2019

Assessing the Invasive Potential of Najas minor in Maine

Kaitlin M. Volk
Colby College

Assessing the Invasive Potential of *Najas minor* in Maine

An Honors Thesis

Presented to

The Faculty of The Department of Biology

Colby College

in partial fulfillment of the requirements for the

Degree of Bachelor of Arts with Honors

by

Kaitlin Volk

Waterville, ME

May 18, 2019

Advisor: Catherine Bevier ______________________

Reader: Chris Moore ______________________

Reader: Judy Stone ______________________

Abstract

Najas minor (European naiad), a relatively new invasive aquatic plant to the state of Maine, is thought be spreading more aggressively than previous invasive aquatic plants have. Once established, *N. minor* grows into dense monocultures that replace all native plant species, leading to a disruption in ecosystem functioning, a loss in food and habitat for invertebrates and fish, and a decrease in the recreational value of a waterbody. Understanding the natural history of *N. minor* – how it is dispersed and how long seeds can survive – is important for understanding the invasive potential of the species. However, no previous studies have assessed the effectiveness of waterfowl as dispersal vectors of *N. minor* or if *N. minor* seeds can remain dormant in the sediment for multiple years. In this study, I fed *N. minor* seeds to captive domesticated mallards to see if seeds could survive passage. I collected and searched excreta for surviving seeds and tested the viability of all seeds recovered. I recovered a median of 10.7% of seeds from the excreta, 50% of which were still viable, suggesting that waterfowl can disperse *N. minor*. My dormancy study did not yield any results. I then created a spatially-explicit probabilistic model in R to assess the potential distribution of *N. minor* in Maine in twenty five and fifty years. While wildlife can disperse invasive species to new areas, human activities are still the biggest pathway for the spread of invasive species, and preventative actions should be taken whenever possible.

1. Project Overview

Najas minor is a relatively new and locally isolated invasive aquatic plant in Maine. Despite its small number of occurrences, the Maine Department of Environmental Protection's (DEP) inexperience with managing this invasive species combined with information gleaned from communications with other organizations about the species has left them concerned over the threat *N. minor* poses to the state of Maine. Management strategies for invasive species are often split into two categories: prevention and control. Prevention of the spread of *N. minor* is inherently more difficult than other invasive aquatic plants because of the small inconspicuous seeds that are the primary reproductive strategy of *N. minor*. These tiny seeds easily cling to the outside of boats and trailers and survive in motor intakes, wet wells, fishing gear, and anything else water may come in contact with.

Methods of controlling *N. minor* are likely to be as difficult as prevention. *Najas minor* easily fragments due to its brittle nature, which is where one of its common names "brittle naiad" comes from. Manual removal efforts (i.e., the use of divers or diver-assisted suction harvester (DASH) units) causes *N. minor* to break apart. These fragments contain seeds that are then dispersed throughout the waterbody on water currents. Once *N. minor* becomes established in an area, it forms very dense patches that can produce tens of millions of seeds per hectare (Lansdown 2014). Some of these seeds will likely germinate the following spring, but some seeds of *N. minor* might have the capacity to remain dormant for multiple seasonal cycles in the sediment and germinate years after their initial development. If *N. minor* seeds have this capacity, it would seriously complicate any attempt to remove the invasion both by manual and chemical means.

The Maine DEP has reasons to suspect that *N. minor* seeds possess this dormancy capacity. In 2001, shorefront property owners on Pickerel Pond in Limerick, Maine found another invasive aquatic plant, commonly known as hydrilla (*Hydrilla verticillata*), in the pond. Beginning in 2003 and continuing until 2010, the Maine DEP administered annual herbicide treatments in the pond to combat the dense and aggressive invasion (Schaffner 2019). During those seven years of herbicide treatments state biologists from the DEP conducted dive surveys to monitor the effects of the herbicide treatment on native species and to look for and remove any hydrilla plants. The biologists saw a complete disappearance of native *Najas* species that were present in the pond prior to the herbicide treatments. All hydrilla plants were gone by 2010 and herbicide treatments were halted. The native *Najas* species were found again in Pickerel Pond during surveys of the plant community immediately following the end of herbicide treatments. The DEP biologists hypothesize that the immediate reemergence of *Najas* sp. in Pickerel Pond was a result of germinating seeds that had remained dormant in the sediment during the duration of herbicide treatments, and *N. minor* might share this ability (pers. comm.). The immediate reappearance of the *Najas* sp. could also be caused by the dispersal of *Najas* seeds by local waterfowl, or a combination of both dormant seeds and waterfowl seed dispersal. While studies have been conducted on the dispersal of *N. marina* by waterfowl (Agami and Waisel 1986, 1988), no such studies have been conducted using *N. minor* seeds. A better understanding of the potential for seed dormancy and waterfowl dispersal in *N. minor* is important for understanding how the species could spread in the future and the relative effectiveness of traditional preventative and management strategies.

In this study I conducted experiments to determine the ability of *N. minor* seeds to be dispersed by waterfowl and to remain dormant for multiple growth and dormancy cycles. Given

the DEP's observations of the native *Najas* sp., I hypothesized that *N. minor* seeds can remain dormant for at least two dormancy cycles and predicted that they can remain dormant for over seven cycles. I also hypothesized that waterfowl are a vector for the spread of *N. minor* and predicted that a portion of seeds will survive the digestive process of captive mallards. Using results from these experiments, I developed a model that also considered *N. minor* natural history and island biogeography theory to project the potential future distribution of *N. minor* in Maine in 25 and 50 years. I hypothesized that the distance of a waterbody to an *N. minor* infestation and the surface area of a waterbody will both strongly effect the probability of the waterbody becoming invaded. Additionally, I hypothesized that in 50 years *N. minor* will be found in more waterbodies than variable-leaf milfoil (*Myriophyllum heterophyllum*) is currently found in (n = 32). Here I provide an overview of *N. minor* and its natural history to both better inform the model and the invasive potential of the species, but also in the hope that people will learn to identify the plant so that the invasive species can be better managed. The results of this study could help inform the Maine DEP in their fight to eradicate *N. minor* and other invasive aquatic plants from Maine. Understanding the dispersal mechanisms and reproductive strategies of an invasive species is key for preventing future invasions and managing current ones. The model could also help identify lakes that are at the highest risk of a *N. minor* invasion so that monitoring, other preventative methods, and funds could be targeted at those high-risk lakes.

2. The Natural History of European Naiad (*Najas minor*)

As the rate at which goods move around the world has increased, so too has the rate of the spread of invasive species (Figuerola and Green 2002). As of 2001, the estimated global number of known invasive species was over 120,000 (Pimentel et al. 2001). Some of these species, such as the autumn olive tree (*Elaeagnus umbellata*) in the U.S. and the cane toad

(*Rhinella marina*) in Australia, have been purposefully introduced for aesthetic or pest control reasons, while others, such as the emerald ash borer (*Agrilus planipennis*) in Canada and green crab (*Carcinus maenas*) in the U.S., have been introduced accidently as hitchhikers on traded goods. For example, the autumn olive tree was used by the Maine Department of Transportation to quickly and beautifully reforest roadsides (pers. comm.), and the emerald ash borer was introduced to Canada in imported wood packaging (CFIA 2014). Regardless of how they get there, invasive species have detrimental impacts on the ecosystems they enter. They typically compete with native species for resources, reduce biodiversity, reduce the health and resiliency of the ecosystem, disrupt human recreation, and cost billions in damages. Estimates of the cost of controlling invasive aquatic plants in the United States are about $100 million dollars annually, with additional economic losses ranging from $10 million to $10 billion dollars (Les et al. 2015). Invasive aquatic plants grow in dense patches that can choke waterways, making it impossible to swim, boat, or fish in certain areas. In extreme cases, invasive aquatic plants have completely stopped the flow of some streams (pers. comm.). Given the economic, environmental, and recreational costs of invasive species, their management is extremely important. It is only through the thorough understanding of the life history of each invasive species within its new ecosystem that effective management strategies can be developed. Currently, not nearly enough is known about *N. minor* as an invasive species to be confident about developing and implementing management plans.

