The northern range extension of the invasive bivalve *Corbicula fluminea*

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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December 2015

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Abstract

The Asian clam *Corbicula fluminea* – one of the world's most invasive freshwater species – was previously believed to be limited to warm temperate habitats, but has extended its North American range northward into cold temperate waters in recent years. Using lab experiments, I show that *C. fluminea* tolerance to low oxygen varies across populations; therefore, risk assessments that are based on environmental tolerance data derived from only a single population are unreliable. Using climate suitability modeling techniques, I identify regions in North America at risk of *C. fluminea* invasion under current and future climate conditions. Finally, I experimentally quantify variation in shell degradation rates across different habitat conditions, to evaluate the feasibility of using spent shells as an indicator of establishment success. These results highlight the importance of accounting for phenotypic variation and context dependence when identifying ecosystems at risk of invasion.

Résumé

La petite corbeille d'Asie *Corbicula fluminea* est une des espèces dulcicoles les plus envahissantes au monde. On croyait jadis que sa distribution était limitée aux cours d'eau des régions tempérées chaudes mais elle a récemment élargi son aire de répartition en Amérique du Nord vers le nord, dans des régions tempérées froides. À l'aide d'expériences en laboratoire, j'ai démontré que la tolérance à l'hypoxie froide varie entre populations. Ainsi, les évaluations de risques basées sur les données de tolérances environnementales dérivées d'une seule population ne sont pas fiables. En utilisant des techniques de modélisation de la qualité du climat, j'ai identifié des régions d'Amérique du Nord potentiellement à risque de faire face à une invasion de *C. fluminea* sous les conditions climatiques actuelles et futures. Finalement, j'ai quantifié les taux de dégradation des coquilles de *C. fluminea* entre différent types d'habitats afin d'évaluer la faisabilité d'utiliser ce trait en tant qu'indicateur de succès d'établissement. Mes résultats soulignent l'importance de considérer la variation phénotypique et la dépendance contextuelle lors de l'identification des écosystèmes à risque d'invasion de petites corbeilles d'Asie.

Contribution of authors

The research presented in this thesis is the product of my own work, and was conducted under the supervision of Prof. Anthony Ricciardi who provided invaluable guidance and support. I designed and executed the experimental and analysis protocols for all three chapters of this thesis, and produced this written report. Prof. Ricciardi provided extensive input on each of these chapters during all phases of execution, including the conceptualization of novel research questions, experimental design and editorial feedback on all drafts. He will therefore be an author on all resulting publications.

Prof. Andrew Hendry and Prof. Bruce Conn supported the design and execution of the hypoxia tolerance experiment described in Chapter 1. Prof. Hendry provided essential laboratory resources and feedback on experimental design, and Prof. Conn supplied test animals. Therefore, both Prof. Hendry and Prof. Conn will co-author the publication resulting from the manuscript presented in Chapter 1.

Acknowledgments

This thesis is the fruit of the support and guidance of many people. Prof. Ricciardi has been an essential figure in the development of both this project, and my career as an ecologist. His unwavering support, professional guidance and dedication to excellence have provided me with a strong skill set with which to pursue future challenges. I will always be grateful for the rewarding years I spent under his tutelage in Redpath Museum.

I would also like to thank my current and past lab members, including Suncica Avlijas, Dustin Raab, Josie Iacarella, Katie Pagnucco, Emilija Cvetanovska, Rowshyra Castañeda, Kayla Hamelin and Jaime Grimm. All provided essential support and feedback, including assistance with fieldwork, experiments, analyses and editing. Your experience and input was much appreciated and elevated the quality of my work. Other graduate students including Kerri Lynn Ackerly, Lidia Della Venezia, Tanja Prsa and Katharine Yagi provided invaluable assistance with experimental design, analysis and editing.

Undergraduate research assistants and field crews provided important support in the field and while conducting experiments. I would like to thank Rachael Ryan, Valeria Sokolenko, Jean-Michel Matte, Sandrine Vigneron, Samuel Plamondon, Kelly McNichols-O'Rourke, Mark Loza, Alexandra Sylvestre, Andrew Darcy, Julie Vanden Byllaardt and Brydon MacVeigh.

I would also like to thank my committee members Prof. Lauren Chapman and Prof. Melania Cristescu for sharing beneficial feedback, and Prof. Andrew Hendry, Prof. Tim Moore and Caroline LeBlond for providing essential experimental resources and support. Prof. D. Bruce Conn, Dr. Jeremy Farrell, Dr. Sandra Nierzwicki-Bauer and Dan Hua assisted my project by supplying clams and environmental data for my hypoxia tolerance experiment, and Dr. Todd Morris provided resources to support sampling at McGeachy Pond.

I must acknowledge the generous support of private citizens in the Montreal area who facilitated my shell degradation experiment, including Mr. David Speak at Beaconsfield Yacht Club, Mr. Richard St-Hilaire at Valleyfield

Marina, Mr. David Fleet and staff at the Royal St. Lawrence Yacht Club, and Ms. Julia Atack at Baie D’Urfé Yacht Club.

I would like to thank the Canadian Aquatic Invasive Species Network for funding this project and supporting the dissemination of my results at international conferences. The Quebec Centre for Biodiversity Sciences also provided financial support for conference participation, as well as extensive assistance with statistical analyses. McGill University has facilitated my development as a scientist for the past six years, and I will look back fondly at my time spent at this excellent institution.

Finally, I would like to thank my fireteam partner Kaiyang Wang for his love and support throughout this endeavour, and my family for always believing in me.

