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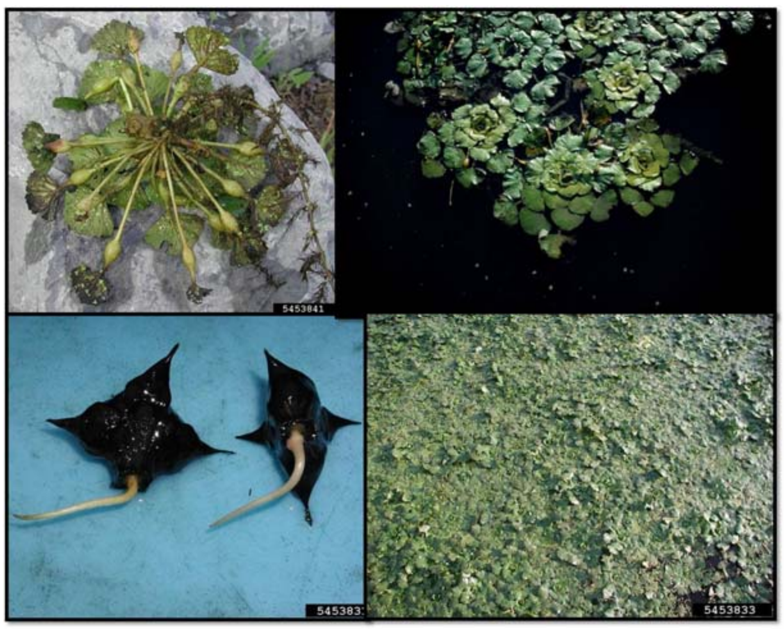
Version 1

Weed Risk Assessment for *Trapa natans* L. (Lythraceae) – Water chestnut



Top left: An upside-down plant showing the inflated petioles that keep individual leaves buoyant. Top right: Growth form in water. Bottom left: Barbed nuts of *Trapa natans* Bottom right: *Trapa natans* infestation (source: Leslie J. Mehrhoff, University of Connecticut, Bugwood.org).

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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, 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.

Trapa natans L. – Water chestnut

Species Information

Family: Lythraceae (NGRP, 2015; Hummel and Kiviat, 2004)

Synonyms: *Trapa natans* has, at times, been split into numerous, narrowly defined species (Weakley, 2015; Mabberley, 2008), including *Trapa bispinosa* (Agrawal and Mohan Ram, 1995) and *Trapa bicornis* (Hummel and Kiviat, 2004). However, currently the genus *Trapa* is recognized as including just one polymorphic species (Weakley, 2015). For this analysis, we treated *Trapa natans* as a single species and included the synonyms above when searching the literature.

Common names: Water chestnut, water caltrop, water nut, singhara nut, bull nut (Hummel and Kiviat, 2004).

Botanical description: *Trapa natans* is a rooted aquatic herb (Agrawal and Mohan Ram, 1995; Shalabh et al., 2012) that grows in water at a depth of 1.2-1.6 m, with a maximum growth depth of about 2 m (Dement'eva and Petushkova, 2010). Floating leaves are arranged in a rosette, with serrated upper leaves up to "5 cm wide and broadly rhomboid, triangular, deltoid or broadly ovate" (Mikulyuk and Nault, 2009). For a full botanical description, see Hummel and Kiviat (2004).

Initiation: In accordance with Part 413 of Michigan's Natural Resources and Environmental Protection Act, the Michigan Department of Agriculture and Rural Development (MDARD) was tasked with evaluating the aquatic species currently on Michigan's Prohibited and Restricted Species List (MCL 324.41302, 1994). The USDA Plant Epidemiology and Risk Analysis Laboratory's (PERAL) Weed Team worked with MDARD to evaluate this species.

Foreign distribution: *Trapa natans* has a very broad native distribution that includes many countries in Africa, Europe, and Asia (NGRP, 2015; GBIF, 2015). This species has become naturalized in India (Reshi and Rashid, 2012), Japan (Kadono, 2004), and Singapore (Keng and Keng, 1990), and was first detected in Canada in southern Quebec in 1998 (Darbyshire, 2003), where it is currently considered invasive (OIP, 2015). *Trapa natans* is extensively cultivated in Asia for consumption (Raju, 1999; von Mueller, 1888; Mabberley, 2008) and medicinal purposes (Shalabh et al., 2012), but it is not known to be cultivated elsewhere.

U.S. distribution and status: *Trapa natans* is present and has naturalized in several states: California, Connecticut, Delaware, Massachusetts, Maryland, New Jersey, New Hampshire, New York, Pennsylvania, Vermont, and Virginia (Kartesz, 2015). This species is regulated in Alabama, Arizona, Connecticut, Illinois, Indiana, Michigan, Oregon, South Carolina, Vermont, Washington, and Wisconsin (NPB, 2015). We found no evidence that *T. natans* is cultivated in the United States to any extent, including within botanical gardens. At Lake Champlain the states of New York and Vermont, the U.S. Fish and Wildlife Service, the Army Corps of Engineers, and the Lake Champlain Basin Program collaborated on a management program from the 1960s until the early 2000s to eradicate the species (Naylor, 2003). Maryland's Department of Natural Resources has also established a management and control program that focuses on preventing *T. natans* from establishing in new areas, and controlling the weed mechanically in its present range (Naylor, 2003).

