Weed Risk Assessment for *Nitellopsis obtusa* (Desv.) J. Groves (Characeae) – Starry stonewort

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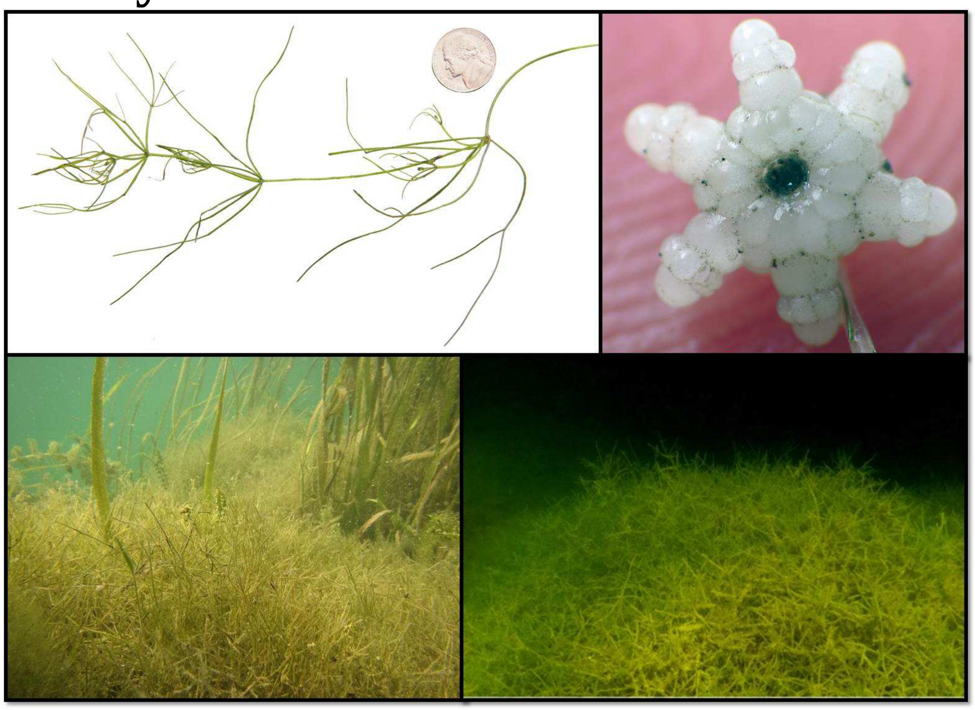

Top left: *Nitellopsis obtusa* growth form (source: Paul Skawinski, UW-Extension Lakes Program). Top right: *Nitellopsis obtusa* bulbil structure, with unique star-like shape (source: Paul Skawinski, UW-Extension Lakes Program). Bottom left: *Nitellopsis obtusa* growth (source: Paul Skawinski, UW-Extension Lakes Program). Bottom right: *Nitellopsis obtusa* infestation (source: Scott Brown, ML&SA).

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

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

We emphasize that our WRA process is designed to estimate the baseline—or unmitigated—risk associated with a plant species. We use evidence from anywhere in the world and in any type of system (production, anthropogenic, or natural) for the assessment, which makes our process a very broad evaluation. This is appropriate for the types of actions considered by our agency (e.g., State regulation). Furthermore, risk assessment and risk management are distinctly different phases of pest risk analysis (e.g., IPPC, 2015). Although we may use evidence about existing or proposed control programs in the assessment, the ease or difficulty of control has no bearing on the risk potential for a species. That information could be considered during the risk management (decision making) process, which is not addressed in this document.

***Nitellopsis obtusa* (Desv.) J. Groves – Starry stonewort**

Species Information

Family: Characeae (Naz et al., 2010), Phyllum: Charophyta (Soulié-Märsche et al., 2002). Although this species technically isn't a plant, because ecologically it is functioning as a plant (a macroscopic photosynthetic taxon) and because it is viewed as a weed (Wisconsin Department of Natural Resources, 2015a; Pullman & Crawford, 2015; Ford-Stewart, 2015b, c), we evaluated it using the same WRA process that we have used for other aquatic weeds of concern to Michigan. We note that the PPQ WRA model was not specifically designed for algae, but felt that because this species is a photosynthetic macroscopic lifeform similar to plants, it would be adequate.

Synonyms: No synonyms were found.

Common names: Starry stonewort (Pullman & Crawford, 2010; Stewart, 2004; Sleith et al., 2015).

Botanical description: Resembling a plant, *Nitellopsis obtusa* is a macroalgae (Schloesser et al., 1986; Geis et al., 1981) with long, variable-length, relatively straight branches arranged in whorls that attach at acute angles to stem nodes (Kipp et al., 2015). This species grows in both deep and shallow freshwater systems, clear water and eutrophic waters (Pullman & Crawford, 2010). For a full botanical description, see Kipp et al. (2015) or Bharathan (1983).