2.1 Overview of Najas minor

There are 39 species within the genus *Najas* distributed globally, with the greatest diversity in tropical and sub-tropical climates. All species of *Najas* are water-pollinated, reproduce from seeds, and are true annuals, meaning individual plants only live for one growing

season and die after seed production (Zalewska 1999, Volunteer Lake Monitoring Program (VLMP) 2019). Unlike some other invasive aquatic plants, *N. minor* cannot reproduce vegetatively following fragmentation (Zalewska 1999). Identifying *Najas minor* can be difficult because the plant is inconspicuous and exhibits phenotypic plasticity (Les et al. 2015). *Najas minor*, known commonly as European naiad and less commonly as spinyleaf naiad, brittle water-nymph, lesser naiad, slender naiad, brittleleaf naiad, slender-leaved naiad, brittle naiad, and minor naiad (USDA 2019), is often misidentified as *Najas gracillima* or *Najas flexilis* (Les et al. 2015). The stems of *N. minor* are narrow and can grow to lengths of 2.5 m with leaves every 0.5-5.8 cm, becoming quite bushy at the top (Zalewska 1999, VLMP 2019). Leaves are long and slender, being 0.1-0.5 cm wide and 0.5-3.5 cm long, and become recurved and stiffened with age. Teeth are visible to the naked eye on both sides of each leaf. Like all other naiads, the leaves of *N. minor* have a sheath at the base (Zalewska 1999). The sheath of *N. minor* is blocky, 1-3 mm wide, and has five to eight teeth per margin (VLMP 2019). The blocky sheath, visible teeth, and stiffly recurved leaves are characteristics commonly used to distinguish *N. minor* from the three naiads native to Maine *N. gracillima*, *N. canadensis,* and *N. flexilis*. The leaves of these three native species are still toothed, but magnification is required to see the teeth, and the shoulders of the sheath are more rounded than the sheath of *N. minor*. The seeds of *N. minor* are slightly recurved are 1.5-3 mm long and 0.5-0.7 mm wide (Zalewska 1999). The seeds change color from light green to a dark purple/brown, hardening as they mature, and a single plant will often contain seeds at various levels of maturity. The seeds form in the leaf axils from July to September. In the late summer and early fall the stems of *N. minor* become brittle and break apart, causing the seeds to disperse in the leaf axil in the water currents. The seeds then germinate each spring and the cycle continues (VLMP 2019).

2.2 Native range and preferences of Najas minor

The native range of *N. minor* includes much of Europe, Asia, and northern Africa. Interestingly, populations of *N. minor* in Western Europe have been declining and *N. minor* is categorized as endangered in Japan and rare in Hong Kong (Zalewska 1999, Les et al. 2015) even though *N. minor* is often transported from waterbody to waterbody in contaminated rice planting stocks within its native range (Les et al. 2015). *Najas minor* grows best in freshwater ponds, lakes, and slow-moving streams with substrates of sand/gravel, and will grow in water up to 5 m deep. *Najas minor* also appears to be tolerant of moderate turbidity and eutrophication (VLMP 2019), and prefers a conductivity above 180 υS/cm and an alkaline pH between 7.4 and 8.0 (Zalewska 1999, June-Wells et al. 2013). This tolerance of turbidity and eutrophication, which are usually indicators of poor water quality, could increase the invasive potential of the species by broadening the range of lake conditions into which the species can establish.

2.3 Najas minor *as an invasive species*

Najas minor was first introduced into the U.S. in the 1930s and is now widespread across North America where it is considered a nonnative and invasive species since it outcompetes native aquatic plant species and disrupts human recreation (Les et al. 2015). *Najas minor* alters or eliminates habitat for native fish and aquatic invertebrates, alters nutrient cycles through its increased growth and decomposition rates, and chokes waterways (Pimentel et al. 2001). *Najas minor* has also been shown to have some anti-microbial and anti-biofilm capabilities, suggesting that it could be altering the surrounding microbial community (Topuzovic et al. 2015). The oldest verifiable record of *N. minor* in North American comes from an herbarium specimen collected in 1932 from Lake Cardinal in Ohio just four years after Lake Cardinal was created. The presence of a large private lake community and water gardening store suggests that water

gardening may have been the source of the new invasion (Les et al. 2015). However, the true source of *N. minor* in North America is still unknown. In 1934 *N. minor* was discovered in the Hudson River at the mouth of the Mohawk River in New York. Over time the plant spread up and down the Hudson and was intentionally introduced in at least two other New York lakes, likely to create a food source for local waterfowl (Les and Mehrhoff 1999, Les et al. 2015). In 1974, *N. minor* made its first appearance into New England. *Najas minor* is now established in 26 U.S. states, including Texas, Oklahoma, and California, and the majority of states east of and adjacent to the Mississippi River (USDA 2019, Lansdown 2014).

Of eighteen invasive aquatic plants in New England, *N. minor* is one of only two species where the initial means of introduction is completely unknown (Les and Mehrhoff 1999). Genetic analysis of *N. minor* populations across the U.S. suggests that there were at least two separate introductions of the plant and the presence of disjunct sites, especially in California, Iowa, Wisconsin, and West Virginia, suggest several more introduction events (Les et al. 2015). The two unique populations identified by Les et al. (2015) have different geographical distributions within the U.S., suggesting that the two populations have different ecological tolerances. If individuals from these two groups were to hybridize, it could increase the invasive potential of *N. minor* in North America (Les et al. 2015). It is unclear if the two groups have hybridized or from which group populations in Maine originate.

Possible sources of the original introductions for both of these genotypes include shipping contamination from Europe, improper disposal of aquarium plants, and escape from cultivation for waterfowl feed, which was advocated in the 1930s given that species of *Najas* are a common food source for waterfowl (Les and Mehrhoff 1999). Ducks are a likely primary dispersal agent for current *N. minor* population given that 20 genera of ducks are known to feed

on *Najas* sp. and that one duck can consume over 4,000 seeds (Les and Mehrhoff 1999). However, the transportation of seeds and plant material containing seeds on boats and fishing gear from one waterbody to another by humans likely plays a much larger role in the spread of *N. minor* across North America.

Najas minor is a relatively new invasive aquatic plant in Maine and is still only found in a very limited number of waterbodies. Current invasions of *N. minor* are restricted to three waterbodies in two watersheds along the Maine/New Hampshire border. For comparison, the most common invasive aquatic plant in Maine, variable-leaf milfoil, is found in over thirty water bodies across much of southern Maine (Schaffner 2019). It is important to note that variable-leaf milfoil has been in Maine for almost 50 years, whereas *N. minor* has been in Maine for only a decade. The small seeds of *N. minor* make it incredibly hard to decontaminate gear/boat, increasing its chances of being transported between waterbodies. Scientists at the University of Connecticut report that *N. minor* is spreading faster than other invasive aquatic plants in New England, particularly in Connecticut (pers. com.). Currently, much emphasis is placed on the threat and damage from Eurasian milfoil (*M. spicatum*), which is thought to be more aggressive than variable-leaf milfoil, because of its aggressiveness and ugly, conspicuous, snake-like appearance. However, reports of *N. minor* spreading faster than other invasive aquatic plants suggests that *N. minor* is a more aggressive invasive species that will require more attention and care to control than other species have previously.

Najas minor was discovered in Legion Pond in 2009 by a local citizen (Schaffner 2019). Five years later, members of the Maine DEP and New Hampshire Department of Environmental Services (NH DES) founds plants in Spaulding Pond in the towns of Lebanon, North Berwick, and Sanford during a survey of the variable-leaf milfoil infestation in the pond. Shortly

afterwards a volunteer plant patroller surveying Northeast Pond in the towns of Acton and Milton found *N. minor* in 2015 (Schaffner 2019). The Maine DEP started herbicide treatments in Northeast Pond in the summer of 2017 to try to decrease the density of the thick mats of *N. minor* there. Manual removal is also being used to control the invasion in both Northeast Pond and Spaulding Pond through the use of a DASH unit. In a DASH unit, divers remove invasive plants from the sediment and feed them into a suction hose, which transport the plants out of the water where they can be bagged and hauled away. New England Milfoil, a business specializing in invasive aquatic plant management, has been hired to conduct the manual removal. Because of the small seeds of *N. minor*, New England milfoil uses a completely separate set of gear for *N. minor* and all other invasive aquatic plants.