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

1. *Trapa natans* analysis

Establishment/Spread Potential *Trapa natans* is invasive in the United States (Hummel and Kiviat, 2004), where it exhibits "explosive growth" (Ding and Blossey, 2005). Within its introduced range, it naturalizes and spreads, and it grows very quickly within waterways. *Trapa natans* has a very dense growth habit (Tall et al., 2011; Swearingen et al., 2002; ISSG, 2005; Strayer et al., 2003). It is prone to both natural (Swearingen et al., 2002; Hummel and Kiviat, 2004; Pemberton, 2002) and human-mediated (Dement'eva and Petushkova, 2010; Hummel and Kiviat, 2004) dispersal spreading via fish nets (Dement'eva and Petushkova, 2010), boats (Hummel and Kiviat, 2004), water currents (van der Pijl, 1982; Pemberton, 2002), birds (Swearingen et al., 2002; Hummel and Kiviat, 2004), and animals (Swearingen et al., 2002; Hummel and Kiviat, 2004). The seeds have a high germination rate of up to 87 percent in the field (Kurihara and Ikusima, 1991). We had a low amount of uncertainty for this risk element.

Risk score = 18 Uncertainty index = 0.07

Impact Potential *Trapa natans* poses the biggest impact within natural systems. It alters nutrient regimes (Tall et al., 2011; Caraco and Cole, 2002) and prevents up to 95 percent of light from penetrating through the water column (Tall et al., 2011; Groth et al., 1996), which inhibits photosynthesis at lower levels and prevents oxygenation of deeper waters. Further, *T. natans* displaces native macrophytes (Strayer et al., 2003; Hummel and Kiviat, 2004) and reduces species diversity (Pemberton, 2002; Countryman, 1977; Hummel and Kiviat, 2004). This species also poses a danger to the public, including injury from stepping on the barbed fruits (Kaufman and Kaufman, 2007; Hummel and Kiviat, 2004) and drowning in its thick growth (Hummel and Kiviat, 2004). This species also reduces the recreational use of areas that it has invaded (Pemberton, 2002; Swearingen et al., 2002; Ding et al., 2006; Hummel and Kiviat, 2004). We

[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).

found no evidence of impacts in agricultural systems. We had a very low amount of uncertainty for this risk element.
Risk score = 3.4 Uncertainty index = 0.06

Geographic Potential Based on three climatic variables, we estimate that about 82 percent of the United States is suitable for the establishment of *Trapa natans* (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 *Trapa natans* represents the joint distribution of Plant Hardiness Zones 3-13, areas with 0-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savanna, 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. *Trapa natans* inhabits temperate to tropical water bodies in sluggish areas with slower water flow (Hummel and Kiviat, 2004).

Entry Potential We did not assess the entry potential of *Trapa natans* because it is already present in the United States (Ding et al., 2006; Countryman, 1977).

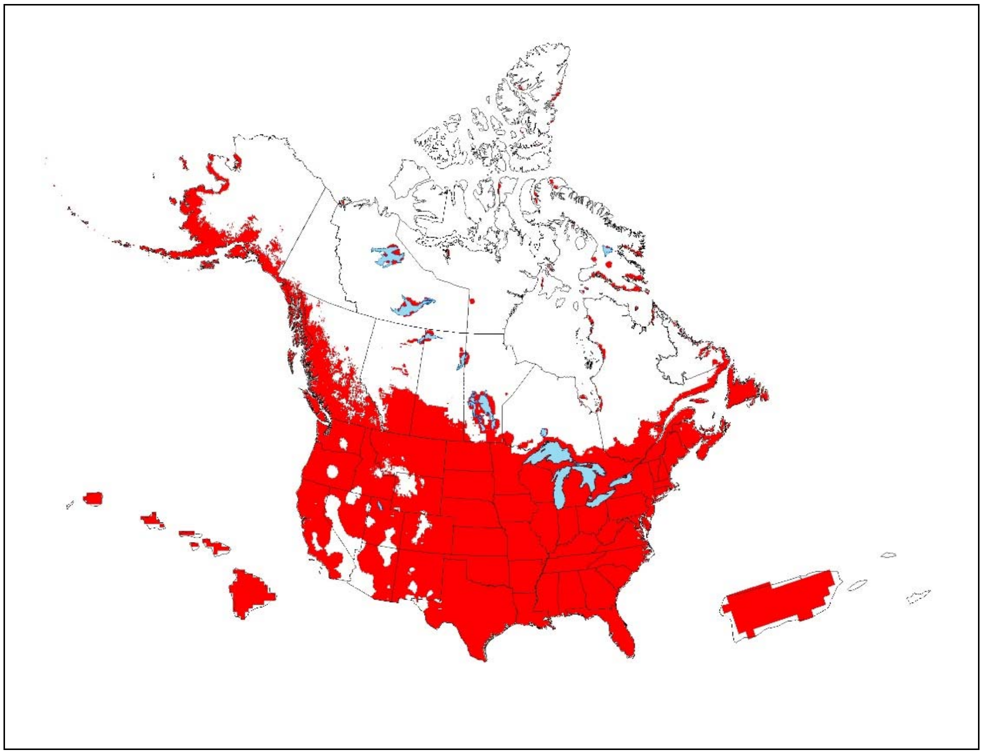

Figure 1. Potential geographic distribution of *Trapa natans* in the United States and Canada. Map insets for Hawaii and Puerto Rico are not to scale.