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

Foreign distribution: *Nitellopsis obtusa* is native to Asia (i.e. China, India, Iran, Japan, Myanmar, and Uzbekistan) and most of Europe (Soulié-Märsche et al., 2002; Geis et al., 1981; Schloesser et al., 1986). This species does not appear to have been introduced elsewhere, besides the United States (see below).

U.S. distribution and status: *Nitellopsis obtusa* was first identified in the United States within the St. Lawrence River, New York, by Geis et al. (1981). Since then, the species has spread to Indiana, Michigan, Minnesota, and Wisconsin (Sleith et al., 2015; Minnesota Department of Natural Resources, 2015a; Wisconsin Department of Natural Resources, 2015a; Kipp et al., 2015). It is generally accepted that *N. obtusa* was introduced into the United States via ballast water (Geis et al., 1981; Schloesser et al., 1986; Kipp et al., 2015), as this species can maintain permanent populations in water with a salinity of up to 5 parts per thousand (ppt), and can tolerate salinity fluctuations of up to 17 ppt for a week (Kipp et al., 2015). For reference, seawater has a salinity of about 35 ppt. This species does not appear to be cultivated to any extent. *Nitellopsis obtusa* is regulated as a noxious weed in Michigan (National Plant Board, 2015) and Wisconsin (Wisconsin Department of Natural Resources, 2015b).

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

1. *Nitellopsis obtusa* analysis

Establishment/Spread Potential *Nitellopsis obtusa* is a shade tolerant (Pullman & Crawford, 2010; Schloesser et al., 1986) aquatic macroalgae (Naz et al., 2010; Schloesser et al., 1986). *Nitellopsis obtusa* has a dense mat-forming growth habit (Pullman & Crawford, 2010) and grows up to 2 m tall in dense beds (Sleith et al., 2015). Meadows often form dense benthic barriers of up to eight feet thick (Brown, 2015). It has colonized more than 900 acres of a lake in three years under optimum conditions (Crawford, 2011). This species fragments easily (Sleith et al., 2015) and is easily transported to different lakes via boats and trailers (Pullman & Crawford, 2010). We had greater than average uncertainty here because information about reproduction and dispersal of this species is limited.
Risk score = 16 Uncertainty index = 0.24

Impact Potential *Nitellopsis obtusa* acts as a benthic barrier to prevent plant growth, reducing habitat complexity (Pullman & Crawford, 2010), which eliminates and reduces of niche (nursery) habitat, as well as loss of woody habitat complexity (sunken snags/stumps) is a direct result of dense *N. obtusa* growth when mats create a benthic barrier (Pullman & Crawford, 2010). This benthic barrier impedes fish from building nests, resulting in reduction in nesting areas, density of nests, and complete elimination of fish spawning activity (Pullman & Crawford, 2010). In anthropogenic systems, *N. obtusa* fouls boat motors and impedes swimming and fishing (Sleith et al., 2015). This species has also caused the closure of public boat launches (Ford-Stewart, 2015a). We had an average amount of uncertainty for this element because all of the evidence for the natural systems sub-element originated from a single paper discussing the impacts of *N. obtusa* in Michigan water bodies.
Risk score = 2.7 Uncertainty index = 0.16

Geographic Potential Based on three climatic variables, we estimate that about 49.7 percent of the United States is suitable for the establishment of *Nitellopsis obtusa* (Fig. 1). This predicted distribution is based on the species' known distribution

[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).

elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *Nitellopsis obtusa* represents the joint distribution of Plant Hardiness Zones 4-11, areas with 20-100 inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical savanna, humid subtropical, marine west coast, humid continental warm summers, humid continental cool summers. Note that in this weed risk assessment it was not clear if *Nitellopsis obtusa* occurs in Plant Hardiness Zone 10 or areas with 60-70 inches of precipitation. For this prediction, we assumed these environments are suitable for it.

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 turbidity and salinity, may further limit the areas in which this species is likely to establish. *Nitellopsis obtusa* grows in both deep and shallow freshwater systems, clear water and eutrophic waters (Pullman & Crawford, 2010). It can survive in waters with salinity levels of up to 5 ppt, and can tolerate salinity fluctuations of up to 17 ppt for a week (Kipp et al., 2015).

Entry Potential We did not assess the entry potential of *Nitellopsis obtusa* because it is already present in the United States (Geis et al., 1981; Sleith et al., 2015; Schloesser et al., 1986).

