reviewed a draft version of a weed risk assessment for *Hydrocharis morsus-ranae* prepared by the Michigan Department of Agriculture and Rural Development (MDARD, 2015) and determined that it represents a potential candidate for listing as a Federal Noxious Weed (FNW). The final assessment by MDARD was completed on August 18, 2015, and the PPQ FNW Policy Manager requested that PERAL obtain and update this assessment in preparation for federal rule-making.

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

FOREIGN DISTRIBUTION: *Hydrocharis morsus-ranae* is native to a large portion of Europe, including an area ranging from Portugal, western France, and the British Isles northward to southern Scandinavia and southward to northern Italy (Cook and Lüönd, 1982). Sporadic observations of *H. morsus-ranae* have come from Iran (Assadi and Wendelbo, 1977), parts of Turkey, the Caucasus, Kazakhstan, and central Siberia (Cook and Lüönd, 1982), though none of these specimens have been preserved in herbaria (GBIF Secretariat, 2017). The species has also recently been observed in the Kashmir Himalaya (Ganie et al., 2016). *Hydrocharis morsus-ranae* is present in parts of Canada and is considered one of five invasive alien plants that have caused major impacts on natural ecosystems there (Catling and Porebski, 1995), though it is not regulated as a noxious weed in any Canadian

[1] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area"] (IPPC, 2017).

province (Kartesz, 2017). It escaped cultivation in 1932 following its intentional introduction to ponds in the Central Experimental Farm of Ottawa (Dore, 1968), and has since spread into adjacent waterways of Ontario and Quebec, including the upper Saint Lawrence River valley and portions of the shorelines of Lake Ontario, Lake Erie, and Lake Huron (GBIF Secretariat, 2017). *Hydrocharis morsus-ranae* is sold by companies within its native range in Europe and is a popular garden plant in the United Kingdom: the Royal Horticultural Society [RHS (2017)] lists eight nurseries selling it. We found no evidence of *H. morsus-ranae* retail sales in Canada.

U.S. DISTRIBUTION AND STATUS: *Hydrocharis morsus-ranae* specimens have been collected from Vermont, New York, and Michigan (GBIF Secretariat, 2017). It has also been reported in Pennsylvania, New Jersey, Ohio, and Washington (Kartesz, 2017), though no specimens have been collected from these states (GBIF Secretariat, 2017). *Hydrocharis morsus-ranae* is not currently categorized as a federal noxious weed, but is classified as a Class B state noxious weed in Vermont, an invasive aquatic plant in Maine, a wetland and aquatic quarantine weed in Washington, and an A list noxious weed in California (7 CFR § 360, 2016; NRCS, 2017). Methods for controlling *H. morsus-ranae* populations following establishment have mostly proven ineffective or impractical (Hackett et al., 2014). Nevertheless, several control trials have been carried out where this species has invaded the Northeast. One trial at Town Farm Bay on Lake Champlain in Charlotte, VT removed a total of 42.5 tons of vegetative matter from a ~45 acre wetland over three years using 5,468 labor hours (LCA, 2011). Thirteen of eighteen total zones met target *H. morsus-ranae* cover percentages after the removal period, while the remaining five zones had coverage no more than 10% above target. A longer-term manual removal trial targeted a quarter-acre area on the Grasse River in New York, removing 36 five-gallon buckets of vegetative matter in 2007, with repeated annual removals and continually diminishing *H. morsus-ranae* cover since then (Quirion et al., 2016). 2016 was the first year of the project in which no frog-bit was observed at the trial site. We found evidence of limited retail sale of *H. morsus-ranae* in the United States. Plant Information Online (University of Minnesota, 2016) lists one nursery in Baltimore, MD selling this species, and it is offered for both wholesale and retail sale according to the company website (WWGNP, 2017). A search on eBay found one seller located in Tennessee willing to ship live plants within the United States (eBay, 2017a), and one seller in Ukraine willing to ship turions worldwide, including to the United States (eBay, 2017b). Figure 1 shows the current known U.S. distribution of *Hydrocharis morsus-ranae*.