2. Results

Model Probabilities: P(Major Invader) = 89.6%
P(Minor Invader) = 10.1%
P(Non-Invader) = 0.3%

Risk Result = High Risk

Secondary Screening = Not Applicable

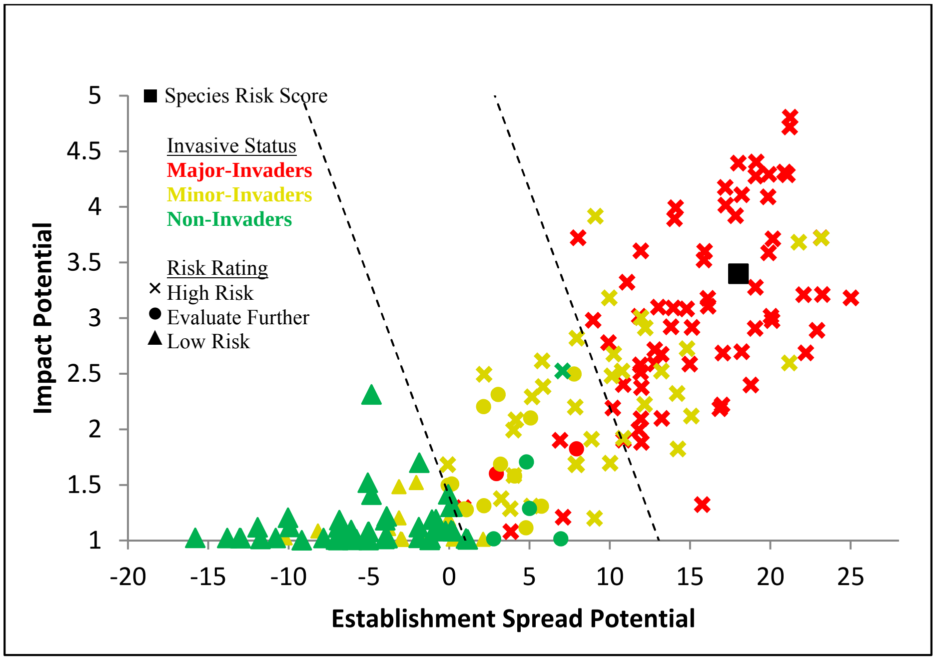

Figure 2. *Trapa natans* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

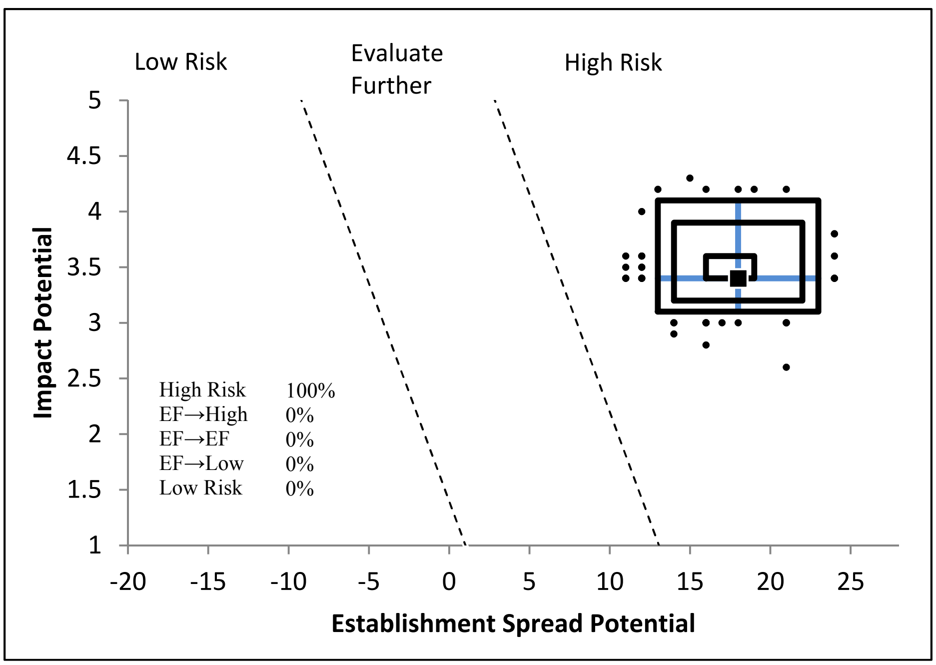

Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for *Trapa natans*. The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Trapa natans* is High Risk (Fig. 2). When compared with the species of known weeds used to validate the WRA model, this species ranked among other High Risk weeds. Our categorization of High Risk is well supported by the uncertainty analysis (Fig. 3). *Trapa natans* has been the focus of several management and eradication programs, most notably within Lake Champlain in the northeastern United States and within Maryland, near the Chesapeake Bay (Naylor, 2003). In Lake Champlain, more than $5 million was spent on control between 1982 and 2003 (Kaufman and Kaufman, 2007), and the states of New York and Vermont, the U.S. Fish and Wildlife Service, the Army Corps of Engineers, and the Lake Champlain Basin Program have collaborated on this management program for decades. The Maryland Department of Natural Resources' 2003 management plan outlined a $27,000 plan for control and management, with additional funds allocated for prevention and educational activities (Naylor, 2003). In the Chesapeake Bay region alone, $2.8 million has been spent in the past 20 years for control and monitoring programs (Eyres, 2009).