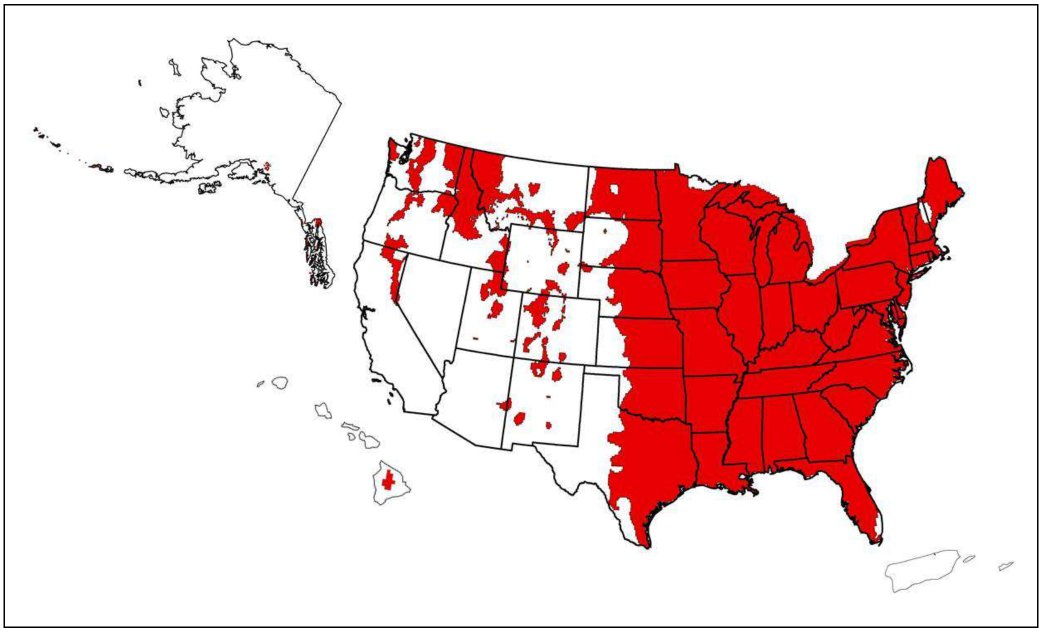

Figure 1. Predicted distribution of *Nitellopsis obtusa* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.

2. Results

Model Probabilities: P(Major Invader) = 82.6%
P(Minor Invader) = 16.7%
P(Non-Invader) = 0.6%

Risk Result = High Risk

Secondary Screening = Not applicable

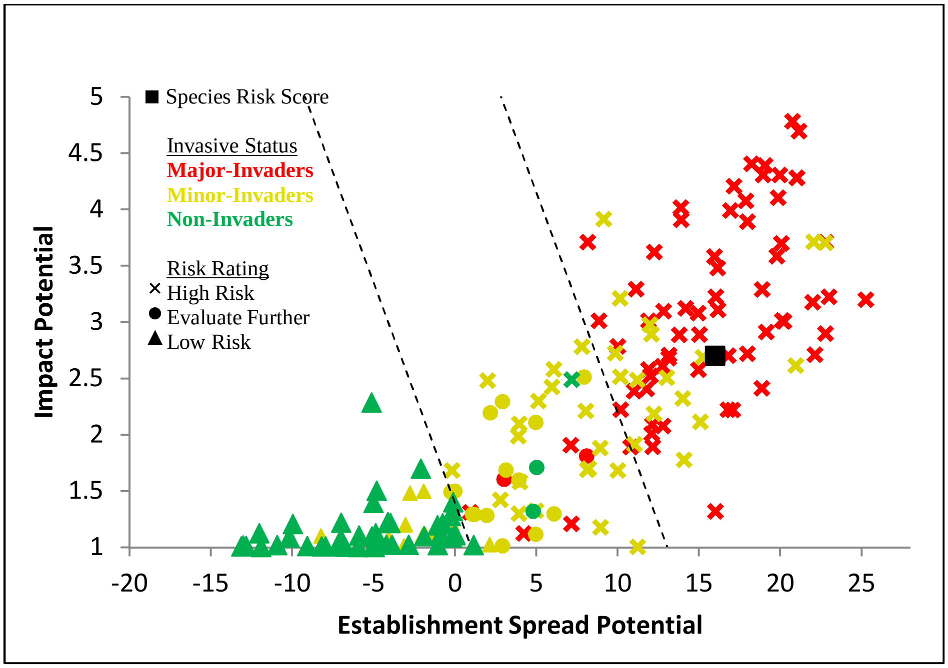

Figure 2. *Nitellopsis obtusa* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