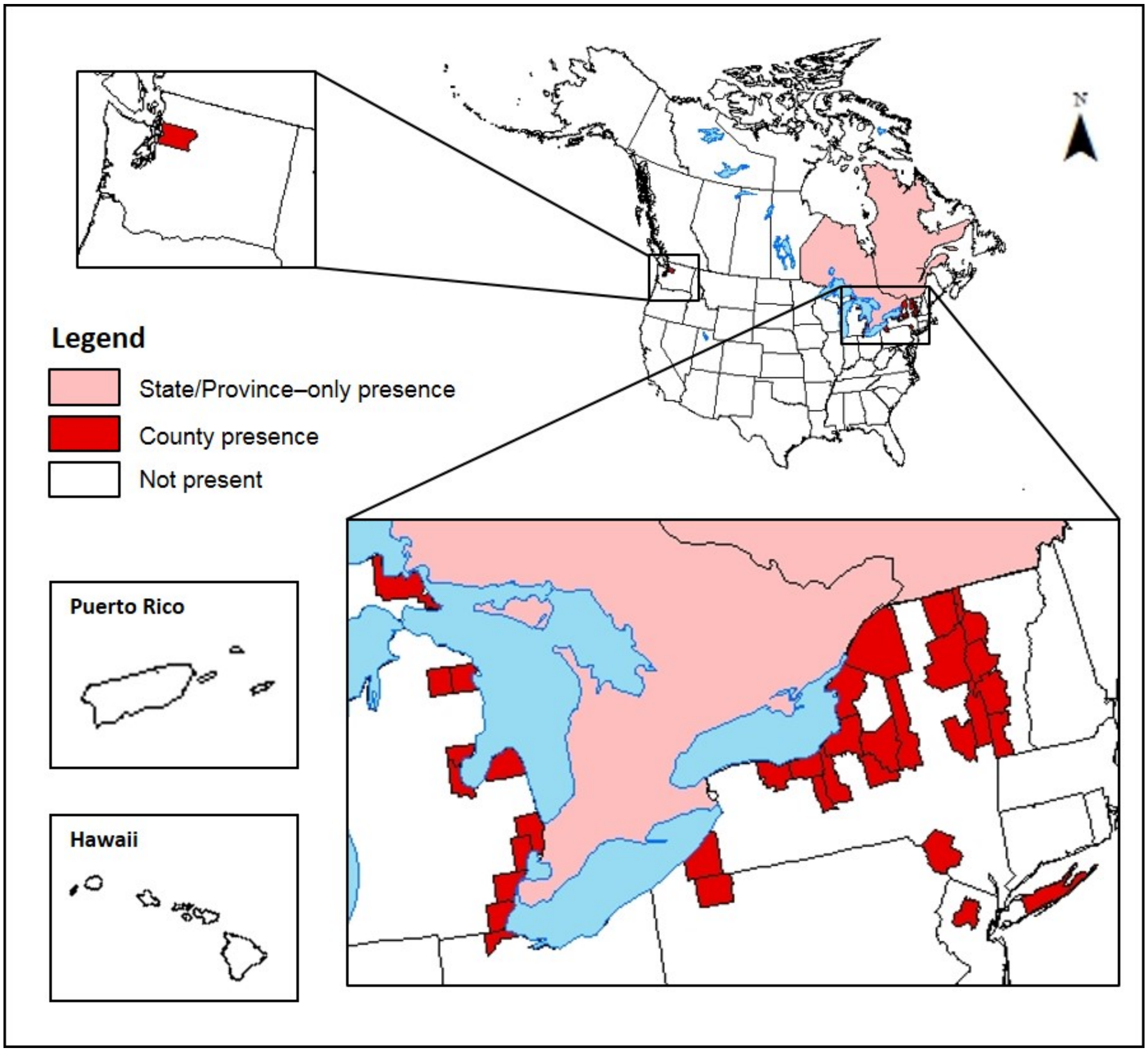

Figure 1. Known naturalized distribution of *Hydrocharis morsus-ranae* in the United States and Canada. The records shown here were obtained primarily from other species distribution databases (EDDMapS, 2017; Kartesz, 2017; NRCS, 2017) and were not independently verified by PERAL. Scales differ for Hawaii, Puerto Rico, and the continental United States and Canada.

3. Analysis

ESTABLISHMENT/SPREAD POTENTIAL

Hydrocharis morsus-ranae has consistently been labeled as invasive in areas of Canada and the United States where it has naturalized (Catling and Porebski, 1995; ISSG, 2005; Zhu et al., 2015). Once established, *H. morsus-ranae* spreads rapidly, primarily by the seasonal production of turions, which overwinter on the bottom of water bodies, rising to the surface and establishing new clones in the spring (Catling et al., 2003). The stoloniferous growth from a single *H. morsus-ranae* turion can produce over ten ramets, and each ramet can in turn produce over ten turions by the following autumn (i.e. the germination of one turion can lead to the production of 100 turions in a single growing season), enabling rapid vegetative reproduction over time (Scribailo and Posluszny, 1984). A plant growing from a single turion may grow to cover an area of 1 m^2 in a single season (Cook and Lüönd, 1982). Plant matter is spread on the feet and bills of ducks and other waterfowl (Catling and Dore, 1982), and by human activity, such as transportation on boats and boat trailers (Catling et al., 2003). We had average uncertainty for this risk element.