Chemical control of *T. natans* is difficult. The concentration of herbicide necessary to control growth is harmful to native flora and fauna (Hummel and Kiviat, 2004). Hand pulling is normally the most effective treatment for smaller populations (Groth et al., 1996; Countryman, 1977), whereas mechanical harvesters are used for large populations (ISSG, 2005). In addition, biocontrol methods with the leaf beetle *Galerucella birmanica* have shown promising results in experimental tests (Ding et al., 2006). This species produces barbed nuts (Swearingen et al., 2002; Ohwi, 1984; Pemberton, 2002) that pose a significant hazard to swimmers, boaters, and fishermen (Kaufman and Kaufman, 2007; Hummel and Kiviat, 2004; Swearingen et al., 2002), as well as those involved with the hand removal of the species. This species has been used in phytoremediation experiments (Sweta et al., 2015) and may be intentionally planted for remediation purposes. It can also alter ecosystem processes by removing large amounts of nitrogen from aquatic systems (Tall et al., 2011).

There is little dispute that *T. natans* is a serious pest within the United States (Gupta, 2011; Groth et al., 1996; Ding and Blossey, 2005; Ding et al., 2006; Countryman, 1977; Pemberton, 2002), but it is important to note that it is declining in other areas of the world. For instance, in Europe and the former Soviet Union, *T. natans* has been in natural decline (Dement'eva and Petushkova, 2010; Gupta, 2011). It is not clear what has caused this decline, but loss of habitat is thought to have contributed (Gupta, 2011). In the United States, the "complete decline" of *Trapa natans* has been observed in the Potomac River, but in this case, the decline was preceded by extensive management using underwater mowing (Orth and Moore, 1984; Carter and

Rybicki, 1994). Although there have been some instances of decline, it continues to have significant impacts in the United States.

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Appendix A. Weed risk assessment for *Trapa natans* L. (Lythraceae). 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	*Trapa natans* has a very broad native distribution that includes many countries in Africa, Europe, and parts of Asia (NGRP, 2015; GBIF, 2015). This species has been introduced and became naturalized elsewhere (NGRP, 2015), including India (Reshi and Rashid, 2012), the United States (Hummel and Kiviat, 2004), Japan (Kadono, 2004), and Singapore (Keng and Keng, 1990). This species was first reported for the United States in 1886 (Wibbe, 1886), and since then it has spread to several northeastern states (Kartesz, 2015; Pemberton, 2002). It was first detected in Canada in southern Quebec in 1998 (Darbyshire, 2003) and is expected to spread down the St. Lawrence River system (De Lafontaine and Costan, 2002). *Trapa natans* is considered one of the worst invasive aquatic species in India (Reshi and Rashid, 2012), where the species is categorized as invasive (i.e., spreading) (Khuroo et al., 2007; Jaryan et al., 2013). *Trapa natans* exhibits "vigorous spread" in Japan (Kurihara and Ikusima, 1991). After its initial introduction in Massachusetts, *T. natans'* "explosive" spread (Ding and Blossey, 2005) extended the species' introduced range throughout the northeastern United States and as far south as Chesapeake Bay (Ding et al., 2006). Within Lake Champlain, (located within the borders of New York, Vermont, and Quebec) total *T. natans* biomass increased tenfold within two years following the abandonment of the control program; eight "bushels" (286 lbs) were hand pulled in 1967, control ceased in 1968, and 80 "bushels" (1.5 tons) were then pulled in 1969 (Groth et al., 1996; Countryman, 1977). Alternate answers for the uncertainty simulation are both "e."
ES-2 (Is the species highly domesticated)	n - low	0	This species is sometimes used in food or used as a source of starch (Raju, 1999; von Mueller, 1888; Mabberley, 2008). *Trapa bicornis* and *Trapa bispinosa*, which are cultivated for food in Asia, have seeds with two stout horns (Keng and Keng, 1990) and are considered to be agricultural selections of *T. natans* (Pemberton, 2002). Researchers are evaluating the potential use of *Trapa natans* in a variety of areas, including phytoremediation (Sweta et al., 2015) and human nutrition (Stoicescu et al., 2012). However, we found no evidence that the species as a whole is highly domesticated or has been bred to reduce traits associated with weed potential.
ES-3 (Weedy congeners)	n - low	0	The genus *Trapa* includes just this one polymorphic species, which, at times, has been split into numerous, narrowly defined species (Weakley, 2015; Mabberley,