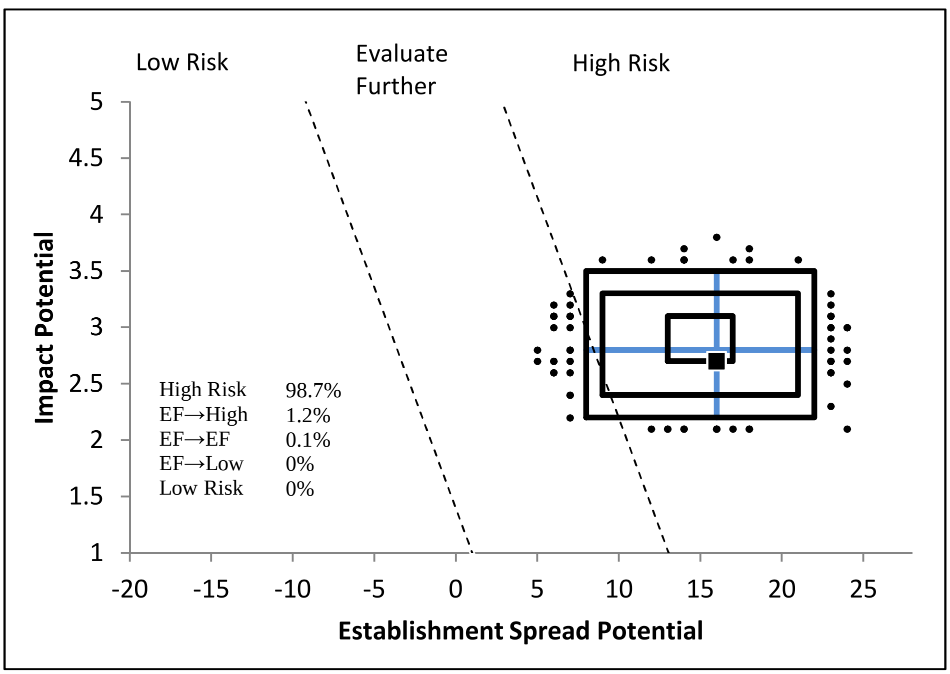

Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for *Nitellopsis obtusa*. The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Nitellopsis obtusa* is High Risk. (Figure 2). The uncertainty analysis of this species shows that 99.3% of simulated outcomes were within the high risk region (Figure 3), indicating that our result of High Risk is robust. Literature gaps resulted in greater range of uncertainty; much of our evidence came from a handful of sources, and having a greater variety of resources about its biology and impacts would help decrease uncertainty.

There is limited information available about the biology of *Nitellopsis obtusa*. Much of the existing literature is dedicated to the study of the family Characeae. In particular, we need additional information about its reproductive and dispersal mechanisms in the Great Lakes region. This species has been present in Michigan since 1983 (Sleith et al., 2015), and has only recently been discovered in Minnesota and Wisconsin (Minnesota Department of Natural Resources, 2015a; Wisconsin Department of Natural Resources, 2015a). The overwhelming response to this species has been to treat and control growth and spread, and little effort has been given to fully understanding its growth cycle. Understanding the effect of the various dispersal mechanisms (bird dispersal, water currents, boats and trailers, etc.) would allow decision makers and states to develop more effective methods for reducing its spread (Pullman, personal communication).

Despite the clear indications of impact, the fact that the evidence all came from a single source caused us to increase the uncertainty for each question in the Impact sub-element. This highlights the need for more conclusive research and studies into the impacts that this species may have on natural systems in other states in which it has been introduced, in addition to a focus on control and eradication. For the purpose of this WRA, we will There is a current "belief" that this species is difficult to treat and kill with chemical herbicides (Pullman, personal communication), however, it is susceptible to many herbicides, most notably common copper and endothall based herbicides (Pullman & Crawford, 2010; Pullman, personal communication). Although this species is susceptible to agaecides, these chemicals are not very effective in dense populations because the chemicals treat only the uppermost layers of vegetation and cannot penetrate dense growth effectively (Pullman, personal communication). Indiana Department of Natural Resources (DNR) is aggressively treating *N. obtusa* in several lakes using various herbicides to determine best control practices, with mixed results (Fischer, 2015). Wisconsin DNR is using a combination of chemical and physical control, including hand pulling and diver assisted suction harvesting, to control *N. obtusa* populations (Wisconsin Department of Natural Resources, 2015a). Minnesota DNR began treatment of a *N. obtusa* population blocking the main public access point in Lake Koronis in October of 2015, using a copper and endothall herbicide mixture. (Minnesota Department of Natural Resources, 2015b). An ongoing study from the The Nature Conservancy, Central Michigan University, and several other organizations seeks to identify the most effective methods of control, which may address some of these literature gaps.