Introduction

Freshwater ecosystems are highly vulnerable to the impacts of human activities, and their biodiversity is declining at rates proportional to tropical forests (Ricciardi and Rasmussen 1999). Numerous stressors threaten their function and diversity, including pollution, habitat loss and biological invasions (Dudgeon et al. 2006). Invasive species may alter physicochemical conditions, displace native species and homogenize communities, and impede their provision of ecosystem services (Rahel 2002; Strayer 2010). Invasive bivalves, in particular, can transform ecosystems by restructuring the local habitat through the formation of shell beds, and by influencing transparency, nutrient cycling and sedimentation through their filtration activities (Darrigran 2002).

A model invader: Corbicula fluminea

One of the world's most invasive bivalves, the Asian clam *Corbicula fluminea*, is native to Asia, Africa and Australia and has achieved a global distribution (Morton 1986). It was introduced to the west coast of North America in the early 20th century and subsequently spread throughout warm temperate habitats in the continental USA (Figure 1; Counts 1981; McMahon 1982; U.S. Geological Survey 2015). Since the 1980s, *C. fluminea* has invaded European waterways, likely via human-mediated introductions followed by natural dispersal within drainage systems (Araujo et al. 1993; Ayres 2008; Brancotte and Vincent 2002; Caffrey et al. 2011; Ciutti and Cappelletti 2009; Elliot and zu Ermgassen 2008; Paunović et al. 2007). It can reach densities exceeding 10,000m^{-2}, and exert significant ecological impacts (McMahon 2000). *C. fluminea* shell bed formation increases habitat complexity and alters benthic invertebrate community composition and abundance (Ilarri et al. 2014; Werner and Rothhaupt 2007; 2008a). Magnified by high local densities, the clam's intense filtration activity can cause ecosystem-level regime shifts by increasing water clarity and reducing algal populations, thus initiating trophic cascades that result in increased macrophyte growth and altered bird and fish community composition (Beaver et al. 1991; Cohen et al. 1984; Phelps 1994; Pigneur et al. 2014a). In addition to suspension feeding, *C. fluminea* are active pedal feeders whose activities reduce sediment bacteria abundance and organic matter, and increase sediment bioturbation (Hakenkamp and Palmer 1999; Hakenkamp et al. 2001; Majdi et al. 2014). Economic impacts are also substantial; clam biofouling may interfere with or damage human infrastructure, especially water supply systems (McMahon 2000; Rosa et al. 2011).

Corbicula *taxonomy in native and invasive ranges*

Müller first described the genus in 1774, identifying three species in its native Asiatic range: *Corbicula fluminea, Corbicula fluminalis* and *Corbicula fluviatilis* (the latter species *C. fluviatilis* was later synonymized with *C. fluminea*; Morton 1977). These taxa were differentiated in the native range by habitat preference: *Corbicula fluminea* typically occurred in fresh waters, whereas *C. fluminalis* was considered an estuarine species (Morton 1986). By the early 20th century, 69 species of *Corbicula* had been speciously identified in the native range based on slight differences in morphology and habitat preference, likely driven by high phenotypic plasticity (Figure 2; Morton 1977; Morton 1986). Although some revisions have since been made, there is still a poor understanding of how many genetically distinct groups of *Corbicula* exist in the native range and little consistency with which names are applied.

When research on the highly invasive North American *Corbicula* populations began in earnest in the mid-1970s, some identified it as *Corbicula manilensis* (see, for example, Aldridge and McMahon 1978; Thompson and Sparks 1977). By consensus, *C. manilensis* was dropped in favour of *C. fluminea*, because the shell morphology and habitat preferences of North American populations were similar to that which was described for the clam identified as *Corbicula fluminea* in the native range (Britton and Morton 1977). Genetic analyses suggest that North American *C. fluminea* populations comprise only two homozygous lineages that differ in nacre colour and shell form; the most common “Form A” morphotype has white nacre with purple highlights, whereas the rarer “Form B” has purple nacre (Britton and Morton 1986; Hillis and Patton 1982; Lee et al. 2005; McLeod 1986; Pigneur et al. 2014b; Siripattrawan et al. 2000).

Evaluating the origin of invasive *C. fluminea* populations is challenging because populations in the native range have high genetic diversity, and employ numerous sexual and asexual reproductive strategies (Morton 1986; Smith et al. 1977). In contrast, invasive populations in North America display much lower genetic variability within and between populations because they reproduce asexually and are likely descended from small founding populations (Britton and Morton 1977; Smith et al. 1977). Some genetic analyses have begun linking North American clams to specific source populations in the native East Asian range (Pigneur et al. 2014b); however, further work is required to determine with certainty which populations in the native range correspond to those that have invaded North America.

A strictly subtropical bivalve?

Given *C. fluminea*’s predominantly subtropical native distribution, this species has long been thought to be limited to warm temperate water bodies (see, for example, Abbott 1977; French and Schloesser 1996; McMahon 1982). Thermal tolerance data are lacking for populations within the native range. However, a lower thermal limit of 2^{o}C was observed for an invasive *C. fluminea* population from Texas, which supported the perception that the species does not recruit in seasonally ice-covered water bodies in its North American range (Mattice and Dye 1975). Several subsequent field studies seemed to corroborate their presumed intolerance of winter water temperatures (French and Schloesser 1991; reviewed in McMahon 1982; Werner and Rothhaupt 2008b). Indeed, virtually all northern populations in cold temperate regions were limited to thermally modified habitats such as the discharge plumes of generating stations (Scott-Wasilik et al. 1983; Ward and Hodgson 1997; White et al. 1984). A dense population established in the thermal plume of a nuclear power station in the St.

Lawrence River (Simard et al. 2012) became extirpated immediately after the plant was decommissioned (Emilija Cvetanovska, pers. comm.).