Question ID	Answer - Uncertainty	Score	Notes (and references)
			2008). None of these species are considered a significant weed (Randall, 2012).
ES-4 (Shade tolerant at some stage of its life cycle)	n - negl	0	*Trapa natans* grows in full sun environments (University of Wisconsin Sea Grant Institute, 2015; Hummel and Kiviat, 2004) and does not tolerate any shade (Golden, 2015). The species has both emergent and submerged leaves occurring on a single plant, but the plant has not been described to be completely submergent (Bitonti et al., 1996).
ES-5 (Plant a vine or scrambling plant, or forms tightly appressed basal rosettes)	n - negl	0	*Trapa natans* is neither a vine nor does it form tightly appressed basal rosettes; it is a rooted aquatic herb (Agrawal and Mohan Ram, 1995; Shalabh et al., 2012).
ES-6 (Forms dense thickets, patches, or populations)	y - negl	2	In parts of the Hudson River during the summer months, *Trapa natans* forms dense populations (Tall et al., 2011). Plants can form dense mats (Swearingen et al., 2002), sometimes covering several miles (ISSG, 2005). *Trapa natans* often occurs at densities between 100-1,000 g dry weight/m^2 (Strayer et al., 2003) and may grow to densities of up to 50 plants per square meter (Tsuchiya and Iwaki, 1984).
ES-7 (Aquatic)	y - negl	1	*Trapa natans* is an aquatic species (Mabberley, 2008) with a floating rosette of leaves and a central stem that is rooted (Ohwi, 1984; Pemberton, 2002; Groth et al., 1996).
ES-8 (Grass)	n - negl	0	This species is not a grass; it is a member of the Lythraceae family (NGRP, 2015; Hummel and Kiviat, 2004).
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 contain nitrogen-fixing species (Martin and Dowd, 1990). Furthermore, this is not a woody plant, but rather a rooted aquatic herb (Agrawal and Mohan Ram, 1995; Shalabh et al., 2012).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	*Trapa natans* produces viable seeds (Cozza et al., 1994). Populations are persistent through spontaneous dissemination of seeds (von Mueller, 1888). Kurihara and Ikusima (1991) found an 87 percent germination rate in the field.
ES-11 (Self-compatible or apomictic)	y - negl	1	Floral biology of *Trapa natans* favors self-pollination (Kadono and Schneider, 1986), and self-pollination is possible before the flower opens (Hummel and Kiviat, 2004). Insect movement within the flower results in the anther sacs being “pushed” against the stigma, facilitating self-pollination (Kadono and Schneider, 1986). Caging experiments conducted by Kadona and Schneider (1986) indicate that *Trapa natans* is “both self- and cross-compatible as well as apomictic.”
ES-12 (Requires specialist pollinators)	n - negl	0	Flowers are insect pollinated (Swearingen et al., 2002; Mikulyuk and Nault, 2009), but the specific pollen vector is unknown (Hummel and Kiviat, 2004). Field experiments and observations conducted by Kadono and Schneider (1986) show that “insects captured and examined for pollen revealed minimum amounts. These observations suggest that insects play a minimum role as cross-pollinators.” We are answering no, due to the

Question ID	Answer - Uncertainty	Score	Notes (and references)
			majority of literature pointing to insect pollination without a specific pollinator, we used negligible uncertainty.
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	*Trapa natans* plants are annuals (Swearingen et al., 2002; ISSG, 2005; Pemberton, 2002) and reproduce naturally only by seed (Countryman, 1977). Parent plants produce seeds by late June and die by fall, killed by the first frost (Countryman, 1977; Hummel and Kiviat, 2004). Seeds generally germinate the next year (Cozza et al., 1994), but seeds may remain dormant in the seed bank and remain viable for 3 to 12 years (Mabberley, 2008; Pemberton, 2002; Kurihara and Ikusima, 1991; Hummel and Kiviat, 2004). Most seeds germinate within two years (Mabberley, 2008). While this species can regenerate from vegetative fragments (Kaufman and Kaufman, 2007; ISSG, 2005), *T. natans* does not naturally fragment (Agrawal and Mohan Ram, 1995). Therefore, we answered "b," and alternate answers for the uncertainty simulation were both "c."
ES-14 (Prolific reproduction)	n - low	-1	*Trapa natans* often grows to densities of up to 50 plants per square meter (Tsuchiya and Iwaki, 1984), and very high density beds can produce about 100 rosettes/m^2 (Hummel and Kiviat, 2004). Seeds stored under natural conditions (in lakes) had a germination rate of about 80 percent (Cozza et al., 1994). Single-seeded fruit germinate early in the spring and can produce 10 to 15 plant rosettes, each of which can produce 15 to 20 seeds (ISSG, 2005). Very high density beds tend to be less sexually productive than low density beds (Hummel and Kiviat, 2004; Groth et al., 1996), yet calculating that each rosette can produce 15 to 20 seeds, these very high density beds would produce 1,500 to 2,000 seeds, which falls below our threshold of 5,000. Therefore, we answered no.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	*Trapa natans* may be introduced to new sites via fish nets (Dement'eva and Petushkova, 2010). Barbs on fruit can cling to nets, wooden boats, clothing, construction equipment, and other vehicles (Hummel and Kiviat, 2004).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	n - high	-1	We found no evidence that this species is dispersed as a contaminant of agricultural, forestry, or horticultural products. It does not seem likely that seeds or vegetation would be dispersed in this manner, due to seed and fruit morphology (see ES-17).
ES-17 (Number of natural dispersal vectors)	3	2	Fruit and seed description for questions ES-17a through ES-17e: Fruit are woody with 2-4 sharp barbs that are derived from the calyx and bear a single seed (Swearingen et al., 2002; Ohwi, 1984; Pemberton, 2002). Fruits are buoyant (Swearingen et al., 2002) and weigh 6 grams (Mikulyuk and Nault, 2009).
ES-17a (Wind dispersal)	n - negl		We found no evidence that propagules are wind dispersed, and given the size and weight of the fruits, it would be nearly impossible for them to disperse in this manner.
ES-17b (Water dispersal)	y - negl		While the flowers are borne above water, as the plant meristem develops, the fruit end up developing in the water (Pemberton, 2002). When mature, the fruit detach from the plants and float for some time, eventually falling