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Appendix A. Weed risk assessment for *Nitellopsis obtusa* (Desv.) J. Groves (Characeae). 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 - mod	5	*Nitellopsis obtusa* is native to Asia (i.e. China, India, Iran, Japan, Myanmar, and Uzbekistan) and most of Europe (Soulié-Märsche et al., 2002). Beyond this range, it has only been introduced within the United States, where it was first identified in the St. Lawrence River in 1978, and Lake St. Clair in 1983 (Sleith et al., 2015). Since then, *N. obtusa* has been confirmed in Indiana, Michigan, Minnesota, New York, Pennsylvania, and Wisconsin (Sleith et al., 2015; Minnesota Department of Natural Resources, 2015a; Wisconsin Department of Natural Resources, 2015a; Kipp et al., 2015). In New York, the total geographic range of *N. obtusa* has not expanded, but the density of populations has increased, with more water bodies within the geographic range becoming "infested" (Sleith et al., 2015). In Michigan beginning in 2006, a rapid expansion of *N. obtusa* throughout inland lakes in Michigan was observed (Kipp et al., 2015). Under optimum conditions, *N. obtusa* can colonize more than 900 acres of a lake in three years (Crawford, 2011). Given its rapid spread in the United States, we answered "f", with moderate uncertainty, as data regarding the spread of this species is unavailable for most of the states in which it occurs. Alternate answers are both "e."
ES-2 (Is the species highly domesticated)	n - mod	0	We found no evidence that this species is highly domesticated.
ES-3 (Weedy congeners)	n - low	0	*Nitellopsis obtusa* is the only extant member of the genus *Nitellopsis* (Soulié-Märsche et al., 2002). The related genus *Nitella* (Pullman & Crawford, 2010) has 53 species (Sakayama, 2008), none of which appear to be considered major weeds. Species of *Nitella* are "rarely of importance as weeds" (DiTomaso et al., 2013)
ES-4 (Shade tolerant at some stage of its life cycle)	y - low	1	Seems to show no preference for shade or full sun (Pullman & Crawford, 2010). Occurs in the St. Lawrence river at 4.8 m depth and 6% light transmittance (Schloesser et al., 1986). Tolerant of low light conditions (Haas, 1994).
ES-5 (Plant a vine or scrambling plant, or forms tightly appressed basal rosettes)	n - low	0	*Nitellopsis obtusa* is neither a vine nor does it form tightly appressed basal rosettes. This species is a macroalgae with branching stems (Schloesser et al., 1986; Geis et al., 1981)
ES-6 (Forms dense thickets, patches, or populations)	y - negl	2	*Nitellopsis obtusa* has a dense mat-forming growth habit (Pullman & Crawford, 2010) and grows up to 2 m tall in dense beds (Sleith et al., 2015). Meadows often form dense benthic barriers of up to eight feet thick (Brown, 2015).
ES-7 (Aquatic)	y - negl	1	*Nitellopsis obtusa* is a macroalgae (Naz et al., 2010; Schloesser et al., 1986)
ES-8 (Grass)	n - negl	0	This taxon is not a grass, but rather is a member of the

Question ID	Answer - Uncertainty	Score	Notes (and references)
			family Characeae (Naz et al., 2010; Brown, 2015)
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	We found no evidence that this species fixes nitrogen, nor is it in a family known to have N-fixing capabilities (Martin and Dowd, 1990). Further, this is not a woody plant, but rather a macroalgae (Schloesser et al., 1986; Geis et al., 1981)
ES-10 (Does it produce viable seeds or spores)	y - mod	1	*Nitellopsis obtusa* is spread via oospores (Pullman & Crawford, 2010), which can remain dormant yet viable for decades (Stewart, 2004). The anecdotal evidence found indicates that oospores are viable, and in the absence of definitive literature, we are using moderate uncertainty. The majority of the literature we found focuses on the viability of bulbils, a somatic structure used in vegetative reproduction.
ES-11 (Self-compatible or apomictic)	n - negl	-1	*Nitellopsis obtusa* is a dioecious species (Sleith et al., 2015; Naz et al., 2010; Bharathan, 1987), meaning that male and female reproductive structures occur on different plants.
ES-12 (Requires specialist pollinators)	n - negl	0	We found no specific information about fertilization for *N. obtusa*. However, members of the Characeae family accomplish fertilization by means of water (von Marilaun et al., 1904) with the spermatozoids swimming and entering the female gametangium (area where gametes are produced) (Soulié-Märsche & García, 2015). Because an intermediary species is not needed for fertilization, we answered no with 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 - high	1	*Nitellopsis obtusa* is a macroalgae (Naz et al., 2010; Schloesser et al., 1986) that reproduces both sexually and asexually. Sexually-produced propagules are called oospores, while asexually-produced propagules are called bulbils (Naz et al., 2010). Bulbils have no dormancy period (Bharathan, 1987). Bulbils have been observed on all parts of the plant at all times of the year in Michigan lakes, but are particularly common on the plant parts that are closest to the sediments in the late fall and early spring (Pullman & Crawford, 2010). We found no information regarding when new individuals reach maturity and produce their own bulbils. No information was found regarding the generation time of oospores. Further, fragments of this species are capable of moving the species around (Sleith et al., 2015; Pullman & Crawford, 2010). Further research is necessary to determine resprouting potential of fragments. It seems unlikely that an aquatic algae would have a minimum generation time of more than three years. Thus, without additional information, we conservatively answered "b", with "a" and "c" as alternate answers for the Monte Carlo simulation.
ES-14 (Prolific reproduction)	? - max	0	We found no evidence regarding volume of propagule production, thus we answered unknown.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	This species fragments easily and may be spread via boat motors and trailers (Sleith et al., 2015; Pullman & Crawford, 2010).
ES-16 (Propagules likely to	n - mod	-1	We found no evidence that this species spreads as a