In 1980, two live *C. fluminea* were collected in thermally unmodified waters of Lake Erie offshore Sterling State Park in Michigan, but there is no evidence that a population persisted at this site (Clarke 1981). However, in the early 1990s populations were identified in the Connecticut River and Highline Lake in Colorado that persist through winter, although no winter water temperature or clam mortality data was published (Kreiser and Mitton 1995). Populations were also detected in the Erie Canal and in lakes in Pennsylvania and New York in the late 1990s (U.S. Geological Survey 2015). High-density *C. fluminea* populations were found in Lake Tahoe, Nevada, in 2008 and Lake George, New York, in 2010 (Lake George Asian Clam Rapid Response Task Force 2011; Wittmann et al. 2012). Additional overwintering populations have been discovered in water bodies in upper New York State, including in Owasco Lake and Seneca River (pers. obs.; U.S. Geological Survey 2015). These findings suggest that at least some *C. fluminea* populations can tolerate temperatures colder than 2^{o}C. Thermal tolerance testing of clams from a remnant reach of the Rhine River supports this assertion, finding that some clams from this local population survive at 0^{o}C (Müller and Baur 2011). Further research confirmed that some North American populations can tolerate prolonged exposure to temperatures near 0^{o}C, and that cold tolerance varies among invasive North American populations (Cvetanovska 2015).

In 2008, *C. fluminea* was detected in Sookes Watershed on Vancouver Island (Kirkendale 2008). This marked the first time a population had been detected in thermally unmodified Canadian waters since 1924, when shells were collected on Vancouver Island (there is no evidence that this historical population persisted; Counts 1981). It has since been found in the lower reaches of the Coquitlam and Fraser rivers (Matthias Herborg, British Columbia Ministry of Environment, pers. comm.).

Canadian government scientists detected another population in McGeachy Pond, a satellite of Lake Erie in southwestern Ontario, in 2010 (Todd

Morris, Fisheries and Oceans Canada, pers. comm.); the population was confirmed in 2013 (pers. obs.). Intriguingly, this population persists despite being subject to multiple environmental stressors that were previously considered intolerable. McGeachy Pond is a eutrophic 15-hectare wetland created by the construction of a berm intended to protect agricultural fields from Lake Erie storm events. It is permanent with no natural inflow or outflow, and likely fed by groundwater, precipitation and runoff from the surrounding fields.

In addition to summer hypoxia events, the pond has winter ice cover, subjecting resident biota to cold temperatures, ice scouring and winter hypoxia events. Therefore, populations persisting in McGeachy Pond must be able to withstand low oxygen conditions at high and low temperatures. *C. fluminea* was thought to be intolerant of hypoxic or anoxic conditions (see, for example, McMahon 2000), and displays low growth rates and elevated mortality during seasonal hypoxia events (Belanger 1991; Ilarri et al. 2011). The presence of a persistent population in this habitat suggests that the species can tolerate a broader range of conditions than previously thought.

Research objectives

Given the apparent northern range extension of an invasive subtropical bivalve into cold temperate habitats previously believed to be intolerable, my research sought to quantify variation in environmental tolerances among conspecific *C. fluminea* populations, and identify regions at risk of invasion given this species' climatic requirements. In Chapter 1, I experimentally test variation in hypoxia tolerance among invasive North American *C. fluminea* populations. In Chapter 2, I explore empirical risk assessment methods by projecting *C. fluminea* climate suitability across North America under current and future climate conditions. In Chapter 3, I evaluate the feasibility of inferring *C. fluminea* establishment success using spent shells, by testing the rates and stages of shell degradation across a range of environmental conditions. Specific predictions are described in each chapter.

Overall, this thesis aims to inform *C. fluminea* risk assessment in North America, and explores the importance of using multiple lines of evidence to predict habitat suitability (and therefore invasion risk) in a particular water body.

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Figures

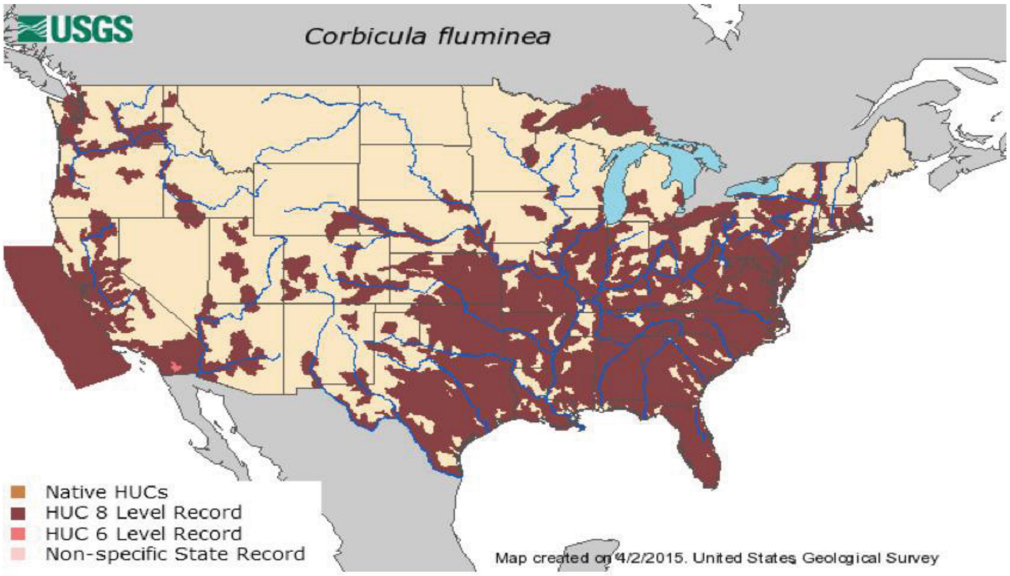

Figure 1: Watersheds known to be invaded by *C. fluminea* in the continental United States (U.S. Geological Survey 2015).