Risk score = 14 Uncertainty index = 0.18

IMPACT POTENTIAL

Hydrocharis morsus-ranae primarily affects natural ecosystems. This species can contribute to hypoxic water conditions (Catling et al., 2003; Zhu et al., 2014) and can heavily shade water columns (Catling et al., 2003; Zhu et al., 2015) in areas where it has formed dense mats. It has been shown to reduce biodiversity in areas of establishment (Catling et al., 2003; Nault and Mikulyuk, 2009). As early as 1965, it had formed pure stands that required mechanical removal in several areas near where it was originally introduced in Ottawa (Cook and Lüönd, 1982). We had low uncertainty for this risk element, as the species' effect on ecosystem processes, habitat structure, and biodiversity have been well characterized.

Risk score = 3.2 Uncertainty index = 0.13

GEOGRAPHIC POTENTIAL

Based on three climatic variables, we estimate that about 61 percent of the United States is suitable for the establishment of *Hydrocharis morsus-ranae* (Fig. 2). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and general 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: Mediterranean, humid subtropical, marine west coast, humid continental warm summers, humid continental cool summers, subarctic, and tundra.

The analysis for the area of the United States shown to be climatically suitable (Fig. 2) for species establishment considered only three climatic variables. Other variables, such as turbidity, water pH, novel climatic conditions, or plant genotypes may alter the area in which this species is likely to establish. *Hydrocharis morsus-ranae* is adapted to a wide range of climates, a fact reflected in its distribution throughout the majority of Europe (Cook and Lüönd, 1982). It inhabits shallow, slow-moving waters and is commonly found along shorelines of rivers, lakes, ponds, marshes and wetlands, in addition to man-made environments such as canals and irrigation ditches (Nault and Mikulyuk, 2009). Zhu et al. (2008) hypothesized that gradual global warming will further facilitate *H. morsus-ranae* invasion of oligotrophic and mesotrophic lakes.

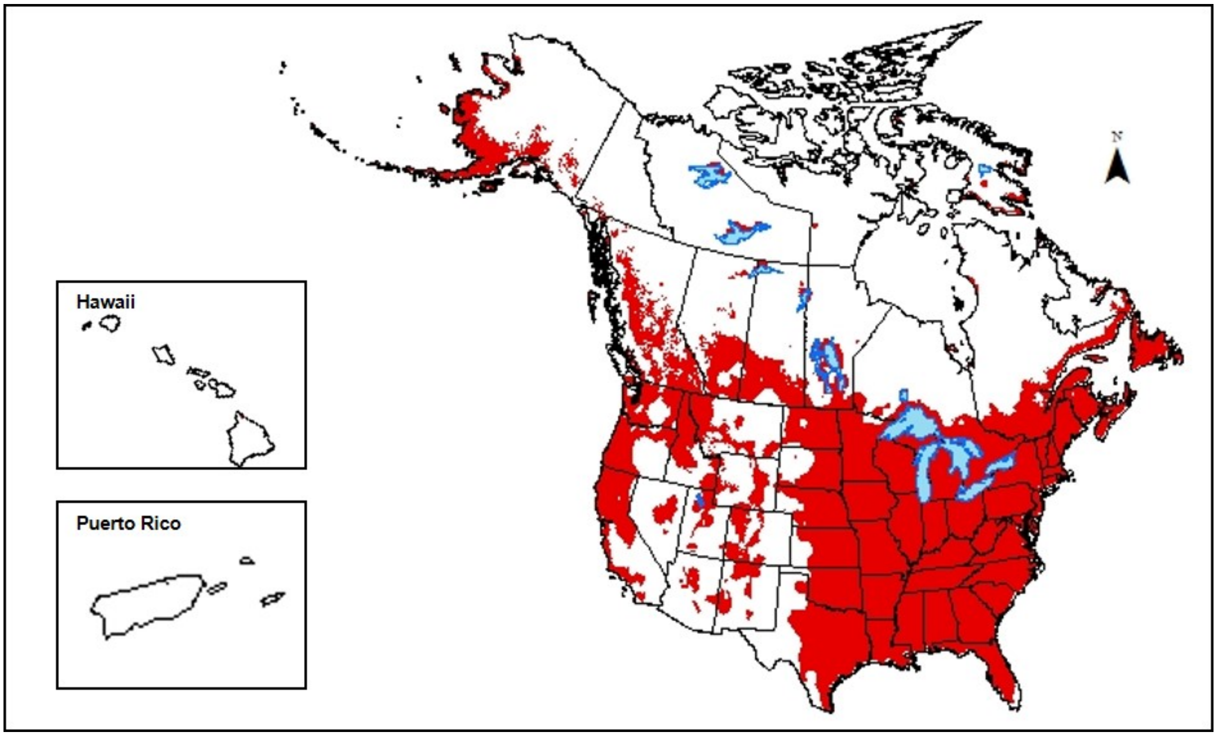

Figure 2. Potential geographic distribution of *Hydrocharis morsus-ranae* in the United States and Canada. Map insets for Hawaii and Puerto Rico are not to scale.