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
disperse in trade as contaminants or hitchhikers)			contaminant in trade. Oospores may be moved in soil or water, but we found no direct evidence of this. Therefore, we are answering no.
ES-17 (Number of natural dispersal vectors)	3	2	Propagule description for questions ES-17a through ES-17e: Bulbils are released below the soil surface and have no natural dispersal mechanisms (Bharathan, 1987). Oospores are 286 µm long, 272 µm wide (Naz et al., 2010)
ES-17a (Wind dispersal)	n - negl		We found no evidence that propagules are wind dispersed. Propagules are released below the surface of the water, as this species is entirely submerged (Schloesser et al., 1986; Haas, 1994). Because plant tissues are not exposed to the wind, we answered no with negligible uncertainty.
ES-17b (Water dispersal)	y - negl		Bulbils are released underwater below the soil surface (Bharathan, 1987) and disturbance of the sediment may move bulbils around (Wisconsin Department of Natural Resources, 2015a). Further, *N. obtusa* fragments easily (Sleith et al., 2015) and may be dispersed via water currents. Oospores may also be transported via water.
ES-17c (Bird dispersal)	y - high		Oospores are transported easily on bird feathers (Pullman & Crawford, 2010). Without further evidence, we answered with high uncertainty.
ES-17d (Animal external dispersal)	y - high		Oospores are easily transported on the fur of aquatic mammals (Pullman & Crawford, 2010). Without further evidence, we are answered with high uncertainty.
ES-17e (Animal internal dispersal)	? - max		We found no evidence regarding this method of dispersal for *N. obtusa* . However, bulbils of other members of the family Characeae (i.e. *Chara aspera* and *C. hornemannii*) fed to ducks remained intact but did not germinate (Proctor, 1962). Therefore, we answered unknown.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	y - mod	0	We found no evidence regarding seed banks, nor of long-term viability of oocytes or bulbils. Pullman and Crawford (2010) list spore and bulbil viability as one of the areas of research needed for *N. obtusa*. Oospores may be viable for decades, however further research is required regarding viability time (Broads Authority, 2008). Haas (1994) states that oospores of members of the family Characeae have very resistant wall structures that allow the oospore to remain viable for years. Stewart (2004) states that "stoneworts" produce durable spores, which can remain dormant yet viable for decades, allowing them to persist through periods when conditions are unsuitable for growth. We are answering yes, with moderate uncertainty, given the evidence presented for related species.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	? - max	1	Boat traffic can cause significant fragmentation (Pullman & Crawford, 2010). *Nitellopsis obtusa* fragments easily and may be spread as debris on boats and trailers (Sleith et al., 2015). These fragments act as disseminules that contribute to the spread of the plant within a lake and from lake to lake (Pullman & Crawford, 2010). However, without conclusive evidence regarding regeneration potential of fragments, we are answering unknown.
ES-20 (Is resistant to some herbicides or has the potential	n - mod	0	We found no evidence that this species is resistant to herbicides. Furthermore, it is not listed by Heap (2013) as

Question ID	Answer - Uncertainty	Score	Notes (and references)
to become resistant)			a weed that is resistant to herbicides. *Nitellopsis obtusa* appears to be highly sensitive to common copper and endothall based herbicides (Pullman & Crawford, 2010), but dense growth of the species may make it very difficult to treat with chemicals (Pullman, personal communication).
ES-21 (Number of cold hardiness zones suitable for its survival)	8	0	
ES-22 (Number of climate types suitable for its survival)	5	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 exhibits allelopathy under natural conditions. Berger and Schagerl (2004) found that *N. obtusa* exhibits allelopathic behavior toward cyanobacteria; however these tests were conducted in a laboratory setting under controlled conditions with *N. obtusa* extracts. We answered no, with moderate uncertainty, given the laboratory findings.
Imp-G2 (Parasitic)	n - negl	0	We found no evidence that this species is parasitic. Furthermore, *N. obtusa* does not belong to a family known to contain parasitic species (Heide-Jorgensen, 2008; Naz et al., 2010; Brown, 2015).
Impacts to Natural Systems			All of the evidence used in this sub-element is derived from a single, albeit exhaustive, source. Consequently, we used higher uncertainty than we normally would.
Imp-N1 (Changes ecosystem processes and parameters that affect other species)	? - max	0.4	*Nitellopsis obtusa* may act as a benthic barrier that contributes to the accumulation of phytotoxins, such as volatile fatty acids (VFAs), and may render the sediments inhospitable for plant growth until the conditions change (Pullman & Crawford, 2010).
Imp-N2 (Changes habitat structure)	y - mod	0.2	*Nitellopsis obtusa* reduces structural habitat complexity by physically and possibly chemically preventing the growth of aquatic macrophytes (Pullman & Crawford, 2010). Elimination and reduction of niche (nursery) habitat may result in increased mortality of juvenile fish species of both native and non-native species. Heavy *N. obtusa* growth also results in the loss of woody habitat complexity (sunken snags/stumps) beneath mats (Pullman & Crawford, 2010). These dense mats fill in and cover these woody complexities.
Imp-N3 (Changes species diversity)	y - low	0.2	Biomass has declined significantly in areas where *N. obtusa* has colonized (Pullman & Crawford, 2010). *Nitellopsis obtusa* directly impacts fish spawning habitat by the formation of a thick mat that serves as a physical barrier effectively impeding access to substrates for nest creation resulting in reduction in nesting areas, density of nests, and complete elimination of spawning activity in the areas of *N. obtusa* dense growth (Pullman & Crawford, 2010).