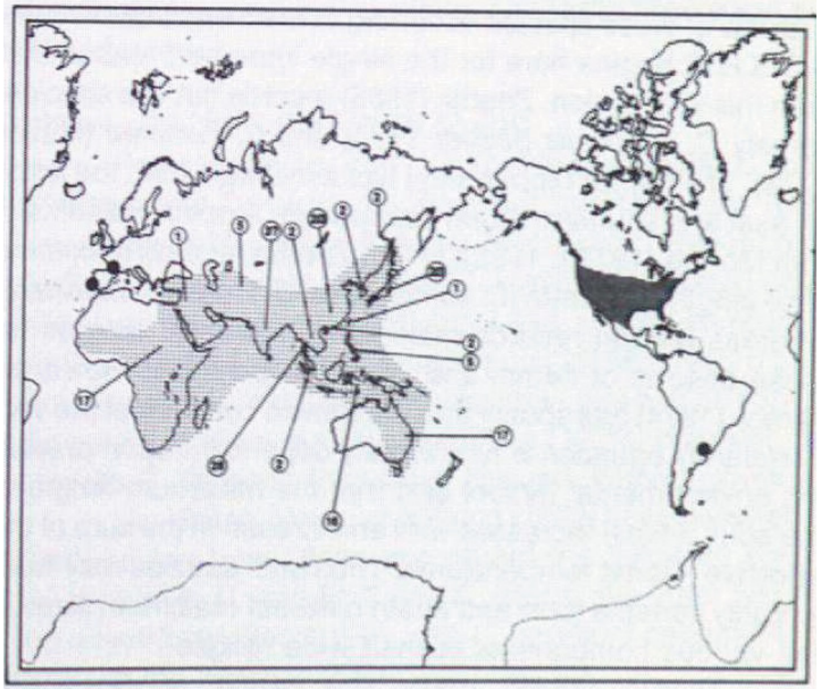

Figure 2: Distribution of *Corbicula* (after Britton and Morton, 1982; Zhadin, 1948; from Morton, 1986). Grey indicates the genus's native range (with numbers indicating the number of species), black indicates invaded ranges as of 1986.

CHAPTER 1

Interpopulation variation in hypoxia tolerance of the Asian clam *Corbicula fluminea*

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Abstract

Cold temperate habitats with seasonal ice cover were thought to be resistant to invasion by the Asian clam *Corbicula fluminea*, owing to the clam's putative thermal and oxygen limits. Experiments showing variation in cold tolerance among invasive *C. fluminea* populations in North America, and recent discoveries of populations established in cold temperate water bodies, suggest that northern habitats are at higher risk of invasion than previously assumed. Here, I conduct a novel experiment testing interpopulation differences in hypoxia tolerance at temperatures approaching summer and winter conditions in hypoxic cold temperate water bodies. I tested clams from invasive populations in Lake George (New York), Lake Cheston (Tennessee) and the Nolichucky River (Tennessee). Populations exposed to 5% dissolved oxygen saturation at ~5^{o}C for 49 days in experimental chambers showed significant variation in survivorship, as well as differing size-tolerance relationships. These results caution against basing risk assessments on environmental tolerance data from a single population in the invaded range.

Introduction

Corbicula fluminea has continued to spread into cold temperate water bodies in North America since the 1990s (U.S. Geological Survey 2015), despite previous experimentally-derived tolerance data (Mattice and Dye 1975) supporting the assertion they could not persist in these habitats (see, for example, McMahon 1982). Recent evidence shows that some populations can survive exposure to 0°C, and there is significant variation in cold tolerance among North American conspecific populations, likely driven by phenotypic plasticity (Cvetanovska 2015; Müller and Baur 2011). *C. fluminea*'s tolerance to stressors imposed in marginal habitats at its range edge, such as McGeachy Pond, should be re-evaluated since the presence of a population at this site contradicts previous research that suggested the species could not persist in hypoxic environments (such as, for example, Belanger 1991; Johnson and McMahon 1998; McMahon 2000).

In field studies, low dissolved oxygen has been shown to limit *C. fluminea* growth and density, and, when paired with warm temperatures, may trigger mass mortality events (Belanger 1991; Ilarri et al. 2011). However, experimental evidence for *C. fluminea* hypoxia tolerance is scarce and inconclusive. Two published studies tested hypoxia tolerance of a single population in Texas, under a variety of treatment and acclimation temperatures. Johnson and McMahon (1998) measured a mean LT_{50} of 20 days for clams exposed to 5% dissolved oxygen (DO) saturation at 5°C, and 5.7 days for clams exposed to 5% DO at 25°C. This reinforced the view that this species was highly sensitive to low-oxygen conditions. However, Matthew and McMahon (1999) observed no mortality in their hypoxic (2-3% DO) treatment group at 5°C throughout an 84 day exposure period, and measured an LT_{50} of 10.8 days at 25°C. These differing results suggest that experimental tolerances are highly influenced by slight variations in protocol. Similarly, previous respiration studies are contradictory in their assessment of *C. fluminea*'s sensitivity to hypoxia. McMahon (1979) found that clams from Texas were oxygen conformers (could not maintain internal oxygen levels with decreasing DO concentration), and their ability to maintain internal oxygen levels deteriorates with higher acclimation and treatment temperatures. However, a more recent study showed that clams collected from a site in France were able to maintain oxygen consumption rates down to DO levels of 2kPa (Tran et al. 2000). These inconsistent experimental results could reflect either sensitivity to laboratory conditions, or variation amongst *C. fluminea* populations driven by phenotypic plasticity or adaptation.