Question ID	Answer - Uncertainty	Score	Notes (and references)
			to the sediment layer, where the barbs help anchor the seeds in the hydrosoil (van der Pijl, 1982; Pemberton, 2002). Nuts and rosettes that are broken off can float to other areas on currents (Swearingen et al., 2002).
ES-17c (Bird dispersal)	y - high		Fruit cling to birds (Swearingen et al., 2002). Barbs cling to the plumage of Canada geese (Hummel and Kiviat, 2004). We answered yes, but with high uncertainty given that the size and weight of the fruit will most likely limit this kind of dispersal over long distances.
ES-17d (Animal external dispersal)	y - high		Fruit cling to animals (Swearingen et al., 2002). Barbs cling to mammal fur (Hummel and Kiviat, 2004). We answered yes, but with high uncertainty given that the size and weight of the fruit will most likely limit this kind of dispersal over long distances.
ES-17e (Animal internal dispersal)	n - low		We found no evidence that this species is dispersed internally; moreover, the woody barbs and husk of the fruit will most likely deter animals from eating it.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	y - negl	1	Seeds are viable for up to 12 years (Mabberley, 2008), although most will germinate within the first two years (Swearingen et al., 2002). In one experiment, some seeds remained dormant until the second year, at which time they germinated at the same rates as seeds that were dormant for only one winter season; this study suggests that plants are producing seeds that are physiologically heteromorphic (i.e., seeds of the same generation have different growth functionality) (Cozza et al., 1994). Seed longevity is three years under natural conditions (Kurihara and Ikusima, 1991). Seeds that do not germinate in the spring after they are released become part of the seed bank and may germinate at a later date (Kurihara and Ikusima, 1991). Seed banks may persist 10-12 years in sediment (Hummel and Kiviat, 2004).
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	y - negl	1	*Trapa natans* fragments will reestablish a plant (Kaufman and Kaufman, 2007; ISSG, 2005). When raking or pulling plants, the floating, uplifted plants and plant parts can spread the plant to new locations (Swearingen et al., 2002; Groth et al., 1996). Detached ramets can produce further ramets and seed, which may develop at any point downstream of the parent plant (Groth et al., 1996). The plant is commonly fragmented by mechanical removal and control methods (Kaufman and Kaufman, 2007) and cutting from boats, ropes, etc. (Hummel and Kiviat, 2004).
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - low	0	We found no evidence this species is resistant to herbicides. Furthermore, it is not listed by Heap (2015) as a weed that is resistant to herbicides. The herbicide 2,4-dicholorophenoxyacetic acid (2,4-D) has been used successfully to treat *T. natans* infestations; however, the high concentrations used are detrimental to both native plants and other wildlife (Hummel and Kiviat, 2004).
ES-21 (Number of cold hardiness zones suitable for its survival)	11	1	

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-22 (Number of climate types suitable for its survival)	10	2	
ES-23 (Number of precipitation bands suitable for its survival)	11	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, *T. natans* does not belong to a family known to contain parasitic plants (Heide-Jorgensen, 2008; Hummel and Kiviat, 2004).
Impacts to Natural Systems			
Imp-N1 (Changes ecosystem processes and parameters that affect other species)	y - negl	0.4	Dense mats of *T. natans* block 95 percent of light from entering the water column, thereby inhibiting photosynthesis and oxygenation at lower levels (Tall et al., 2011; Groth et al., 1996). Emergent plants of *T. natans* vent oxygen directly into the atmosphere, depleting oxygen from the surrounding water (Tall et al., 2011) and causing hypoxia and anoxia. In a study conducted in Hudson River tidal areas, Caraco and Cole (2002) measured dissolved oxygen (DO) levels of native plants beds and *Trapa natans* beds from July to August. DO in native macrophyte beds (*Vallisneria americana*) never declined below 5 mg/L, and varied between 6.3 and 11.8 mg/L, while beds of *Trapa natans* had DO levels lower than 2.5 mg/L, with measurements that varied between 0 and 6 mg/L. Furthermore, decaying plants reduce oxygen levels in the water, which increases the chance for fish kills (Kaufman and Kaufman, 2007; Swearingen et al., 2002). Because aquatic species with floating leaves deliver oxygen directly into the atmosphere, fixed carbon is retained in the aquatic system (Pierobon et al., 2010; Strayer et al., 2003; Goodwin et al., 2008). In the tidal portion of the Hudson River, beds of *Trapa* remove significant amounts of nitrogen each year because the low oxygen levels they create when the tide runs out, promoting microbial activity which denitrify the system through the production of nitrous oxide and nitrogen gas (Tall et al., 2011). In fact, although the large *Trapa* beds in this system represent only 2.7 percent of the total area of the tidal Hudson, they remove between 70 and 100 percent of the total nitrogen in this river (Tall et al., 2011).
Imp-N2 (Changes habitat structure)	y - low	0.2	*Trapa natans* displaces submerged native vegetation in the Hudson River (Strayer et al., 2003). *Trapa natans* can cover 100 percent of the water's surface and block 95 percent of sunlight, shading out all submerged vegetation (Hummel and Kiviat, 2004). Only tall, emergent species are able to grow in water chestnut beds and are unaffected by its interspecies competition (Hummel and Kiviat, 2004).
Imp-N3 (Changes species diversity)	y - negl	0.2	*Trapa natans* can dominate ponds, shallow lakes, and river margins, displacing native vegetation due to heavy shading of submersed and other floating plants