2.4 Waterfowl as dispersal agents

Aquatic habitats like the lakes and ponds that *N. minor* inhabit, typically exist in a heterogeneous landscape spatially isolated from one another by large tracts of land. Despite this, aquatic species are usually widespread, suggesting that passive dispersal of seeds or other reproductive bodies is a requirement for non-mobile aquatic plants (Figuerola and Green 2002, Wongsriphuek et al. 2008). Waterfowl, including dabbling and wading ducks, have long been considered vectors for the short- and long-distance dispersal of aquatic organisms including aquatic plants, invertebrates, snails, and mollusks. Ducks are common in aquatic systems in large numbers, are known to eat seeds, and migrate for long distances between breeding, molting, and wintering areas (Wongsriphuek et al. 2008). Additionally, ducks can serve as a potential mechanism for rupturing the seed coat, which is required for the germination of many aquatic plant species, through chemical and mechanical digestion. For instance, the seeds of *N. marina*, another species in the *Najas* genus, have very low rates of germination when the seed coat

remains intact, but germination rates drastically increase after the seed coat has been mechanically cracked (Agami and Waisel 1986, 1988). Other mechanisms to rupture the seed coat could include ingestion by fish and abrasion by the sediment. Support for ducks as vectors for the dispersal of aquatic plants mostly comes from anecdotal reports, but a number of laboratory and field experiments have since been conducted (Agami and Waisel 1988, Figuerola and Green 2002, Wongsriphuek et al. 2008, Kleyheeg at al. 2016). None of the studies have included *N. minor*, however.

The majority of these studies have focused on mallards, as mallards are extremely common, have large populations, and inhabit a very wide range of habitats (Wongsriphuek et al. 2008). Ducks can disperse aquatic plants in one of two ways. First, seeds or vegetation can attach to duck feet or plumage as the ducks move about normally. The ducks will then fly to another waterbody where the hitchhiking seed or vegetation can detach and establish. This form of external dispersal is known as epizoochory. The first demonstration of epizoochory was conducted by Darwin when he dipped a duck foot into a tank of pond snails and then waved the foot in the air to simulate flight (Kleyheeg et al. 2018).

Second, seeds can be ingested by ducks as a regular part of their diet. Some of these seeds will survive ingestion and be dispersed to new waterbodies into which the ducks defecate. This form of internal dispersal is known as endozoochory (Kleyheeg et al. 2018). Mallards, like other dabbling ducks, are highly opportunistic tactile feeders that filter out unseen seeds from the bottoms of aquatic habitats, making them prime candidates for both epizoochory and endozoochory (Wongsriphuek et al. 2008, Kleyheeg et al. 2018). Small seeds that float and have hook-like structures are best at epizoochory as they can easily attach to a duck's plumage and

stay attached during flight (Figuerola and Green 2002). The seeds of *N. minor* sink and lack any hook-like structures, so dispersal by epizoochory is unlikely.

Endozoochory is thought to be more frequent and more important than epizoochory for duck-mediated seed dispersal and has been more thoroughly studied (Kleyheeg et al. 2018). Diet studies on migratory waterfowl have documented large numbers of seeds from a wide variety of plant species, including 445 different plants in European studies (Figuerola and Green 2002, Kleyheeg et al. 2016). Ducks can ingest seeds both voluntarily, as is the case for ducks that directly forage on seeds, or involuntarily as they are feeding on vegetation, invertebrates, or other preferred food items. Involuntary ingestion is likely more conducive to dispersal as the duck lacks the machinery required to crush and breakdown seeds (Figuerola and Green 2002). In my personal experience working with *N. minor* seeds I have observed multiple types of seeds on the same plant just, as Agami and Waisel (1988) did in their work with *N. marina*. Both of these species of *Najas* simultaneously have seeds with soft seed coats and seeds with hard seed coats. Agami and Waisel (1988) hypothesized that the soft seeds, which are easily digestible, could be "bait type" seeds used to attract foraging ducks while the hard seeds, which are hard to digest, are the "dispersal type" seeds used for the spread and propagation of the species. *Najas* seeds are known to be eaten by at least nineteen species of waterfowl, and could be an important food source since the seeds are 10% protein and loaded with carbohydrates (Agami and Waisel 1986).

Once a seed is ingested by a duck, it passes through a series of digestive organs. It is first retained in the crop (an elastic portion of the esophagus) before entering the proventriculus (stomach) where it is mixed with acidic gastric juices. Next, it enters into the gizzard where chemical and mechanical breakdown is initiated from the muscles and ingested grit. Any surviving seeds will enter the small intestine where enzymes are added to breakdown starch, fat,

and proteins, and the bacterial flora breakdown fiber and carbohydrates. Lastly, the seeds enter the colon where water and nutrients are further extracted before finally exiting out of the cloaca as excreta (Kleyheeg et al. 2018). Seeds are generally resistant to each of these processes individually but can be very susceptible when all of the processes are combined. For instance, scarification from mechanical breakdown in the gizzard increases the damage caused by the gastric juices (Kleyheeg et al. 2016). The potential for mechanical destruction of seeds in the gizzard has made it the limiting factor for dispersal in most species (Kleyheeg et al 2018). The size of the gizzard is negatively correlated with seed recovery, meaning that larger gizzards destroy more seeds so fewer seeds are recovered after ingestion. Different duck species have different gizzard sizes and strengths, resulting in different dispersal capacities. Wild mallards tend to have well-developed gizzards (Wongsriphuek et al. 2008).

The gizzard size, type of grit in the gizzard, intestine and colon length, and diet of the individual duck or duck species will all affect its ability to disperse seeds (Figuerola and Green 2002). Intestine and colon length help determine the time period seeds are subjected to the digestive process, known as the retention time. Longer retention times mean a longer exposure to the gastric juices and elevated body temperature that could damage the seed, but also mean that the seeds could be transported further distances (Charalambidou et al. 2005. Kleyheeg et al. 2018). The length of the intestines and the size of the gizzard both change seasonally in response to a seasonal change in diet and are capable of changing within days of the new diet (Wongsriphuek et al. 2008). In autumn and winter mallards feed on a high fiber diet based on seeds which is possible because the gizzard is larger and intestines longer. In the spring and summer mallards feed on invertebrates/animal protein, which are digestible with a smaller gizzard and shorter intestine (Charalambidou et al. 2005, Kleyheeg et al. 2018). This means that

seeds are more likely to successfully pass through the digestive system intact during late summer and early fall, which is when *N. minor* produces the most mature seeds (Kleyheeg et al. 2018). This plasticity in digestive function, however, likely varies amongst species of waterfowl and seems to also vary between sexes. Males on average have a larger gizzard, longer intestines, and more grit mass than females (Kleyheeg et al 2016).

In addition to the traits of the digestive system of ducks, the traits of different seeds also affect their chance of surviving the digestive process. The size, seed coat thickness, fiber content, and seed coat permeability have all been studied for their role in seed survival/resistance to digestion and the results of experiments on viability have been mixed (Figuerola and Green 2002, Kleyheeg et al. 2018). Some results indicate that resistance is higher for small seeds with hard seed coats while others have found no relationship between resistance, size, and hardness (Figuerola and Green 2002, Wongsriphuek et al. 2008, Kleyheeg et al. 2018).

Regardless of what affects the likelihood of a seed surviving the digestive process of a duck, seeds successfully pass through ducks in large quantities. Kleyheeg et al. (2016) identified 4,548 seeds of 66 taxa in 76 wild mallards, although only 1.4% of those seeds were from fully aquatic plants. On average, each duck had 45 ± 15 seeds, but one duck was found with over a thousand seeds in its digestive tract demonstrating the potential ducks have to be significant seed dispersers. In laboratory experiments seeds of various plant species took anywhere from 4 to 72 hours to be excreted (Agami and Waisel 1986, Charalambidou et al. 2005, Wongsriphuek et al. 2008). These retention times, especially the longer ones, would allow for a considerable dispersal distance as retention time and how far the duck is capable of flying both factor into dispersal distance (Figeurola and Green 2002).

Ducks can cover tremendous distances over relatively short periods of time, making them ideal long-distance and short-distance dispersers. Depending on the species, ducks have been documented flying at speeds from 48-78 km/h. Greenwing teal have been documented flying over 1200 km in under 24 hours and Pintails have been tracked to move 1,000 km every 72 hours (Figuerola and Green 2002). Mallards can fly 90 km/h during migration and could disperse seeds over 720 km away (Wongsriphuek et al. 2008). During non-migratory seasons, such as the summer breeding season, mallards and other waterfowl are still capable of long-distance seed dispersal through successive short dispersal events as they move from one local waterbody to the next (Figuerola and Green 2002). Understanding waterfowl as a dispersal agent will allow a better understanding of the ecology of aquatic plants, which in turn will improve conservation efforts when invasive plants are involved (Wongsriphuek et al. 2008). However, it is important to remember that humans have become the most influential agent for the dispersal of any species, including invasive ones, and have greatly accelerated the rate of this dispersal (Figuerola and Green 2002).