As shown by Cvetanovska (2015), the use of experimentally derived environmental tolerance data in risk assessment must be interpreted with care for highly plastic species. *C. fluminea*'s ability to persist in stressful habitats may be another indication of high phenotypic plasticity, consistent with significant morphological variation between populations (Morton 1977; Prezant and Chalermwat 1983; Prezant et al. 1988). This should be an important consideration for mechanistic models, which are being used with increasing frequency to predict species' ranges, including invaders (Gallien et al. 2010; Kearney and Porter 2009; Sutherst and Bourne 2009).

I aimed to test the hypoxia tolerance of several invasive *C. fluminea* populations at temperatures approaching their putative thermal limits. Given burgeoning detections of *C. fluminea* in unlikely water bodies, I hypothesized that this species varies in its tolerance to hypoxia across populations. I tested the hypoxia tolerance of three disparate populations from its invaded North American range at warm and cold temperatures, to simulate summer and winter hypoxia events. I predicted that *C. fluminea* from sites that are exposed to seasonal hypoxia would exhibit higher survivorship under low dissolved oxygen regimes.

Methodology

Experimental design

C. fluminea were obtained from Lake Cheston (Tennessee), the Nolichucky River (Tennessee) and Lake George (New York; Figure 1.1). These three *C. fluminea* populations are morphologically distinct (Table 1.1) – but all individuals exhibited characteristics (white nacres with purple highlights) of the Form A clonal lineage (Lee et al. 2005). Form A is the most common form in North America, and occurs in the regions where the experimental specimens were collected, although these particular populations have not been included in published genetic analyses (Lee et al. 2005; McLeod 1986; Pigneur et al. 2014).

The three sites are environmentally distinct and were selected to cover a large range of climatic and dissolved oxygen regimes. Lake George is a deep, cold temperate lake that is well oxygenated and seasonally ice-capped. Its population is likely the youngest among the three, as it was detected only in 2010 (Wick 2014). Lake Cheston is an isolated pond in the warm temperate region of Tennessee near the campus of Sewanee University, and therefore is highly impacted by human activities including fishing and run-off, and experiences summer low-oxygen events (Bruce Conn, Berry College, pers. comm.). The Nolichucky River is a warm temperate winding river located in eastern Tennessee, approximately 275km from Lake Cheston. It is not known to experience hypoxia events, and the lowest DO level recorded between 1996 and 2010 at United States Geological Survey gauge #03467609 (located 27km downstream of the clam collection site) was 5.6 mg/L, which is well above standard hypoxia thresholds (2 mg/L; U.S. Geological Survey 2015). I therefore predicted that Lake Cheston clams would be the most tolerant of low dissolved oxygen conditions, owing to their natural exposure to seasonal hypoxia; and, further, that Lake George clams would be more tolerant to hypoxia at low temperatures than the Nolichucky River clams, owing to their natural exposure to prolonged periods of cold temperatures.

Experimental temperature and DO levels were selected to expose clams to stressful conditions that would cause moderate mortality, such that any survivorship differences between populations would become apparent. Temperatures 25^{o}C and 5^{o}C were selected because all three populations would have experienced these levels in summer and winter (Bruce Conn, Berry College, unpub. data; Jeremy Farrell, Darrin Fresh Water Institute, pers. com.; U.S. Geological Survey 2015). Additionally, these temperatures approach the thermal limits of the species without causing elevated mortality or respiration stress at high DO levels, and would also allow the results of this study to be compared to prior *C. fluminea* hypoxia tests (Johnson and McMahon 1998; Matthews and McMahon 1999; Mattice and Dye 1975). Furthermore, they are similar to water temperatures commonly experienced during summer or winter hypoxia events. The hypoxic treatment was set at 5% DO saturation (approximately 0.4 mg/L at 25°C and 0.6 mg/L at 5°C), which is well below the thresholds at which the species can no longer maintain oxygen consumption rates and internal oxygen levels (Tran et al. 2000). Oxygen levels were measured as percent oxygen saturation instead of concentration, because it is partial pressure that determines diffusion rates across the gills of *C. fluminea* (Grieshaber et al. 1994). Dissolved oxygen concentration is a function of DO partial pressure, temperature and barometric pressure, and so is slightly higher (+0.2 mg/L) in the cold treatment than in the warm treatment (Grieshaber et al. 1994).

Clams were collected during the summer of 2014 and transported to McGill University in Montreal. Within 36 hours, the animals from Lake George were transported in lidded tubs filled with lake water in an air-conditioned vehicle, and those from Tennessee were shipped by courier, in plastic bags filled with damp paper towel on ice packs in packed boxes. Upon arrival, clams were immediately transferred into aquaria kept at 16-18°C, in densities of less than 800 clams/m^2 for the small size classes (Lake George and small Lake Cheston clams) and 450 clams/m^2 for the larger size classes (Nolichucky River and large Lake Cheston clams). Neither of these densities approach the maximum field densities found in their natural habitats. Lake George clam bed densities are approximately 1,500 clams/m^2 (Jeremy Farrell, pers. com.) and stable populations from the southeastern United States are commonly reported to have densities of 3,000-14,000 clams/m^2 (McMahon 2000). During the holding period, clams were regularly fed *Sprillium* algae; 20% of the water was replaced on alternate days. Ammonia, pH, nitrate and nitrite levels were monitored and water-changing schedules were adjusted accordingly to ensure waste did not accumulate. Clams that died during the holding period were immediately removed from the tanks. Experimentation was started within 2 to 6 weeks of collection.