Question ID	Answer - Uncertainty	Score	Notes (and references)
			(Pemberton, 2002) and outcompeting native plants for sunlight (ISSG, 2005; Countryman, 1977; Swearingen et al., 2002). In the Hudson River, *Trapa natans* has replaced the native submerged species water celery (*Vallisneria americana* Michx.) and clasping pondweed (*Potamogeton perfoliatus* L.), as well as the introduced species Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Hummel and Kiviat, 2004). *Trapa natans* is of little use to wildlife (Swearingen et al., 2002; Countryman, 1977) and crowds out desirable aquatic plants that provide food and shelter to fish and waterfowl (Countryman, 1977). Displacement of submersed plants by *T. natans* is believed to cause the loss of many animal species and their replacement by more tolerant, more common, and in some cases non-native species (Hummel and Kiviat, 2004; Pemberton, 2002; Countryman, 1977; Hummel and Kiviat, 2004).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species?)	y - low	0.1	There is concern that *T. natans* populations in the Connecticut River will spread into the tidal marshes of that area, which have exceptional significance for rare plants and animals (Hummel and Kiviat, 2004). This species greatly reduces sunlight (Tall et al., 2011; Groth et al., 1996) and depletes oxygen in the water column it occupies, which may lead to deaths of native wildlife (Kaufman and Kaufman, 2007; Swearingen et al., 2002). Further, *T. natans* outcompetes and crowds out native species (ISSG, 2005; Countryman, 1977; Swearingen et al., 2002). The displacement of native species is believed to have replaced native wildlife populations as well (Hummel and Kiviat, 2004). These effects on natural ecosystems and native populations indicate that this species is likely to have a very serious impact on T&E species in areas that it invades.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions?)	y - negl	0.1	*Trapa natans*' predicted distribution in the United States includes globally outstanding ecoregions as defined by Ricketts et al. (1999). *Trapa natans* is already present as a noxious weed in areas of Pennsylvania and Maryland (Pennsylvania Sea Grant, 2015) that occur in a globally outstanding ecoregion (Ricketts et al., 1999). *Trapa natans* may move to nearby counties in globally outstanding ecoregions via the dispersal methods discussed in ES-17. Given the range of ecosystem and habitat impacts described under Imp-N1 and Imp-N2, this species is likely to affect globally outstanding ecoregions.
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	*Trapa natans* is a weed of natural areas in Australia (Randall, 2007). Control methods are described in several sources (Swearingen et al., 2002). The Nature Conservancy has organized teams of volunteers to pull rosettes from the environment in the eastern United States (Pemberton, 2002). Hand removal of small populations is best because it uproots easily and helps prevent additional spread (ISSG, 2005). Chemical and machine removal is more effective for large populations (ISSG, 2005). The U.S. Department of Agriculture's Agricultural Research Service has sponsored research to identify suitable

Question ID	Answer - Uncertainty	Score	Notes (and references)
			biological control agents (Pemberton, 1999). Field experiments by Ding et al. (2006) showed promise for biocontrol of *T. natans* in natural areas by *Galerucella birmanica*, a leaf beetle. In Lake Champlain, more than $5 million was spent on control between 1982 and 2003 (Kaufman and Kaufman, 2007). The Maryland Department of Natural Resources' 2003 management plan outlined a $27,000 plan for control and management, with additional funds allocated for prevention and communication efforts (Naylor, 2003). Alternate answers for the uncertainty simulation are both "b."
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Negatively impacts personal property, human safety, or public infrastructure)	y - negl	0.1	*Trapa natans* may have played a role in the drowning deaths of a woman and two children in the Hudson River in July 2001 due to entanglement (Hummel and Kiviat, 2004). Nuts that wash up on the shoreline are hazardous to walkers and bathers due to the sharp spines (Kaufman and Kaufman, 2007). Specialized methods for control are needed to prevent injury to people (Swearingen et al., 2002). Nuts float to shores where the sharp spines are a nuisance to bare feet (ISSG, 2005). Barbed spine-tips may break off in the skin and have caused infection (Hummel and Kiviat, 2004). The Asian custom of eating raw water chestnut contributes to the ingestion of giant intestinal fluke (*Fasciolopsis buski*) larvae that cause fasciolopsiasis (Hummel and Kiviat, 2004).
Imp-A2 (Changes or limits recreational use of an area)	y - negl	0.1	*Trapa natans* limits recreation and navigation (Pemberton, 2002). Dense growth eliminates or severely impedes most recreational activities such as swimming, fishing from the shoreline, use of small boats, and even duck hunting (Pemberton, 2002; Swearingen et al., 2002; Ding et al., 2006; Hummel and Kiviat, 2004). These large mats make areas inaccessible to fishermen (ISSG, 2005; Ding et al., 2006). Swimming and other beach-related activities are also hindered by the sharp nut hulls that accumulate on shores (Hummel and Kiviat, 2004; Swearingen et al., 2002).
Imp-A3 (Affects desirable and ornamental plants, and vegetation)	n - low	0	We found no evidence that this species affects 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	The Weed Science Society of America classifies this species as a weed (WSSA, 2010), and eradication efforts are currently in place for a population discovered in the Erie Canal (Clay, 2011), including hand pulling and monitoring for any re-establishment of the species in the area. Volunteers in New York utilize hand pulling as a control effort for the population in the Oswego River, near Battle Island, both popular tourist and recreation sites. Volunteers conduct these control efforts annually in an attempt to suppress and eradicate the local population (Yablonsky, 2015). Therefore, we answered "c," and the alternate answers for the uncertainty simulation were both "b."