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
Imp-N4 (Is it likely to affect federal Threatened and Endangered species?)	y - mod	0.1	Threatened and endangered species are likely to be affected by *N. obtusa* given the impacts described under Imp-N2 and Imp-N3 The creation of a benthic barrier by dense *N. obtusa* growth may prevent plants from growing (Pullman & Crawford, 2010), eliminating and reducing nursery habitat for juvenile fish (Pullman & Crawford, 2010). These dense growths also prevent fish from spawning, and outcompete aquatic vegetation (Pullman & Crawford, 2010). This disruption of the ecosystem is likely to impact both habitat and food webs for T&E species.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions?)	y - mod	0.1	The geographic potential for this species (Figure 1) shows that it can establish in areas designated as globally outstanding ecoregions (Ricketts et al., 1999). *Nitellopsis obtusa* can alter habitats (Pullman and Crawford, 2010) and change species diversity (Pullman and Crawford, 2010). Because it can form dense and extensive mats (Pullman and Crawford, 2010) it is likely to affect globally outstanding ecoregions if it were introduced into these areas.
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	Indiana DNR is aggressively treating populations of *N. obtusa* in several lakes using various herbicides to determine best control practices, with mixed results (Fischer, 2015). Wisconsin DNR is using a combination of chemical and physical control, including hand pulling and diver assisted suction harvesting, to control *N. obtusa* populations (Wisconsin Department of Natural Resources, 2015a). The Runyan Lake Association in Tyrone, MI, treats Runyan Lake annually for *N. obtusa* to "preserve the current natural state of the lake as much as possible " (Runyan Lake Inc., 2015). An attempt was made to clear known traditional fish nesting sites with chemical controls during the spawning season in Big Lake, Oakland County, Michigan in 2008 (Pullman & Crawford, 2015). Alternate answers for the Monte Carlo simulation are both "b."
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Negatively impacts personal property, human safety, or public infrastructure)	n - mod	0.1	We found no evidence of this kind of impact.
Imp-A2 (Changes or limits recreational use of an area)	y - low	0.1	*Nitellopsis obtusa* fouls boat motors and impedes swimming and fishing (Sleith et al., 2015). As one of the filamentous algae that frequently detaches from the bottom to form a floating mat, *N. obtusa* contributes both to lake "scum" and mats that wash up on beaches (Kipp et al., 2015).
Imp-A3 (Affects desirable and ornamental plants, and vegetation)	n - mod	0	We found no evidence that *N. obtusa* affects desirable and ornamental 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	c - mod	0.4	Residents and lake management districts have called for the complete closure of boat launches and lakes infested with *N. obtusa* in an effort to prevent the species from spreading, reducing home values, and restricting recreational activity (Ford-Stewart, 2015b, c). Minnesota

Question ID	Answer - Uncertainty	Score	Notes (and references)
weed and evidence of control efforts]			DNR began treatment of a *N. obtusa* population blocking the main public access point in Lake Koronis in October of 2015, using a copper and endothall herbicide mixture. One public boat launch in Little Muskego Lake, WI was closed temporarily to prevent spread of the *N. obtusa* into or out of the lake, as well as to allow management teams to treat the boat launch for *N. obtusa* growth (Ford-Stewart, 2015a). (Minnesota Department of Natural Resources, 2015b). We used moderate uncertainty for this question due to the lack of sources labeling this species as an anthropogenic weed; however this may be due to the fact that *N. obtusa* is a relatively recent introduction outside of Michigan. Alternate answers for the Monte Carlo simulation are b and a.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - low	0	We found no evidence that *N. obtusa* is a pest of agricultural systems or that it occurs in these systems. Consequently, we answered no for most of the questions in this section and used low uncertainty. Further, stoneworts don't grow well in areas with agricultural runoff (Stewart, 2004).
Imp-P2 (Lowers commodity value)	n - low	0	We found no evidence.
Imp-P3 (Is it likely to impact trade?)	n - low	0	We found no evidence.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - low	0	We found no evidence.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - low	0	We found no evidence.
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	We found no evidence this species is considered a weed in production systems. Alternate answers for the Monte Carlo simulation are both "b."
GEOGRAPHIC POTENTIAL			Unless otherwise indicated, the following evidence represents geographically referenced points obtained from the Global Biodiversity Information Facility (GBIF, 2015).
Plant hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	There is no evidence that this species occurs in this hardiness zone.
Geo-Z2 (Zone 2)	n - negl	N/A	There is no evidence that this species occurs in this hardiness zone.
Geo-Z3 (Zone 3)	n - low	N/A	There is no evidence that this species occurs in this hardiness zone.
Geo-Z4 (Zone 4)	y - negl	N/A	The United States: New York (Sleith et al., 2015), Minnesota (Minnesota Department of Natural Resources,