The experiment's cold treatment was conducted in a general laboratory refrigerator, and the warm treatment was located in a temperature-stabilized greenhouse. I used six 25L aquaria filled with 20L of water for each temperature treatment, and randomly assigned 3 tanks each to the "normoxia" and "hypoxia" treatment groups. Atmospheric air and compressed N_2 gas were delivered into the aquaria through gas bubblers to control DO levels. A YSI Pro 2030 was used to measure temperature and DO levels. The tanks were kept in darkness except when the experiments were being administered to, in order to reduce the effects of a light gradient within the cold treatment refrigerator, and to reduce algae growth and temperature fluctuation in the warm treatment. Each aquarium contained 20 Lake George clams, 20 Lake Cheston clams and 10 Nolichucky River clams, resulting in a density of 400 clams/m^2, or 2.5 clams/L. The clams from each population were kept spatially segregated by being placed in plastic cups that were lined thinly with aquarium gravel, both to weigh them down and to permit some natural burrowing behavior. Clams from the same population were grouped together to prevent misidentification, and population group locations within tanks were randomly selected. Given the relatively low clam density in the experimental aquaria, as well as the immediate removal of dead clams and the lack of feeding competition, this clustering was not expected to affect experiment outcomes. It also facilitated maintenance of the aquaria without causing excess turbulence in the water column, which would elevate DO levels. Clams were transferred into the experimental aquaria at 15^{o}C to begin transitioning to the treatment temperatures of 5^{o}C and 25^{o}C. The clams were also not provided with any food during the experiment, which minimized waste and ammonia buildup, and allowed my results to be comparable to other *C. fluminea* hypoxia studies; previous work has shown that this species can persist without food with little mortality for over six months in a laboratory setting (Johnson and McMahon 1998; Matthews and McMahon 1999).

Over a 7-day period, the aquaria temperatures were gradually lowered to 5°C, or increased to 25°C, for each temperature treatment. Over the subsequent 7 days, DO levels in the "hypoxic" treatment tanks were reduced to 5% DO saturation while "normoxic" treatment tanks were kept above 80% DO. After this 14-day adjustment period to treatment conditions, the clams were exposed to 5% or 90% dissolved oxygen levels at 5°C or 25°C for 49 days. DO levels were re-established daily using compressed N_2 gas and aerators. 20% of the water volume in each tank was replaced three times weekly, and the temperature and DO levels of the replacement water were brought as close to treatment conditions as possible using icepacks and N_2 gas before flowing into the tanks. Two temperature loggers placed in tanks in each temperature treatment recorded readings every ten minutes. In the cold treatment, the loggers were placed in tanks on the top and bottom shelves of the refrigerator to capture the temperature gradient within the unit, whereas in the warm treatment they were placed randomly. Ammonia and pH levels were checked regularly.

Daily visual inspections for dead individuals were conducted by searching for gaping clams. Each week, a probe test was performed on all clams in which a dissection needle is gently forced into the posterior valve margins near the hinge (a method used in previous studies: Cvetanovska 2015; Johnson and McMahon 1998; Matthews and McMahon 1999; Mattice and Dye 1975; Müller and Baur 2011); resistance indicates the animal is alive, whereas a dead clam's shell will open upon needle pressure and not re-close. Some live clams also gaped in the hypoxia treatments and would fully extend their foot, presumably to maximize diffusion surface area. On these clams, mortality was tested by lightly probing the exposed tissue with a dissection needle for a response. Upon death, clam size was recorded by measuring the maximum shell length from the posterior to anterior margins.

In the cold treatment, the clams were gradually returned to a recovery condition of 15°C and 95% DO over a period of two weeks, following the 49 day exposure period. A recovery period was included in this experiment because prior research testing *C. fluminea* interpopulation variation in cold tolerance showed that population differences were detectable only during a recovery period (Cvetanovska 2015). Food was introduced once at the beginning of the recovery period to initiate feeding but avoid ammonia buildup, and mortality checks and tank maintenance schedules were continued for the next two weeks. The experiment ended after the two-week recovery period, although mortality was tracked for an additional 3 weeks. In the warm treatment, no recovery period occurred because the experiment was terminated on Day 18 of the exposure period when 100% mortality was reached in all hypoxia treatment tanks.

Statistical analyses

All analyses were conducted in R Studio version 0.98.953 (R Studio 2013). Summary statistics of environmental data in the tanks over time were calculated and compiled using the zoo package (Zeileis and Grothendieck 2005).

To quantify tolerance, two metrics of interest were defined for each clam: mortality and survival time. These two variables were selected because they describe different features of the survival curve. Mortality was defined by the binary variable, TRUE (1) or FALSE (0), describing whether or not the clam died in the experiment. Clams that died during the initial adjustment period were not included in the analysis. Survival time is the continuous variable in days describing how long the clam persisted in the experiment, to a maximum of 77 days including the 49 day exposure period, 14 day readjustment period and 14 day recovery period. Therefore, all statistics were calculated from the beginning of the exposure period (after the two week period of adjustment to treatment conditions) to the end of the recovery period (77 days total).

To evaluate whether populations differ in their tolerance to hypoxia at low temperatures, I tested the effects of treatment (hypoxic or normoxic DO levels), population (Lake Cheston, Lake George and Nolichucky River), size (shell length in millimetres), and their interactions on mortality. The continuous variable ‘size’ was centered on the mean to aid model interpretation. Tank was introduced as a random effect to account for spatial clustering of individuals within replicate tanks, and environmental gradients within the experimental refrigerator. I fitted a binomial generalized linear mixed model with the lme4 package using a backward elimination protocol (Bates et al. 2014; Zuur et al. 2010). Pairwise differences were determined with a contrast matrix post-hoc analysis using the glht function in the multcomp package (Hothorn et al. 2015).