2.6 Dormancy and overwintering

As an annual plant in a seasonal climate, the seeds of *N. minor* delay germination until the spring and go into a dormancy period during the cold winter months. This means that instead of germinating immediately once deposited in the sediment, *N. minor* seeds overwinter in the sediment until conditions are conducive for growth (Handley and Davy 2005). Seed dormancy is considered an adaptive mechanism since it ensures that seeds germinate at the right time and place in order to maximize reproductive success (Wagner and Oplinger 2017). Seed dormancy is often broken by the scarification of the seed coat or by long periods of cold temperatures (Handley and Davy 2005). If exposure to low winter temperatures continues past that required to

break primary dormancy, many species of seeds can enter into secondary dormancy to prevent late germination (Handley and Davy 2005).

Primary dormancy in *N. minor* is broken by warming ambient temperature. The number of non-dormant and viable seeds in the sediment peaks in early spring the year after the seeds were produced. In one study, approximately 70% of viable seeds of *N. marina* germinated the first year after production, 50% of remaining viable seeds germinated the second year after production, and less than 20% of remaining viable seeds germinated the third year (Handley and Davy 2005). This suggests that *N. minor* seeds could survive in the seed bank for multiple years but that their chance of germinating decreases with time and that it's unlikely that seeds older than three years will germinate. Therefore, it is unlikely that *N. minor*, or any aquatic plant, will form a long-term seed bank because the seeds aren't able to dry like they are for terrestrial species (Handley and Davy 2005). This is good news, since the management of *N. minor* would become extremely complicated and costly if seeds could remain dormant in the sediment over multiple years of herbicide treatments. However, the potential for *N. minor* seeds to remain viable for three years still increases its invasive potential and complicates management strategies.

Through two separate laboratory experiments I assess the ability of *N. minor* seeds to survive multiple dormancy cycles and the digestive tract of ducks. I hypothesize that *N. minor* seeds are able to survive multiple dormancy cycles, making seeds able to survive in the seed bank for multiple years, and that a portion of seeds will successfully pass through the digestive tract of ducks, making ducks a dispersal vector for *N. minor*. Additionally, I project the potential future distribution of *N. minor* in Maine using a spatially-explicit probabilistic model that accounts for waterbody surface area, the distance of the waterbody from a *N. minor* infestation,

and the presence of a public boat ramp. I hypothesize that *N. minor* will move further into Maine over the next fifty years. Understanding the overwintering capacity, seed-dispersal pathways, and dispersal patterns of *N. minor* will help create better management strategies for this aggressive invasive aquatic plant.

3. Methods

On September 5, 2018 staff members from the Maine DEP collected two one-gallon Ziploc bags of *N. minor* from Spaulding Pond in Lebanon, ME. Plants were transported from the DEP office in Augusta to Colby College in Waterville, ME. I started seed collection on September 9, 2018 where I used forceps to remove all seeds from the plant matter, taking care to avoid crushing the seeds in the process. I kept the bags of plant matter at 4 °C in the dark to match the natural temperature of lake sediments and the temperature required to break dormancy after cumulative chilling of the seeds (Handley and Davy 2005). I placed the seeds into 1.25 ml plastic vials filled with distilled water with 20-100 seeds per capsule, and maintained under dark conditions at 4 °C. On January 7, 2019 I examined all seeds in the light and divided them into three categories based on their developmental stage reflected by their color and hardness: white to light green and soft, green to dark green and moderately hard, and brown and very hard (Handley and Davy 2005). I separated the seeds into these categories to control for seed maturity in the ability of seeds to survive multiple dormancy cycles and the digestive tract of ducks. After separation into these categories, I divided seeds back into the vials so that each vial had 50 seeds from one category and stored in the dark at 4 °C. Care was taken at every step of the process to minimize the risk of spreading *N. minor* through improper/accidental disposal of the seeds. I left all water used during the seed collection process to evaporate in an autoclave bag in a fume head,

and eventually autoclaved and disposed it. I placed all plant material in a trash receptacle, as deemed appropriate by state biologists at the Maine DEP.

3.1 Experiment 1: Overwintering

To test whether seeds remain dormant and viable for multiple growth and dormancy cycles, and to see if seeds that weren't mature by the end of the summer could mature over time, I placed 25 seeds from each of the three categories (light green, green, dark greenish-brown) in two square weigh boats (10 cm x 10 cm) filled with sand approximately 4 cm deep for a total of 50 seeds for each category. I planted seeds 1 cm apart and 1 cm deep (Blanchette 2017). I placed all containers at the bottom of a 25 gallon glass aquarium filled with aged tap water. I placed two bubblers at opposite ends of the tank to oxygenate the water, and placed the tank in an environmental chamber kept at 25 °C and 12L:12D. This was previously reported to be the optimal temperature and light cycle for germinating *N. marina* (Handley and Davy 2005). After seeds were given the chance to germinate (~two weeks), all seeds that didn't germinate by the end of the two weeks would be placed back into the dark at 4 °C to simulate the winter dormancy period for three weeks and the process would be repeated.

After seven weeks I did not observe any germination so I tried another method to germinate seeds by placing them between pieces of wet paper towel in petri dishes (Hussner et al. 2014). Again, I placed twenty-five seeds from each category in each of two circular petri dishes (9 cm diameter) lined with paper towels for a total of 50 seeds per category. I moistened the paper towel and wrapped the petri dishes in parafilm to reduce evaporation. I placed the petri dishes in the environmental chamber and maintained them at 25 °C eight weeks. For this iteration, I used a 14L:10D light cycle (Agami and Waisel 1986).

3.2 Experiment 2: Waterfowl dispersal

To determine the viability and recovery of seeds eaten by ducks, I used a small population of domesticated mallards at Freshening Farm in Pittsfield, Maine. I used a total of seven individuals, representing four different varieties of domesticated duck: calling ducks, magpie ducks, apple silver yard ducks, and a calling duck x mallard hybrid. All ducks were flightless to ensure *N. minor* seeds were not dispersed to nearby waterways by the ducks. During the experiment, ducks were kept individually in metal mesh dog kennels with hard plastic removable bottoms. Cages were kept near each to reduce stress since ducks are social animals. Each cage contained dishes of food and water. In the first trial each duck received duck and chicken feed integrated with 150 seeds: 50 light seeds, 50 green seeds, and 50 dark seeds. In all future trials I fed ducks 50 dark seeds since only dark seeds successfully passed through in the first trial and the number of light and green seeds was limited. Ducks were housed in the cages for 23 hours (10:00 am to 9:00 am the following morning) to ensure that the ducks had time to eat and pass the seeds. Water and food levels were continuously monitored and replenished over the 23 hours.

At the end of the 23 hours, ducks were returned to their normal group housing, and trays were labeled, collected, and transported back to Colby College. That day I picked through all excreta by hand to search for seeds that successfully passed through the ducks' digestive systems. To do this, I rehydrated and dissolved excreta in dishes of water to make it easier to break them apart and search for seeds. I placed recovered seeds into vials labeled with the duck they came from and maintained the vials in the dark at 4 °C for up to 4 days until I could complete analysis.

To test seed viability, I cut all seeds transversely using a fresh razor blade to expose the inner embryo. Twenty fresh dark seeds, selected at random from the supply of fresh uneaten seeds, were used as a control and also cut. I placed all seeds into vials filled with 0.75 ml of 1% 2,3,5-triphenyltetrazolium chloride solution (Moore 1985, Wagner and Oplinger 2017) and incubated them at 30 °C for 48 hours (Moore 1985). After 48 hours I observed dissected seeds under a dissecting microscope to determine color. Tetrazolium stains any cells that are actively respiring red, which indicates that the cells are alive and viable (Figure 1). In contrast, a yellow stain indicates a non-viable seed (Handley and Davy 2005). Once stained, I categorized seeds as viable if a dark red stain was present at the site where the seed was cut in half and the inner tissue was exposed to the tetrazolium solution. Seeds with a light pink color or that lacked stain all together were categorized as dead. I removed seed coats from each seed half after the tetrazolium treatment to better expose the stain color.