ENTRY POTENTIAL

Hydrocharis morsus-ranae is already naturalized in several sites within the United States (EDDMapS, 2017; Kartesz, 2017; NRCS, 2017). We evaluated this risk element, however, in order to determine how additional material may enter the country. On a scale of 0 to 1, where 1 represents the maximum risk score, *H. morsus-ranae* obtained a score of 0.64. The retail sale of *H. morsus-ranae* within the United States by several vendors (eBay, 2017a, 2017b; WWGNP, 2017) contributed a large portion of this risk element. This species is also spread by human activity, including discarding of aquarium contents into water bodies (Catling and Dore, 1982) and inadvertent transportation on recreational boating equipment (Catling et al., 2003). The presence of *Hydrocharis morsus-ranae* in several bodies of water on the United States-Canada border, including Lake Erie, Lake Ottawa, and the Saint Lawrence River, also makes it highly likely that this species will continue to disperse through the border area via natural pathways. We had low uncertainty for this risk element.

Risk score = 0.64 Uncertainty index = 0.10

4. Predictive Risk Model Results

Model Probabilities: P(Major Invader) = 74.8%
P(Minor Invader) = 24.2%
P(Non-Invader) = 1.0%

Risk Result = High Risk

Secondary Screening = Not Applicable

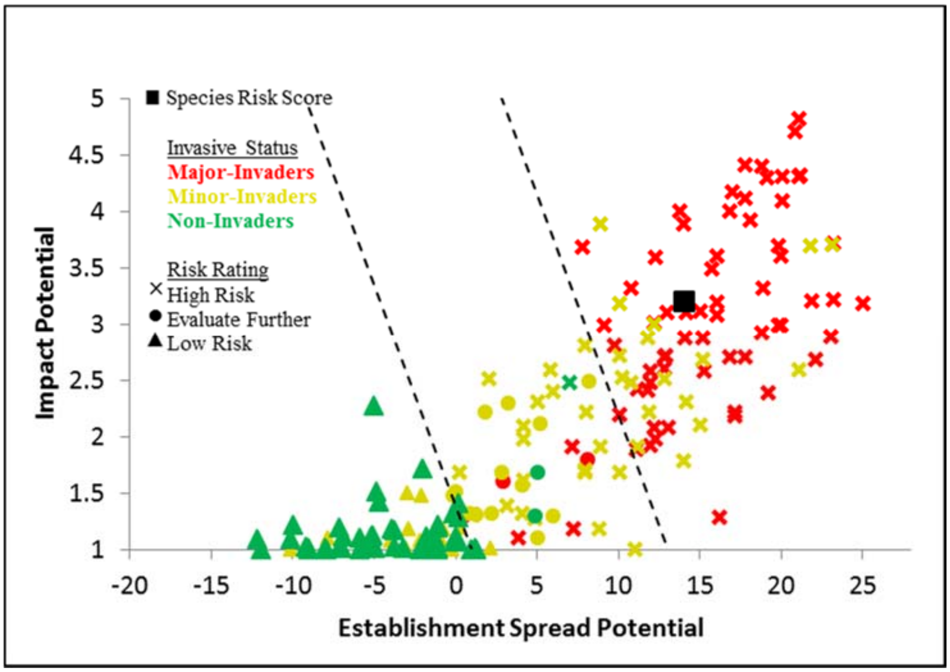

Figure 3. *Hydrocharis morsus-ranae* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