Question ID	Answer - Uncertainty	Score	Notes (and references)
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - mod	0	Although this species is a weed of rice in India (Moody, 1989; Raju, 1999), we found no evidence that it reduces 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?)	n - mod	0	*Trapa bicornis* is regulated in New Zealand, while *Trapa* spp. in general are regulated in Australia and Nauru (APHIS, 2015). Within the United States, *T. natans* is regulated in Alabama, Arizona, Connecticut, Illinois, Indiana, Michigan, Oregon, South Carolina, Vermont, Washington, and Wisconsin (NPB, 2015). While this species is regulated in trade, we found no evidence that *T. natans* is likely to follow a pathway of trade as a contaminant, due to the size and morphology of its seeds. *Trapa natans* is cultivated as a food product within Asia (von Mueller, 1888; Mabberley, 2008; Keng and Keng, 1990), but is unlikely to move as a contaminant.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - mod	0	We found no evidence that this species affects the quality or availability of water.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - negl	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]	b - low	0.2	*Trapa natans* has been identified as a weed of rice in India (Raju, 1999; Moody, 1989). However, we found no evidence that this species is being controlled in this system. Therefore, we answered "b." Alternate answers for the uncertainty simulation were both "a."
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 - mod	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z3 (Zone 3)	y - low	N/A	A few points in Russia. It was noted in one source (Cozza et al., 1994) that this species undergoes a "chilling period" necessary for germination that may adapt some populations of the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4 °C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we answered yes, but with moderate uncertainty.
Geo-Z4 (Zone 4)	y - negl	N/A	A few points each in Canada, Russia, India, and China.
Geo-Z5 (Zone 5)	y - negl	N/A	A few points in the United States and Russia.
Geo-Z6 (Zone 6)	y - negl	N/A	The United States, Japan, Russia, Poland, and Austria.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Geo-Z7 (Zone 7)	y - negl	N/A	The United States, Japan, and France.
Geo-Z8 (Zone 8)	y - negl	N/A	China, Japan, France, Spain, Belgium, Germany, and Italy.
Geo-Z9 (Zone 9)	y - negl	N/A	China, Japan, France, and Greece.
Geo-Z10 (Zone 10)	y - negl	N/A	The United States, South Africa, Namibia, Botswana, Zambia, China, and Japan.
Geo-Z11 (Zone 11)	y - negl	N/A	South Africa, Zambia, and China.
Geo-Z12 (Zone 12)	y - negl	N/A	Sudan, Uganda, Burkina Faso, China, and Thailand.
Geo-Z13 (Zone 13)	y - low	N/A	A few points in Thailand.
Köppen-Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - mod	N/A	One point in Thailand.
Geo-C2 (Tropical savanna)	y - negl	N/A	Zambia, Sudan, Uganda, Burkina Faso, and Thailand.
Geo-C3 (Steppe)	y - negl	N/A	South Africa, Namibia, Botswana, Spain, and China.
Geo-C4 (Desert)	n - low	N/A	We found no evidence that it occurs in this climate class.
Geo-C5 (Mediterranean)	y - negl	N/A	The United States, Turkey, Greece, and Algeria.
Geo-C6 (Humid subtropical)	y - negl	N/A	The United States, South Africa, Zambia, China, and Japan.
Geo-C7 (Marine west coast)	y - negl	N/A	France, Spain, Germany, Belgium, and the Netherlands.
Geo-C8 (Humid cont. warm sum.)	y - negl	N/A	The United States, Japan, and South Korea.
Geo-C9 (Humid cont. cool sum.)	y - negl	N/A	The United States, Canada, China, Japan, and Russia.
Geo-C10 (Subarctic)	y - mod	N/A	A few points in mountainous areas of France and Greece. It was noted in one source (Cozza et al., 1994) that this species undergoes a "chilling period" necessary for germination that may adapt some populations of the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4 °C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we answered yes, but with moderate uncertainty.
Geo-C11 (Tundra)	y - mod	N/A	Two points in mountainous regions in France. It was noted in one source (Cozza et al., 1994) that this species undergoes a "chilling period" necessary for germination that may adapt the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4 °C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we answered yes, but with moderate uncertainty.
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)	y - mod	N/A	A few points in Uganda. There is no reason that this species could not survive in this precipitation band, as long as there is a permanent body of water.
Geo-R2 (10-20 inches; 25-51 cm)	y - negl	N/A	The United States, Botswana, Namibia, Burkina Faso, and Spain.
Geo-R3 (20-30 inches; 51-76 cm)	y - negl	N/A	South Africa, Zambia, Sudan, Burkina Faso, France, and Spain.
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	South Africa, France, Belgium, Germany, and Russia.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	The United States, Canada, Zambia, China, and Japan.
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	The United States, Japan, and France.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	China, Japan, and France.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	China, Japan, Thailand, and France.
Geo-R9 (80-90 inches; 203-229 cm)	y - negl	N/A	China, Japan, and France.
Geo-R10 (90-100 inches; 229-254 cm)	y - negl	N/A	China and Japan.
Geo-R11 (100+ inches; 254+ cm)	y - negl	N/A	China, Japan, Thailand, and Myanmar.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	We did not evaluate the entry potential of this species because it is already present and invasive in the United States (Ding et al., 2006; Countryman, 1977). It was cultivated in Asa Gray's botanical garden at Harvard University, in 1874 (Countryman, 1977). First observed to have escaped in North America in Concord, MA, in 1886 (Ding et al., 2006; Countryman, 1977).
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	

Question ID	Answer - Uncertainty	Score	Notes (and references)
Ent-5 (Likely to enter through natural dispersal)	-	N/A	