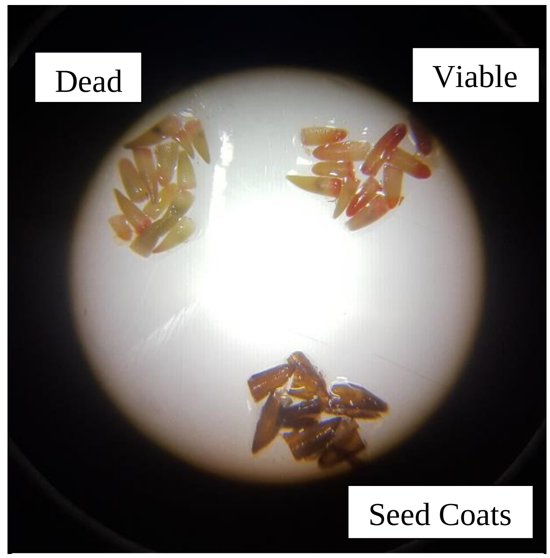

Figure 1. Assessing the viability of N. minor *seeds using tetrazolium to stain living tissue.*

3.3 Modeling the spread of N. minor *across Maine*

I constructed a probabilistic spatial model in R (v. 3.5.3) to model the potential future distribution of *N. minor* in Maine. Lakes and ponds in Maine are differentially vulnerable to invasive plant infestations, so I chose specific characteristics, including the surface area of each waterbody, how close waterbody was to an infested waterbody, whether or not the waterbody currently contained a population of *N. minor,* and if the waterbody had a public boat ramp, to

include in the model. I obtained these data from the Maine DEP database. Given the large number of freshwater lakes and ponds in Maine (n=6044), I restricted the analysis to 1069 lakes and ponds in southern Maine as defined by those with a longitude and latitude less than Great Pond in Belgrade, ME. This site is the furthest north and third furthest east an invasive aquatic plant has been identified in Maine (44.660093, -69.783626).

I used surface area as a proxy for the amount of habitat suitable for *N. minor* since detailed depth data for lakes is not readily available/accessible. Also, according to the theory of island biogeography, large areas are thought to increase the probability of a dispersal event since there is more space for a duck to land on as well as more space for the plant to grow. I included the presence of a boat ramp to include the potential for human-mediated dispersal of *N. minor* seeds. Lakes could either have no ramp, a carry-in ramp, or a trailer ramp. Trailer ramps are the most likely to facilitate the spread of aquatic plants as there is much more equipment that comes into contact with the water, including the boat, motor, anchor, trailer, and tow vehicle. Carry-in ramps only allow for the launching of canoes or kayaks that don't require a trailer. These vessels can still use motors and can still transport aquatic plants/seeds from one waterbody to another, but the probability is lower. Lakes without a ramp have minimal to no risk of human-mediated dispersal. When data on private or personal ramps could not be found, I excluded these ramps from the model, so human-mediated dispersal of *N. minor* may be underestimated in this model. The equation I used to assess the probability of waterbodies becoming invaded is as follows:

$$P_{j,t+1} = S_{j,t}^{D*\lambda} * A(A+\sigma)^{-1} * R \quad (1)$$

Where P is the probability of lake j becoming invaded in the next time step, $S_{j,\,t}$ is the state of lake j in the previous time step, D is a vector of how far the lake is to every other waterbody, λ is

the coefficient for distance, A is the surface area of the lake, σ if the coefficient for area, and R is the presence and effect of a boat ramp.

I ran the model 100 times for 25 and 50 time steps, representing 25 and 50 years in the future. I ran the model multiple times with a low, medium, and high effect of distance and a low, medium, and high effect of area on the probability for dispersal to show how the future distribution of *N. minor* could look given the relative importance of these variables. I held the effect of boat ramps constant using probabilities reported by De Ventura *et al.* (2016) (trailer ramp = 0.5, carry-in = 0.08, no ramp = 0). After the model ran 100 times, I summed the number of times a lake was invaded at t=25 and t=50 to give the probability of that lake becoming invaded in the future. For instance, if a lake had a score of 76, it means that lake was invaded in 76 out of the 100 possible future scenarios. I used these values to produce a final map showing the possible spread of *N. minor* in Maine, with the color of each lake representing the likelihood of its invasion.

4. Results

4.1 Overwintering Experiment

After 22 weeks of seeds being in the environmental chamber, only 1 seed germinated. This seed was of medium maturity. Since no other seeds germinated during the experiment after one simulated dormancy cycle, I could not test for the ability of *N. minor* seeds to remain dormant for multiple cycles. Algae grew in the glass tank and fungus grew on some of the seeds in the petri dishes, so the experiment was terminated.

4.2 Duck-Mediated Dispersal Experiment

I used seven ducks for this experiment. However, the first trial was used as a test run, so I excluded data from the first trial from my analysis. Two of the ducks were only used once in the

first trial, two were used three times in the three subsequent trials, and three were used four times in all trials for a total of 20 trials. This number drops to 15 after samples for the first trial are excluded. I was able to recover intact *N. minor* seeds from the excreta of the domesticated ducks for 14 of the 15 trials. I recovered a median of 10.7% of the seeds fed to the ducks (first quartile (Q1) = 5.4%, third quartile (Q3) = 16.1 %, Figure 2), with a maximum recovery success of 44.4%. Of the seeds recovered, 50% of them were still viable based on the tetrazolium assessment (Q1 = 29.2%, Q3 = 63.4%). The percent of seeds recovered from ducks that were viable was not significantly different from the percent of seeds in the control group that were viable, which had a median of 65% viable (Q1 = 60.0%, Q3 = 77.5%, Figure 3).

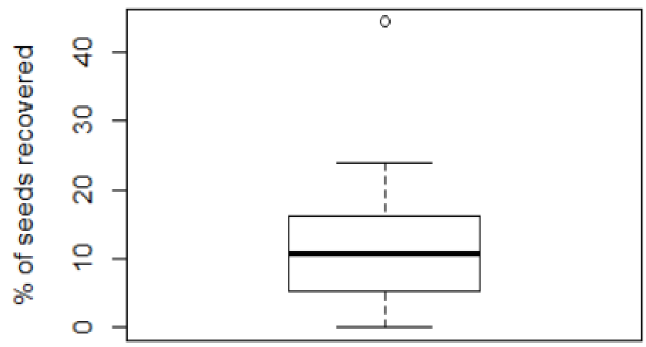

Figure 2. The percent of N. minor *seeds recovered from the excreta of five domesticated ducks (n=15, Q1 = 5.4, Q2 = 10.7, Q3 = 16.1).*

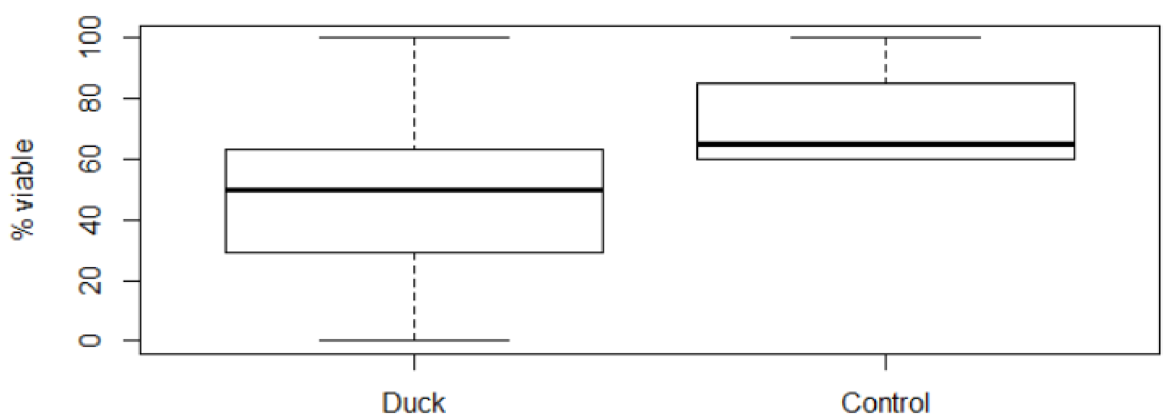

Figure 3. The percent of N. minor *seeds found to be viable using tetrazolium to stain living tissue. "Duck" refers to seeds that were recovered from duck excreta (n = 15) after their ingestion, while "Control" refers to seeds in the control group that were not fed to ducks (n = 4).*

Given that the digestive tract of ducks can vary considerably between individuals and could also be affected by sex and breed, I conducted a multiple linear regression analysis to assess the effect sex and individual variation may have had on the percent of recovered and viable seeds. None of these variables were found to be significant, suggesting that variation between individuals does not affect the likelihood of *N. minor* seed survival (Figure 4). I also ran Chi-squared goodness of fit tests to assess the effect of sex and individual variation on *N. minor* seed recovery. Again, sexes and individuals did not vary significantly in their effects on seed recovery and viability.