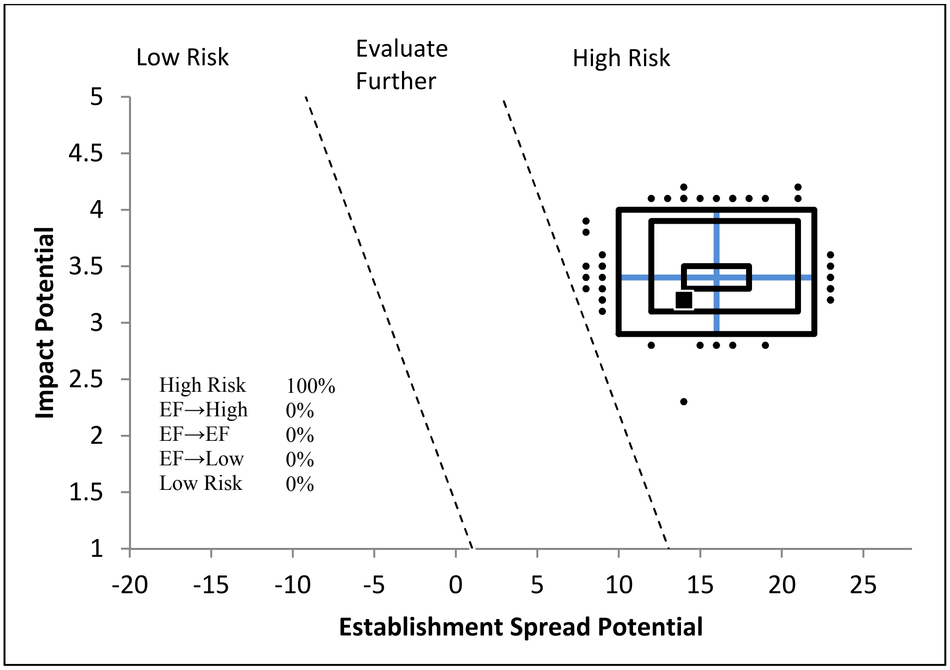

Figure 4. Model simulation results (N=5,000) for uncertainty around the risk score for *Hydrocharis morsus-ranae*. The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

5. Discussion

The result of the weed risk assessment for *Hydrocharis morsus-ranae* is High Risk (Fig. 3). This species is an example of a plant for which introduction to a new area and its subsequent naturalization and spread have been well documented. Therefore, the results of our uncertainty simulation (Fig. 4) unequivocally support the categorization of *H. morsus-ranae* as high risk, with 100 percent of simulation results falling within this category. Following its escape from cultivation in Ottawa in 1932 (Dore, 1968), it rapidly invaded shorelines in and around Lake Erie, Lake Ontario, and the Saint Lawrence river valley (Catling and Porebski, 1995). While turions and whole plants are moved by water, birds, and boats, part of this expansion was also likely due to the transportation of seeds by birds (Catling and Porebski, 1995). *Hydrocharis morsus-ranae* has recently been recorded spreading on and around the shores of Lake Huron in Michigan (Hackett et al., 2014), in Lake Champlain in Vermont and New York, and in portions of Adirondack State Park (Martine et al., 2015). This species is one of five invasive alien plants that have had a major impact on Canadian natural ecosystems (Catling et al., 2003). It has a well-documented ability for rapid vegetative reproduction over successive years, producing turions that overwinter on lake and pond bottoms, floating in the spring to sprout new stolons (Scribailo and Posluszny, 1985). *Hydrocharis morsus-ranae* also has well-documented detrimental impacts in areas where it naturalizes, reducing the biomass of native aquatic plants (Catling et al., 1988). Thick, intertwining mats of this species also hamper water traffic and transportation, and inhibit recreational activities such as fishing and swimming (Catling et al., 2003). In the United States, mechanical removal trials have been carried out at multiple sites (LCA, 2011; Quirion et al., 2016); however, there is no highly effective, large-scale, and economically-viable means of control once *H. morsus-ranae* becomes naturalized within an environment (Hackett et al., 2014).

6. Acknowledgements

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SUGGESTED CITATION

PPQ. 2017. Weed risk assessment for *Hydrocharis morsus-ranae* L. (Hydrocharitaceae) – European frog-bit. United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (PPQ), Raleigh, NC. 22 pp.

DOCUMENT HISTORY

December 15, 2017: Version 1.

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Appendix A. Weed risk assessment for *Hydrocharis morsus-ranae* L. (Hydrocharitaceae)

Below are 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 in which 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 and Lüönd, 1982; NGRP, 2017). NGRP (2017) includes Algeria and Morocco in its native range, though we were unable to find additional evidence of its presence in these countries. In 1932, it was introduced into a Canadian arboretum for cultivation from a botanical garden in Switzerland (Catling et al., 2003; Catling and Porebski, 1995). 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 (Dore, 1968; OISAP, 2012). Catling and Porebski (1995) detail the spread of *H. morsus-ranae* in North America from Ottawa south to New York. From a single turion, an individual plant may grow to cover an area 1 m in diameter in a year (Cook and Lüönd, 1982). It continues to spread south of Canada (Zhu et al., 2014), and has become established in multiple locations along the New York and Vermont shores of Lake Champlain, as well as in at least one area within the interior of the Adirondack State Park (Martine et al., 2015). Within northeastern New York, the invasiveness of *H. morsus-ranae* has been ranked as "very high" (APIPP, 2015). 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 and 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* plants are sold for water garden use within the species' native range, particularly in the United Kingdom (RHS, 2017). It is also sold by at least one nursery in the United States (WWGNP, 2017). However, we found no evidence that it has been bred for any traits resulting in reduced weed potential.
ES-3 (Significant weedy congeners)	y - low	1	The genus *Hydrocharis* contains three species (Cook and Lüönd, 1982). *Hydrocharis dubia* has been identified as a weed (Randall, 2017), though we found no information indicating that it is a significant weed. Because this genus is very small, we expanded the scope of this question to include the Hydrocharitaceae genus