To evaluate whether populations differ in survival time among clams that died, I tested the effects of treatment, population, size and their interactions on the survival time of clams that died during the experiment. Tank was again included as a random effect, and I fitted a linear mixed model using the backward elimination protocol. Pairwise differences were determined using a Tukey HSD post-hoc analysis with the glht function in the multcomp package (Hothorn et al. 2015).

To evaluate the explanatory power of the models, marginal and conditional R^2 values were calculated using the method introduced by Nakagawa and Schielzeth (2013), implemented using the MuMIn package (Bartoń 2015). These values calculate the proportion of the variance in the models that is explained by the fixed effects alone (marginal R^2) and the fixed and random effects together (conditional R^2).

Environmental conditions during the experiment

In all treatment groups, dissolved oxygen levels fluctuated within each 24-hour period between adjustments. For more information on environmental conditions within the cold treatment aquaria, see Appendix A. The warm temperature treatment's DO levels fluctuated dramatically within each 24 hour period between adjustments, likely resulting in elevated mortality in both normoxic and hypoxic warm treatment groups. The resulting spikes in ammonia levels, exacerbated by high temperatures, caused further uncontrollable mortality (Figure 1.2). Therefore, this data was not included in the analysis.

Results

Variation in tolerance among populations and treatments

Clams in the hypoxic treatment tanks were 18.5 times more likely to die than clams in normoxic treatment tanks (Tables 1.2, 1.3). Under the normoxic treatment, Lake George clams experienced some significant mortality (49% survival ± 17% at 77 days), mostly during the recovery period when the temperature was 15^{o}C (Figure 1.3), whereas Lake Cheston and Nolichucky River clams exhibited high survivorship.

Treatment significantly affected mortality, and there was a significant interaction between clam size and population. Post-hoc analysis revealed that the relationship between size and mortality differed significantly between Nolichucky River clams and Lake Cheston clams ($p = 0.043$), and Nolichucky River and Lake George clams ($p = 0.034$; Figure 1.4). Survivorship of clams from Lake George and Lake Cheston increased with increasing size, whereas those from the Nolichucky River showed decreased survivorship with increasing body size. Fixed effects explained 47% of the variation in the model, and the inclusion of the random effect of 'tank' increased the amount of explained variation to 54% (Nakagawa and Schielzeth 2013).

Among clams that died, size and population significantly affected survival time (Figure 1.5). Size had a small positive effect on survival time, and post-hoc analysis revealed that Nolichucky River clams survived for significantly less time than Lake Cheston clams ($p = 0.005$), and marginally significantly less time than Lake George clams ($p = 0.069$; Figure 1.6). Fixed effects explained little variation in the model, which had a marginal R^2 of 0.05 and a conditional R^2 value of 0.32 (Nakagawa and Schielzeth 2013).

Differences in size distributions among populations

The Lake George clams were much smaller than the other two populations, and Nolichucky River clams were skewed to larger size classes (Table 1.4). The smaller size distribution is an inherent trait of the Lake George population, likely reflecting a shorter growing season (also observed for Lake Tahoe clams; Denton et al. 2012). Although significant differences in size distributions were observed among populations, clam size distributions within populations were not significantly different among tanks (Table 1.5).

Discussion

Interpopulation variation in cold hypoxia tolerance

This experiment revealed significant differences in cold hypoxia tolerance among invasive *C. fluminea* populations, largely explained by their contrasting size distributions. Lake Cheston clams were the most tolerant, lending support to the hypothesis that clams from a habitat in which they are regularly exposed to hypoxic conditions are better able to recover from hypoxia events. Much of their increased tolerance is explained by their larger mean body size, suggesting that habitats that foster high growth rates may produce more resilient clams with larger energy stores. Nevertheless, there was a significant difference in survival time among populations that is not explained by their different size distributions. Nolichucky River clams survived for shorter time periods than Lake Cheston clams, despite having large mean body sizes. The Nolichucky River is not known to experience hypoxia events, and therefore these clams were subjected to environmental conditions with which they have no prior experience (U.S. Geological Survey 2015). This supports the hypothesis that among *C. fluminea* populations, there is high variation in tolerance to specific environmental stressors (Cvetanovska 2015; Evans et al. 1977). There is no evidence that the Lake George clams' tolerance was determined by any factor considered here other than size; given that their smaller size typifies the source population, these clams are effectively less tolerant of cold hypoxic conditions.

These results expand upon prior knowledge of *C. fluminea* hypoxia tolerance at low temperatures, which did not test variation across conspecific populations. Matthew and McMahon's (1999) test subjects survived at least 84 days at 2-3% DO and 5^{o}C, whereas Johnson and McMahon (1998) measured a mean LT_{50} of approximately 20 days for clams exposed to 5% DO saturation at 5^{o}C. The mean values of LT_{50} for the three populations exposed to 5% DO saturation at 5^{o}C in this study were 50.3 days for Lake Cheston clams, 43.7 days for Nolichucky River clams, and 30.5 days for Lake George clams (Table 1.2). Thus, in addition to population source and size distribution, experimental design can significantly influence the outcome of tolerance studies for an environmentally sensitive species.

Body size and tolerance to environmental stress

Body size plays an important yet perplexing role in explaining tolerance differences between populations. In general, larger clams are more likely to survive and persist longer than smaller clams. However, in both normoxic and hypoxic treatments, Nolichucky River clams' probability of survival decreased with size, whereas Lake Cheston and Lake George clams had a positive size-tolerance relationship. This may be because the Nolichucky River clams tested in this experiment have a much narrower size range than Lake Cheston and Lake George clams, since smaller size classes were not represented. However, Johnson and McMahon (1998) found a negative relationship between size and survival in their cold hypoxia treatment; their clams' size distributions were wide (13.6mm to 25.7mm) and suggest their sample groups covered a large age range.