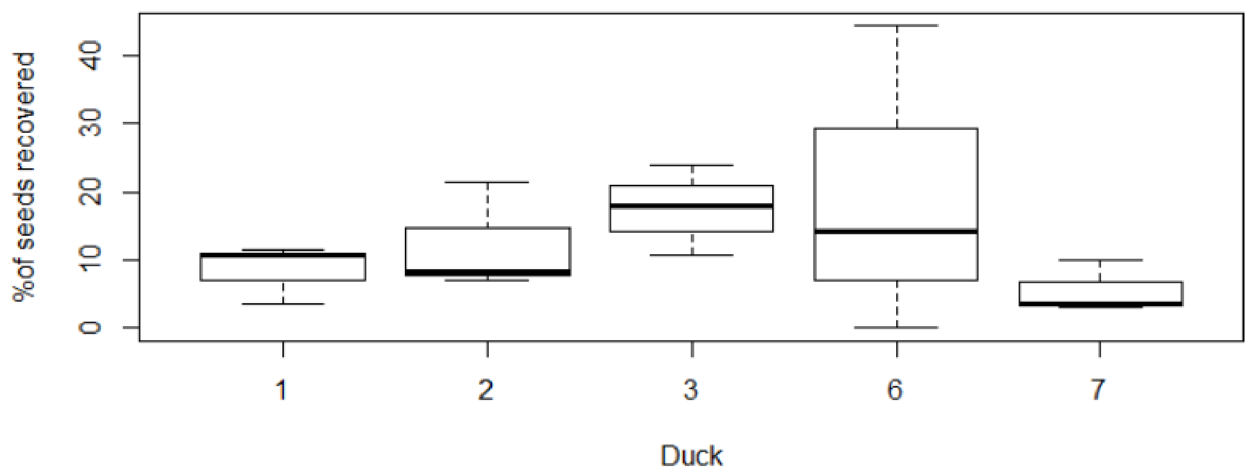

Figure 4. The percent of N. minor *seeds recovered from the excreta of each individual duck across 3 trials. Ducks 4 and 5 were only used in one trial and excluded from the analysis. Differences between individuals are not statistically significant, suggesting that individual ducks do not differ in their ability to digest* N. minor *seeds.*

4.3 Modeling the spread of N. minor *in Maine*

I ran the model with a low, medium, and high effect of waterbody surface area (σ), a low, medium, and high effect of distance (λ), and a constant effect of boat ramp presence and importance on the probability of *N. minor* spreading to new waterbodies. The model was run with these differing strengths of effects because the actual effect of distance and surface area on the probability of new *N. minor* invasions is still unknown. Incorporating different values lends

strength to the model as it considers a wider range of possible future scenarios. As the importance of waterbody surface area increases, larger lakes become more likely to become invaded in the future compared to smaller lakes (Figures 5b, d). As the importance of distance from established *N. minor* invasions increases, waterbodies that are closest to invaded waterbodies become more likely to become invaded compared to far away waterbodies (Figures 5c, d). Regions with red shading are more likely to be invaded by *N. minor* in the future, while regions with yellow shading are less likely to be invaded in the future.

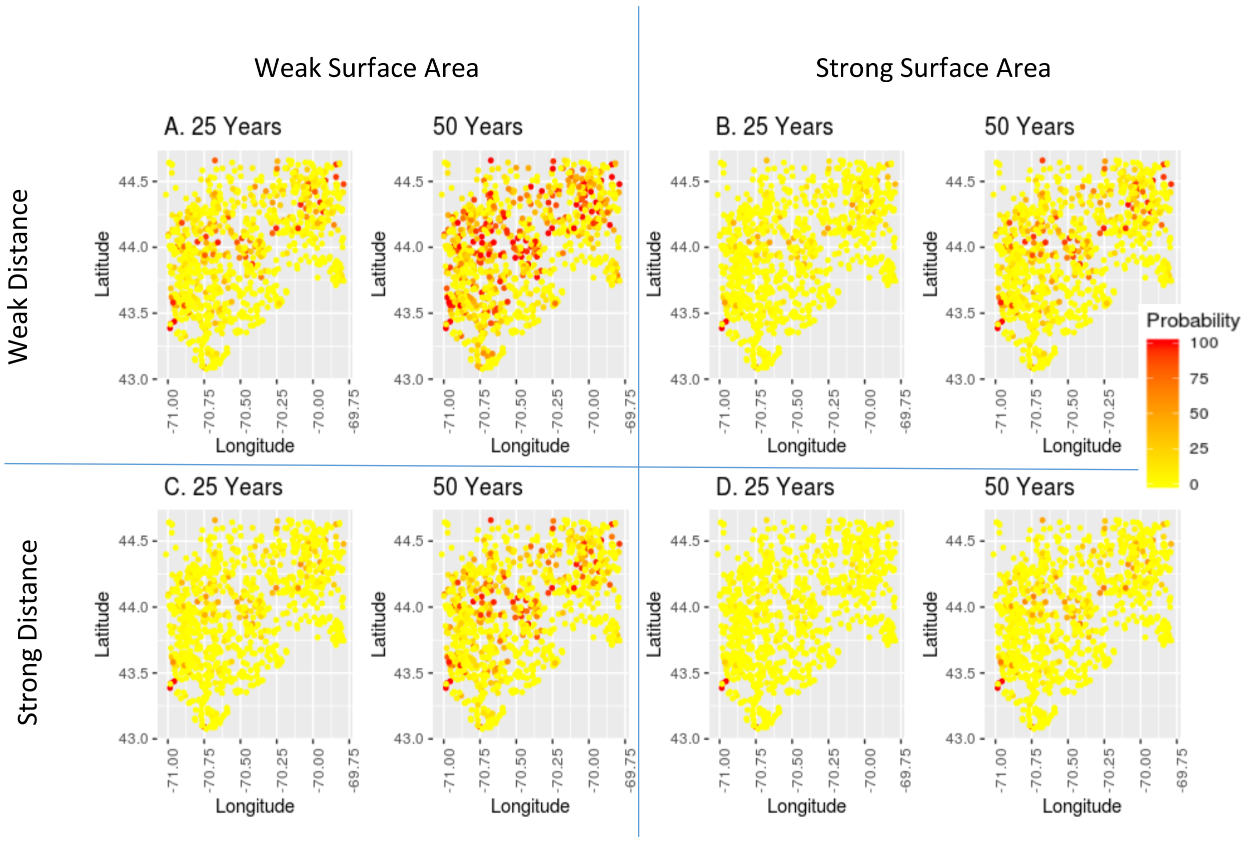

Figure 5. The potential future distribution of N. minor *in southern Maine 25 and 50 years from the present. Red dots indicate waterbodies that became invaded in close to 100 of the 100 potential future states, while yellow dots indicate waterbodies that became invaded in close to 0 of the 100 potential future states. Different values were used in the model to produce the distributions in each quadrant.* ***A)*** *Distance and surface area both have a weak effect on the probability of spread.* ***B)*** *Distance has a weak effect while surface area has a strong effect on the probability of spread.* ***C)*** *Distance has a strong effect while surface area has a weak effect on the probability of spread.* ***D)*** *Distance and surface area both have a strong effect on the probability of spread.*

5. Discussion

Due to a lack of germinating seeds, I was not able to assess the ability of *N. minor* seeds to remain viable for multiple dormancy cycles. I did recover, on average, 10.7% of the seeds I fed to captive ducks. Half of these seeds were still viable and potentially able to germinate, suggesting that ducks are a vector for the dispersal of *N. minor* and could contribute to the plant's past and future movement into Maine. Based on ducks likely staying local or migrating south during peak *N. minor* seed production and on island biogeography, which states that larger and closer "islands" are more likely to become colonized by new species, my model predicts that *N. minor* will move further into Maine in the next 25 and 50 years. The strengths of the effect of distance and surface area affect the probability of a waterbody becoming invaded. Given a strong effect of distance and surface area (Figure 5d), 20 waterbodies have a greater than 50% probability of becoming invaded in 50 years according to the model.