			Limnobium, which is very similar to *Hydrocharis* (Catling et al., 2003; Cook and Lüönd, 1982). *Limnobium* contains two species (Cook and Urmi-König, 1983). One species, *Limnobium laevigatum*, is considered a serious weed in California because it spreads rapidly, forms dense mats, and causes problems for boating, fishing, and public infrastructure (Cal-IPC, 2017). This species is regulated as a state noxious weed by the state of California (CDFA, 2016).
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 a shade level of 70% was an ideal cover to actively 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	0	This species is neither a vine nor a terrestrial herb with a basal rosette (Catling et al., 2003; Cook and Lüönd, 1982).
ES-6 (Forms dense thickets, patches, or populations)	y - negl	2	The dense growth of *Hydrocharis morsus-ranae* 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 plant (Catling et al., 2003; Scribailo and Posluszny, 1986) 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 (Haynes, 2000) 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 nitrogen-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 and Posluszny, 1985).
ES-11 (Self-compatible or apomictic)	? - max	0	The majority of plants are dioecious (Catling and Dore, 1982; Scribailo et al., 1984), with male and female flowers occurring on different plants. However, Scribailo and Posluszny (1984) found that 5-10 percent 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 monoecious individuals, we answered unknown.
ES-12 (Requires specialist pollinators)	n - low	0	Multiple fly species of the families Ephydridae, Syrphidae and Scatopsidae and solitary bees of the family Halictidae (Dialictus spp.) were observed visiting flowers (Scribailo and Posluszny, 1984). Of these, bees and the fly species *Toxomerus marginatus* were the most efficient pollinators, based upon observed flower visitation patterns and insect pollen loads (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	b - low	1	*Hydrocharis morsus-ranae* is a perennial species (Catling et al., 2003) which reproduces both vegetatively and sexually (Cook and Lüönd, 1982). Vegetatively, this species produces turions, modified stolon buds adapted for overwintering (Catling et al., 2003). Turions are produced in the autumn, detach, and sink

annuals; (c) 2 or 3 years; (d) more than 3 years; or (?) unknown]			before winter when plant tissue at the water's surface begins to die back, 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 (Catling et al., 1988; ISSG, 2005). We did not find any evidence suggesting that *H. morsus-ranae* avoids autumn die-back and turion formation even in warmer environments. Turion formation and dormancy is determined by an antagonistic interaction between temperature and photoperiod. Between 15° C and 25° C, the higher the temperature, the shorter the required photoperiod for turion initiation (Cook and Lüönd, 1982). Below 10° C, turions will not form, while above 25° C, they will form continuously (Cook and Lüönd, 1982). In sexual reproduction, fruits mature underwater and detach from the plant in late autumn (Cook and Lüönd, 1982). Seeds undergo a chilling dormancy period similar to that of the turions, whereby germination takes place after water temperature reaches 15° C (Cook and Lüönd, 1982). Alternate answers for the Monte-Carlo simulation were both "a," as plant cuttings and stolons transported to other areas may regenerate into new individuals (Catling et al., 2003; Catling and Dore, 1982; Dore, 1968; Minshall, 1959).
ES-14 (Prolific seed producer)	n - low	-1	In its native range in Poland, plants produce 3000-4000 seeds/m^2 annually (Toma, 2013). In Rondeau Park, Ontario, however, plants produce about 250 seeds/m2. This may be because male plants outnumber female plants by about 8.5:1 in this location (Scribailo and Posluszny, 1984). Because neither estimate met our threshold for prolific seed production, 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	Anchors, ropes, boat motors, etc. break up interwoven mats of ramets, and subsequently transport plant matter between water bodies (Catling et al., 2003; Catling and 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 and Mikulyuk, 2009). Without definite evidence that this species follows a trade pathway, however, we answered unknown with maximum uncertainty.
ES-17 (Number of natural dispersal vectors)	2	0	Propagule 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 dehisce underwater and split to release the seed (Cook and 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 and Posluszny, 1985). Seeds are covered with a gelatinous mass when they emerge from the fruit (Catling and Dore, 1982; Cook and Lüönd, 1982). Vegetative turions (winter buds) 5-7 mm in