Question ID	Answer - Uncertainty	Score	Notes (and references)
			2015b), Michigan (Schloesser et al., 1986; Hackett et al., 2014).
Geo-Z5 (Zone 5)	y - negl	N/A	The United States: New York (Sleith et al., 2015), Wisconsin (Ford-Stewart, 2015 a,b,c), Michigan (Schloesser et al., 1986; Hackett et al., 2014).
Geo-Z6 (Zone 6)	y - negl	N/A	The United States: New York (Sleith et al., 2015) and Michigan (Schloesser et al., 1986; Hackett et al., 2014); Sweden.
Geo-Z7 (Zone 7)	y - negl	N/A	Japan, Germany, Sweden, Finland.
Geo-Z8 (Zone 8)	y - negl	N/A	Japan, Belgium, Sweden, Denmark.
Geo-Z9 (Zone 9)	y - negl	N/A	The United Kingdom, France, Belgium.
Geo-Z10 (Zone 10)	y - mod	N/A	Occurs in Zone 9 and in Zone 11 (GBIF, 2015; Naz et al., 2010), so it follows that *N. obtusa* can also survive in Zone 10.
Geo-Z11 (Zone 11)	y - high	N/A	One location in Bangladesh (Naz et al., 2010).
Geo-Z12 (Zone 12)	n - negl	N/A	There is no evidence that this species occurs in this hardiness zone.
Geo-Z13 (Zone 13)	n - negl	N/A	There is no evidence that this species occurs in this hardiness zone.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C2 (Tropical savanna)	y - high	N/A	One location in Bangladesh (Naz et al., 2010).
Geo-C3 (Steppe)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C4 (Desert)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C5 (Mediterranean)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C6 (Humid subtropical)	y - low	N/A	Japan.
Geo-C7 (Marine west coast)	y - negl	N/A	The United Kingdom, France, Belgium, Germany.
Geo-C8 (Humid cont. warm sum.)	y - negl	N/A	Japan, the United States: Wisconsin (Ford-Stewart, 2015 a,b,c), Michigan (Schloesser et al., 1986; Hackett et al., 2014).
Geo-C9 (Humid cont. cool sum.)	y - negl	N/A	Japan, Sweden, Denmark, Switzerland, Germany, Finland, the United States: New York (Sleith et al., 2015), Wisconsin (Ford-Stewart, 2015 a,b,c), Michigan (Schloesser et al., 1986; Hackett et al., 2014), Minnesota (Minnesota Department of Natural Resources, 2015b).
Geo-C10 (Subarctic)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C11 (Tundra)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
Geo-C12 (Icecap)	n - negl	N/A	There is no evidence that this species occurs in this climate class.
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - negl	N/A	There is no evidence that this species occurs in this precipitation band.
Geo-R2 (10-20 inches; 25-51 cm)	n - low	N/A	There is no evidence that this species occurs in this precipitation band.
Geo-R3 (20-30 inches; 51-76	y - negl	N/A	The United Kingdom, Germany, Sweden, the United

Question ID	Answer - Uncertainty	Score	Notes (and references)
cm)			States: Minnesota (Minnesota Department of Natural Resources, 2015b).
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	The United Kingdom, Belgium, Switzerland, Sweden, Denmark, Finland, the United States: Minnesota (Minnesota Department of Natural Resources, 2015b), New York (Sleith et al., 2015), Wisconsin (Ford-Stewart, 2015 a,b,c), Michigan (Schloesser et al., 1986; Hackett et al., 2014).
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	France, Belgium, the United States: New York (Sleith et al., 2015), Wisconsin (Ford-Stewart, 2015 a,b,c), Michigan (Schloesser et al., 1986; Hackett et al., 2014).
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	The United States: New York (Sleith et al., 2015).
Geo-R7 (60-70 inches; 152-178 cm)	y - low	N/A	Occurs in areas where 50-60 inches of rainfall occur and in areas where 70-80 inches of rainfall occur (GBIF, 2015; Naz et al., 2010; Sleith et al., 2015), so it follows that *N. obtusa* can also survive in areas where 60-70 inches of rainfall occur.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	Japan, Bangladesh (Naz et al., 2010).
Geo-R9 (80-90 inches; 203-229 cm)	y - low	N/A	Japan.
Geo-R10 (90-100 inches; 229-254 cm)	y - low	N/A	Japan.
Geo-R11 (100+ inches; 254+ cm)	n - high	N/A	There is no evidence that this species occurs in this precipitation band. Because there is no reason to believe an aquatic plant couldn't survive in wetter areas, we used high uncertainty.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	Identified in the St. Lawrence River in 1978, and the St. Clair-Detroit River system in 1983 (Sleith et al., 2015; Schloesser et al., 1986).
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	

Question ID	Answer - Uncertainty	Score	Notes (and references)
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	