5.1 Inability to test for overwintering capacity

I could not test the ability of *N. minor* seeds to overwinter for multiple growth and dormancy cycles because seeds did not germinate. While wet storage of seeds, as used in this experiment, increases the percentage of seeds to germinate and decreases the time required to germinate in previous studies, the low levels of dissolved oxygen in the sealed vials used for storage may have inhibited germination (Hussner et al 2014, Wagner and Oplinger 2017). However, *N. minor* seeds *in vivo* are deposited in the sediment as the plant rapidly decomposes (Handley and Davy 2005). Lake sediments tend to have very low levels of oxygen, so seeds of *N. minor* should be able to withstand low oxygen conditions in the vials. *Najas minor* plants were collected in early September, which is approximately two months before they would naturally senesce in early November. Handley and Davy (2005) found that seeds collected earlier in the

growing season had a reduced rate of germination, which could explain why it was so difficult to germinate seeds for this experiment. However, they also found that germination of seeds was fastest after 84-112 days of cold storage. I stored seeds in this experiment in cold conditions for about 90 days, which falls into this range.

5.2 Ducks are a vector for the spread of N. minor

The results of my experiment suggest that seeds of *N. minor* can successfully pass through the digestive tract of a duck intact and potentially germinate once deposited. This means that duck populations in the wild could disperse *N. minor* from an infested waterbody to a clean one through endozoochory. The percentage of *N. minor* seeds recovered after ingestion, 10.7% of seeds, is comparable to recovery rates of other aquatic plant species assessed in other papers. For example, in their study on over forty aquatic plant species, Wongsriphuek et al. (2008) reported recovery rates ranging from 1.9 ± 0.6% to 51 ± 4.7%. Wongsriphuek et al. (2008) also reported no significant difference in recovery percentage between pairs of congeneric species, suggesting that *N. minor* should have a similar recovery rate as *N. marina*. However, in a study on *N. marina* by Agami and Waisel (1986), 26-34% of seeds were recovered after ingestion by mallards, which is significantly higher than what I found.

Results of studies on the capacity for waterfowl to disperse seeds are hard to compare because of differences in experimental design and differences in the ducks used in the experiments. Other studies have force fed seeds to ducks (Charalambidou et al. 2005, Wongsriphuek et al. 2008), while I chose to mix seeds with chicken feed and allow the ducks to eat the seeds at their own pace to avoid putting the ducks into distress, as did Agami and Waisel (1986). To determine passage rate, researchers had collected excreta for 24 to 48 hours, sometimes collecting the excreta every one to four hours (Agami and Waisel 1986,

Charalambidou et al. 2005, Wongsriphuek et al 2008). I collected excreta only after 23 hours and did not collect it at regular time intervals because of time and travel constraints. I based this approach on Wongsriphuek et al (2008), who recovered all seeds within 24 hours. The largest percentage of seeds were recovered within the first 4 hours after feeding. This suggests that I recovered the majority, if not all, of the seeds that survived digestion, but also that my findings should be taken as a conservative estimate.

While I did not find any significant difference between individual ducks in my experiment, other studies have cautioned that variability between individuals should be considered since the digestive tract of ducks has been shown to be very plastic (Figuerola and Green 2002). The time in captivity and the diet of ducks both affect the structure of the digestive track in captive ducks. For example, gizzard size and weight decrease as the time in captivity increases, and captive ducks on a more fiber-rich diet have heavier gizzards than those on a fiber-poor diet (Clench and Mathias 1995). These differences make it difficult to compare results across studies and to estimate the dispersal potential of wild ducks based on *in vitro* experiments on captive ducks. Wild ducks have heavier gizzards than captive ducks, suggesting that more seeds will be destroyed in the robust gizzard of wild ducks (Kleyheeg et al. 2016). However, no studies have ever been conducted on the ability of ducks (captive or wild) to disperse *N. minor* seeds so the results of my study are still valuable in examining potential dispersal pathways for *N. minor* in Maine and beyond.

The tetrazolium test for assessing viability of seeds was helpful, but I was not able to directly test for capacity to germinate. Wongsriphuek et al. (2008) cautions that viability tests overestimate germination ability since not all viable seeds will actually germinate in the wild. They argue that there is an important difference between a seed being alive and a seed actually

growing into a plant, and that by only evaluating the viability researchers are overestimating the reproductive potential of a seed cohort. They suggest that all studies on endozoochory should use germination tests. I chose to test for viability instead of germination because none of the seeds I collected germinated in my overwintering experiment. While germination rates are lower than viability rates, assessing the viability of seeds still provides a useful idea of how many seeds have survived the digestive process and have the potential to germinate.

5.3 Najas minor *will spread further into Maine*

I ran the probabilistic model with varying strengths of the effects of distance to invaded waterbodies and surface area of waterbodies on the probability of *N. minor* spreading. As expected, the potential future distribution of *N. minor* changes as these effect strengths change. *Najas minor* has the largest future distribution when distance to invaded waterbodies and waterbody surface area are less important (Figure 5a). This would be the case if waterfowl are able to disperse *N. minor* seeds over long distances – say during migration -- and if lake size played little to no role in where waterfowl landed and deposited the seeds. *Najas minor* has the smallest future distribution when distance to invaded waterbodies and waterbody surface area were more important (Figure 5d). This would be the case if waterfowl are only able to disperse *N. minor* seeds over short distances – say during the summer breeding season – and if waterfowl preferentially land in larger waterbodies where larger quantities of suitable *N. minor* habitat is available.

Island biogeography informs us that larger "islands" (in this case waterbodies are islands in a larger terrestrial landscape) are more likely to be colonized as they offer a larger target for species to land on and more habitat for them to occupy (MacArthur and Wilson 1976). During the time of peak seed production, ducks are mainly feeding and breeding locally or migrating

south away from Maine, suggesting that local short-distance dispersal events are more likely than long-distance dispersal events. Given these two ideas, the future scenario presented where distance and surface area both have a large effect on the probability of *N. minor* spreading (Figure 5d) is the most likely future scenario. Even so, in this scenario, only 20 of the 1,069 lakes included in the model had a probability of becoming invaded about 50% in 50 years, which is somewhat good news. In the 50 year history of variable-leaf milfoil in Maine, the plant has invaded over thirty waterbodies. *N. minor* is thought to be spreading faster than other invasive aquatic plants, so I expected *N. minor* to become established in more waterbodies than predicted by the model. However, the report of the rate of spread of *N. minor* is from Connecticut, which has different land-use patterns, population density, and environmental policies than Maine. Further studies should be conducted to assess how these variables affect the spread of invasive aquatic species.

The small number of predicted *N. minor* invasions could also be due to the model itself and its limitations. Given my limited time and the limited amount of accessible data, the effects of waterbody area and distance between waterbodies was chosen somewhat arbitrarily. Data on the rate at which ducks move from one water body to the next, the rate at which *N. minor* seeds are consumed by wild ducks, and the amount of suitable habitat in each waterbody would enhance the accuracy of the model to be more reflective of real world conditions. However, the model still offers valuable insight into the spread of *N. minor* in Maine and could be used as a starting point for future modeling projects with *N. minor* or other invasive species.

6. Conclusion

The results of my study indicate that *N. minor* will move further into Maine in the coming decades. The extent of this expansion will depend on how far local duck species travel

between waterbodies in a typical day, the percentage of *N. minor* seeds that survive the digestive process, the amount of available habitat in a given waterbody, and the presence of boat ramps. The recovery of *N. minor* seeds from captive duck excreta suggests that duck-mediated seed dispersal in the wild has the potential to contribute to some of this range expansion. This ability to survive the digestive process, along with *N. minor*'s tolerance of low water quality, large reproductive capacity, and propensity to fragment, increases *N. minor*'s invasive potential and makes it a rather successful invasive species. While I was not able to test for it, it is still possible that *N. minor* seeds can remain dormant for multiple years, which would increase its invasive potential and complicate its management.