			length, ellipsoidal, are produced on stolons and detach and sink underwater (Cook and Lüönd, 1982).
ES-17a (Wind dispersal)	n - negl		Seeds are released underwater, and have no adaptations for wind dispersal (Scribailo and Posluszny, 1985).
ES-17b (Water dispersal)	y - negl		After pollination, flowers are pulled underwater, and fruits are released under water (Cook and Lüönd, 1982; Scribailo and Posluszny, 1984). The fruits sink to the bottom of the body of water once they mature and split open, releasing the seeds in a gelatinous mass (Cook and Lüönd, 1982). These seeds may float to the surface and travel via surface currents (Cook and Lüönd, 1982). Plants produce overwintering turions which are dispersed by water currents (Scribailo et al., 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 beaks or feet, and seeds, which are surrounded by a gelatinous mass, may adhere to birds (Catling and 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). The establishment of *H. morsus-ranae* in isolated bodies of water suggests that transportation of seeds by birds may be important for dispersal (Catling and Dore, 1982; Catling and Porebski, 1995).
ES-17d (Animal external dispersal)	? - max		The gelatinous coating of the seeds of *H. morsus-ranae* (Catling and Dore, 1982) may allow the seeds to attach to other water dwelling creatures (e.g. 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 and Posluszny, 1984), 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 and Dore, 1982; Dore, 1968); however, regeneration rates have not been studied. These fragments generally occur as cuttings on boat anchors, motors, propellers, 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. It is not listed by Heap (2017) as a weed that is resistant to herbicides. *Hydrocharis morsus-ranae* is susceptible to diquat, paraquat, chlorthiamid, terbutryn, 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)	7	2	

ES-23 (Number of precipitation bands suitable for its survival)	8	1	

IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	n - mod	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, *H. morsus-ranae* does not belong to a family known to contain parasitic plants (Heide-Jørgensen, 2008).
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 and Posluszny, 1985). Leaves of free-floating species exchange oxygen directly with the atmosphere rather than with the surrounding water (Pierobon et al., 2010). When the vegetation dies, it sinks and decays, consuming 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 (Catling et al., 2003; Nault and Mikulyuk, 2009; Zhu et al., 2015).
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 and Mikulyuk, 2009). *Hydrocharis morsus-ranae* is dominant where it occurs (Catling and Porebski, 1995; Toma, 2013). Native flora support a greater diversity of native aquatic animals than do the floating mats of *H. morsus-ranae* (Catling et al., 2003).
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 (Catling et al., 1988; Dore, 1968; Roberts et al., 1981). *Hydrocharis morsus-ranae* also outcompetes native vegetation (Catling and Porebski, 1995; Nault and Mikulyuk, 2009; Toma, 2013) 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 - mod	0.1	To date, no specimens of *Hydrocharis morsus-ranae* have been collected from any U.S. globally outstanding ecoregions as defined by Ricketts, et al. (1999). Field observations of this species have been recorded in Washington (Kartesz, 2017), though it is not clear if the locations of these observations overlap with any globally outstanding ecoregions. Based upon the impacts listed in Imp-N1 through Imp-N3, this species could alter globally outstanding habitats in areas where it does not yet occur, in ecoregions including Northern California coastal forests, British Columbia coastal forests, and Appalachian mixed mesophytic forests (Ricketts et al., 1999).
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 and 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 and Porebski, 1995). Mechanical and chemical methods are the most frequent control strategies utilized (Nault and Mikulyuk, 2009). *Hydrocharis morsus-ranae* is susceptible to the herbicides diquat, paraquat, chlorthiamid, terbutryn, and cyanatryn, though chemical control can result in adverse impacts on human health, beneficial and native plant species, and fauna (Catling et al., 2003). Multi-year manual plant removal trials have been conducted at Town Farm Bay on Lake Champlain in Vermont (LCA, 2011) and on the Grasse River in New York (Quirion et al., 2016). Alternate answers for the Monte Carlo simulation are both "b."
Impact to Anthropogenic Systems (e.g., cities, suburbs, roadways)			
Imp-A1 (Negatively impacts personal property, human safety, or public infrastructure)	? - max		*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 answered unknown.
Imp-A2 (Changes or limits recreational use of an area)	y - low	0.1	In Ontario and New York, thick mats impede water traffic, swimming, and 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 and 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 (Cook and Lüönd, 1982; Zhu et al., 2014). It invades many water sources used for recreation and public infrastructure (Catling et al., 1988; Dore, 1968; Nault and Mikulyuk, 2009). Mechanical and chemical control strategies are the most commonly used 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 yield)	n - mod	0	We found no evidence that this species affects crop or 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 and Galatowitsch, 2004); however, there is no direct evidence that this species has been identified as following a pathway of trade. The Southern African country of Namibia requires phytosanitary certificates for the entire family of Hydrocharitaceae (USDA, 2017), and Illinois, Indiana, Maine, Michigan, New Hampshire, Vermont, Washington, and Wisconsin regulate this species (NPB, 2017). 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 (CDFA, 2015; Zhu et al., 2014).
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	*Hydrocharis morsus-ranae* inhabits still, slow-moving waters such as canals (Catling et al., 2003), irrigation ditches, and water-intake pipes (Catling and Porebski, 1995; Zhu et al., 2014); 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 Secretariat, 2017).
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 three points in Canada near the edge of zone 4. Because of potential mapping error, we answered no because we did not 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.
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)	n - high	N/A	This species is reported to occur in Iran and Uzbekistan (Assadi and Wendelbo, 1977; NGRP, 2017), which are mostly represented by desert and steppe habitats. We found no specific evidence that this species occurs in these kinds of habitats, but see no reason why it could not, as long as there was a permanent source of water. However, without additional and more specific evidence we answered this question as no with high uncertainty.
Geo-C4 (Desert)	n - high	N/A	This species is reported to occur in Iran and Uzbekistan (Assadi and Wendelbo, 1977; NGRP, 2017), which are mostly represented by desert and steppe habitats. We found no specific evidence that this species occurs in these kinds of habitats, but see no reason why it couldn't as long as there was a permanent source of water. However, without additional and more specific evidence we answered this question as no with high uncertainty.
Geo-C5 (Mediterranean)	y - negl	N/A	Some points in France and a few in Spain. Two points in Israel. Present in one county in Washington, United States (Kartesz, 2017).
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	Two points in Italy. A few counties in southeastern Michigan, United States (Kartesz, 2017).
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’ reproductive structures (i.e. turions and seeds) undergo as a necessary dormancy period prior to germination (Catling et al., 1988; Cook and Lüönd, 1982; ISSG, 2005).
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	Two 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+ cm)	n - high	N/A	We found no evidence that it occurs in this precipitation band.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	n - negl	0	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 and Dore, 1982). Although this species is already present in the United States, we set this answer to no to evaluate the likelihood that additional material would enter the United States.
Ent-2 (Plant proposed for entry, or entry is imminent)	n - negl	0	*Hydrocharis morsus-ranae* has not been proposed for import into the United States.
Ent-3 [Human value & cultivation/trade status: (a) Neither cultivated or positively valued; (b) Not cultivated, but positively valued or potentially beneficial; (c) Cultivated, but no evidence of trade or resale; (d) Commercially cultivated or other evidence of trade or resale]	d - negl	0.5	This species is traded and sold for aquarium and water garden use, more commonly in Europe (RHS, 2017), but also in North America (eBay, 2017a, 2017b; WWGNP, 2017)
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	y - negl		The species escaped cultivation in Canada in 1939 (Catling and Dore, 1982), and has since become naturalized in areas surrounding the Great Lakes and Saint Lawrence river valley (Catling et al., 2003).
Ent-4b (Contaminant of plant propagative material (except seeds))	? - max		*Hydrocharis morsus-ranae* is spread through the aquarium and gardening industries (Catling et al., 2003); however, we found no specific evidence that it is commonly a contaminant of propagative plant material.
Ent-4c (Contaminant of seeds for planting)	n - mod	0	We found no evidence suggesting that this species is a contaminant of seeds for planting
Ent-4d (Contaminant of ballast water)	? - max		We found no evidence of *H. morsus-ranae* being transported in ballast water; however, this mode of dispersal is feasible given that the species is an aquatic plant.

Ent-4e (Contaminant of aquarium plants or other aquarium products)	y - high	0.04	Catling and Dore (1982) identify the discarding of aquarium contents into water bodies as a likely dispersal pathway, but acknowledge that this can rarely be proven for a given introduction. The species' production of thick, tangled vegetative mats on water surfaces, prolific vegetative reproduction via turions (Catling et al., 2003), and its sale as an aquarium and water garden plant (eBay, 2017b; RHS, 2017) make it a likely contaminant of aquarium products.
Ent-4f (Contaminant of landscape products)	n - mod	0	We found no evidence of this mode of contamination.
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	y - low	0.04	Much of the recent spread of *H. morsus-ranae* along the shore of Lake Ontario, into Lake George, and into Lake Champlain has been attributed to dispersal on boats and boat trailers (Catling et al., 2003).
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	n - low	0	We found no evidence that this species contaminates agricultural commodities, fruits, vegetables, grains, or other products for consumption. We chose low uncertainty as this mode of contamination seems unlikely for this species.
Ent-4i (Contaminant of some other pathway)	a - mod	0	We found no evidence of any other pathways.
Ent-5 (Likely to enter through natural dispersal)	y - low	0.06	*Hydrocharis morsus-ranae* inhabits multiple bodies of water lying on the Canada-United States border. Overall rates of spread for this species from its place introduction in Ottawa to three different points on the Canadian shore of Lake Erie averaged 5.5, 11.9, and 15.6 km/year (Catling and Porebski, 1995). It is highly likely that it spread into the United States through these water bodies.