I expect humans to still be the largest vector for transporting *N. minor*. Preventative measures, such as removing any plant material before boats or gear leave a boat ramp, should be taken in order to reduce the likelihood of spreading an invasive species. Additionally, thoroughly rinsing any surfaces that come in contact with the water and giving them time to completely dry will decrease the likelihood of spreading invasive aquatic species. Avoiding areas where an invasive species is known to be present will also help reduce its spread both between waterbodies and within a waterbody. In the summer of 2019, Maine DEP staff discovered a private waterski course had been set up in the same area as an extremely dense patch of *N. minor* in Northeast Pond. During an herbicide treatment of this patch, multiple boats motored through the treatment area. This sort of boating activity has the potential to fragment *N. minor* plants, which can then drift to new areas of the pond or be carried on the boat to other waterbodies.

The boating activity on Northeast Pond also demonstrates the lack of public knowledge or concern over invasive aquatic plants. More outreach should be conducted in order to inform the public of invasive aquatic plant species, the risk associated with them, and methods of

preventing and controlling them. This will require more funding from the state for the Invasive Species unit of the Maine DEP and will be complicated by the large number of people from out-of-state that recreate on Maine lakes in the summer without being aware of the local aquatic community. Preventative methods are far cheaper and more effective at managing the spread of invasive species than control methods. While there are a number of success stories of invasive aquatic plants being cleared from specific waterbodies, these stories are rare and the removal process spanned multiple years and cost thousands of dollars because of how difficult it is to eradicate an invasive species once it becomes established. However, if an invasion is caught early enough – when only a few plants are present – then rapid removal is often successful at eradicating the invasion. Educating the public about invasive aquatic plants increases the number of eyes aware of or directly looking for these species, thereby increasing the likelihood of an invasion being caught early when it can still be effectively managed. My hope is that this study is a step towards widespread understanding of, and concern for, invasive aquatic plants in Maine and beyond.

Acknowledgements

I'd like to thank Denise Blanchette and John McPhedran of the Maine Department of Environmental Protection for suggesting I study *N. minor*, providing the seeds used in this study, and for their endless encouragement; Don and Sandy Neville for allowing me to use their ducks, watching the ducks while I was gone, and delightful conversations; Cathy Bevier for advising me during this process, reading multiple drafts, and for her endless support; Chris Moore for creating the structure of the model, suggesting I use tetrazolium, and for editing my thesis; Judy Stone for her plant knowledge and for editing my thesis; Marilyn Kelly and Minou Raschid Farrokhi for their help isolating seeds; and the Colby College Biology Department for funding my research.

Works Cited

Agami, M. and Y. Waisel. 1986. The role of mallard ducks (*Anus platyrhynchos*) in distribution and germination of seeds of the submerged hydrophyte *Najas marina* L. *Oecologia* **68**(3): 473-475.

Agami, M., and Y. Waisel. 1988. The role of fish in distribution and germination of seeds of the submerged macrophytes *Najas marina* L. and *Ruppia maritima* L. *Oecologia* **76**(1): 83-88.

Blanchette, D. 2017. The treatment of *Najas minor* to prevent spread from seeds. Maine Department of Environmental Protection, Augusta, ME.

Canadian Food Inspection Agency. 2014. Emerald Ash Borer: Questions and Answers. <http://www.inspection.gc.ca/plants/plant-pests-invasive-species/insects/emerald-ash-borer/faq/eng/1337355937903/1337356019017>. Downloaded May 10 2019.

Charalambidou, I., L. Santamaria, C. Jansen, and B. A. Nolet. 2005. Digestive plasticity in Mallard ducks modulates dispersal probabilities of aquatic plants and crustaceans. *Functional Ecology* **19**: 513-519.

Clench M. H., and J. R. Mathias. 1995. The avian cecum: a review. *Wilson Bulletin* **107**: 93-121.

Borman, S., R. Korth, and J. Temte. 1997. Through the looking glass: A field guide to aquatic plants. Edited by Dorothy Snyder. Merrill, WI: Reindl Printing, Inc.

De Ventura, L., N. Weissert, R. Tobias, K. Kopp, and J. Jokela. 2016. Overland transport of recreational boats as a spreading vector of zebra mussel *Dreissena polymorpha*. *Biological Invasions* **18**: 1451-1466.

Figuerola, J., and A. J. Green. 2002. Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. *Freshwater Biology* **47**: 483-494.

Handley, R.J., and A. J. Davy. 2005. Temperature effects on seed maturity and dormancy cycles in an aquatic annual, *Najas marina*, at the edge of its range. *Journal of Ecology* **93**: 1185-1193.

Hussner, A., P. Heidbuechel, and S. Heiligtag. 2014. Vegetative overwintering and viable seed production explain the establishment of invasive *Pistia stratiotes* in the thermally abnormal Erft River (North Rhine-Westphalia, Germany). *Aquatic Botany* **119**: 28-32.

June-Wells, M., F. Gallagher, J. Gibbons, and G, Bugbee. 2013. Water chemistry preferences of five nonnative aquatic macrophytes species in Connecticut: a preliminary risk assessment tool. *Lake and Reservoir Management* **29**(4): 303-316.

Kleyheeg, E., M. Claessens, and M. B. Soons. 2018. Interactions between seed traits and digestive processes determines the germinability of bird-dispersed seeds. *PLOS ONE* **13**(4): 1:15.

Kleyheeg E., M. Klaassen, and M. B. Soons. 2016. Seed dispersal potential by wild mallard duck as estimated from digestive tract analysis. *Freshwater Biology* **61**: 1746-1758.

Lansdown, R.V. 2014. *Najas minor*. In: The IUCN Red List of threatened species. < https://www.iucnredlist.org/species/164446/43123412>. Downloaded on 12 January 2019.

Les, D.H, E. L. Peredo, N. P. Tippery, L. K. Benoit, H. Razifard, U. M. King, H. R. Na, H. Choi, L. Chen, R. Shannon, and S. P. Sheldon. 2015. *Najas minor* (Hydrocharitaceae) in North America: A reappraisal. *Aquatic Botany* **126**: 60-72.

Les, D. H., and L. J. Mehrhoff. 1999. Introduction of nonindigenous aquatic vascular plants in southern New England: a historical perspective. *Biological Invasions* **1**: 281-300.

MacArthur, R., and E. O. Wilson. 1967. The theory of island biogeography. Princeton, NJ: Princeton University Press.

Moore, R.P. 1985. Handbook of Tetrazolium Testing. The International Seed Testing Association, Zurich.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J, Zern, T. Aquino, and T. Tsomondo. 201. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems, and Environment* **84**: 1-20.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Schaffner, B. 2019. Maine DEP Invasive Aquatic Plant Map. <https://maine.maps.arcgis.com/home/Webmap/viewer.html?webmap=126b9dbc59f44f969f74739bc9bc4ade>. Downloaded on 15 January 2019.

Topuzovic, M. D., I. D. Radojevic, M. S. Dekic, N. S. Radulovic, S. M. Vasic, L. R. Comic, and B. Z. Licina. 2015. Phytomedical investigation of *Najas minor* All. In the view of the chemical constituents. *EXCLI Journal* **14**: 496-503.

United States Department of Agriculture. 2019. *Najas minor* All. Brittle waternymph. In: Plants Profile. < https://plants.usda.gov/core/profile?symbol=nami>. Downloaded 12 January 2019.

Volunteer Lake Monitoring Program. 2019. European naiad, spiny naiad, *Najas minor*. In: Maine's interactive field guide to aquatic invaders and their look alikes. <http://www.mainevlmp.org/mciap/herbarium/EuropeanNaiad.php/mciap/herbarium/EuropeanNaiad.php>. Downloaded 12 January 2019.

Wagner, E. J., and R. W. Oplinger. 2017. Effect of overwinter hydration, seed storage time, temperature, photoperiod, water depth, and scarification on seed germination of some *Schoenoplectus, Polygonum, Eleocharis,* and *Alisma* species. *Aquatic Botany* **136**: 164-174.

Wongsriphuek, C., B. D. Dugger, and A. M. Bartuszevige. 2008. Dispersal of wetland plant seeds by Mallards: Influence of gut passage on recovery, retention, and germination. *Wetlands* **28**(2): 290-299.

Zalewska, J. 1999. The genus *Najas* (Najadaceae) in Poland: remarks on taxonomy, ecology, distribution and conservation. *Fragmenta floristica et geobotanica* **44**(2): 401-422.