Although it is uncertain why a positive size-tolerance relationship (with the exception of Nolichucky River clams) was detected in this experiment in contrast to the negative size-tolerance relationship reported in Johnson and McMahon (1998), similar contradictory size-tolerance relationships have been reported for other environmental stressors. For example, two studies found that cold tolerance increased with body size (Cvetanovska 2015; Müller and Baur 2011). However, Mattice and Dye (1975) detected no significant size effects in their original *C. fluminea* cold tolerance experiments. Similarly, Mattice and Dye's heat tolerance tests (1975) detected no significant size effects, whereas Goss et al. (1977) revealed positive size-heat tolerance relationships. Inconsistent size-tolerance relationships may be another artefact of experimental design or stressors to which clams were subjected in their natural habitat prior to collection.

C. fluminea size-tolerance relationships may also vary depending on the stressor being imposed. In a review of a collection of environmental contaminant tolerance studies conducted on *C. fluminea*, it was reported that smaller clams are more sensitive to tin exposure, and more tolerant of gamma radiation, than larger clams (Doherty and Cherry 1988). Juvenile clams' tolerance to copper also increases with size (Harrison et al. 1984). The effect of size on *C. fluminea*'s tolerance to contaminants can also interact with the intensity of the stressor. Larger clams are more sensitive to chlorine dioxide at low concentrations, whereas smaller clams and larvae are more sensitive to the same chemical control at high concentrations (Goss et al. 1977). *C. fluminea*'s response to environmental stressors may also vary according to how size-correlated traits interact with the mechanism of the stressor. For example, Byrne et al. (1988) reported that the relationship between *C. fluminea* desiccation tolerance and size interacted with temperature. Larger clams are more tolerant to desiccation than smaller clams because they have lower water-loss rates; however, under heat stress they are less tolerant, owing to a slower metabolic rate (Byrne et al. 1988). Again, differences in study protocol likely explain some variation in tolerance, as McMahon (1977) found that body size did not affect *C. fluminea* water loss rates.

Physiological and metabolic consequences of body size in C. fluminea

Lake George clams' elevated mortality in both hypoxic and normoxic treatments compared to the other populations may be due to differences in the metabolic composition of smaller clams. Spann et al. (2011) compared the metabolomes (the small-molecule chemical composition) of larger (~24mm) and smaller (~16mm) invasive *C. fluminea*, detecting higher levels of energy-related metabolites such as glucose, glycogen and turanose in larger animals, and higher levels of nucleotides and amino acids in smaller animals. These differences drive differential responses to low-level cadmium and zinc exposure in large and small clams (Spann et al. 2011). Small clams' amino acid levels decreased, while large clams' levels increased due to the breakdown of glucose and glycogen, which were being consumed (Spann et al. 2011). Amino acids are the main energy source available to small *C. fluminea*, however big clams' access to larger energy stores may increase hardiness (Spann et al. 2011).

Confounding effect of age on size-tolerance relationships

Differences among populations' average body condition prior to the start of experimentation due to advanced age or prior environmental stressors may explain why the size-tolerance relationship was positive for Lake George and Lake Cheston clams, and negative for Nolichucky River clams. I postulate that body condition, and therefore tolerance to environmental stressors, may have a non-linear relationship to body size. Energy stores increase as a clam grows, but its body condition may decrease either near the end of its life cycle at maximum body size (Figure 1.7), or if energy stores are depleted when previously exposed to a stressful environment. Nolichucky River clams used in this experiment had a narrow size distribution that did not include younger/smaller classes, and therefore is skewed toward the older/larger end of the body condition-body size curve where the relationship between condition and size may be negative. Lake George and Lake Cheston clams have a much larger size distribution and include smaller/younger clam classes, and so their ability to withstand environmental stress may increase with size. This provides an alternative explanation to the contradictory size-tolerance relationships that were observed in Nolichucky River clams, and Lake Cheston and Lake George clams.

This hypothesis is further complicated by the relationship between clam size, clam age and habitat. Nolichucky River and Lake Cheston clams likely reach a larger maximum body size than Lake George clams due to the warmer climate and longer growing season in Tennessee than in New York State. For example, within one *C. fluminea* population, individuals in warmer microhabitats achieved larger body sizes than those in cooler microhabitats (Abbott 1977). Therefore, a hypothetical Lake George clam with a body length of 15mm is likely much older than a Nolichucky River clam or Lake Cheston clam of the same size.

It may have the same available energy stores as the other clams, but is more likely to have a poorer body condition, as it may be closer to the end of its lifespan. Therefore, clams of equivalent sizes from different populations may differ in their tolerance to hypoxia due to differences in age.

It was not possible to determine the age of the clams used in this experiment due to variation in shell morphology and spawning periodicity among populations (Britton and Morton 1977; Denton et al. 2012; Doherty et al. 1987).

Environmental tolerances vary between invasive populations

Although hypoxia tolerance was mostly determined by clam size in our experiment, we also found some evidence of variation among populations: Nolichucky River clams survived for less time than Lake George and Lake Cheston clams, and their size-survivorship relationship was negative rather than positive. Very few *C. fluminea* tolerance experiments have tested differences among populations. No evidence of interpopulation variation in potassium tolerance was found among *C. fluminea* from Texas, California and South Carolina (Daum et al. 1977). However, clams collected in California had lower survivorship in salinity shock tests than clams from Texas or South Carolina, and Texas clams could better acclimate to higher salinity conditions (Evans et al. 1977). Cvetanovska (2015) also quantifies variation in cold tolerance among North American invasive *C. fluminea* populations acclimated at 10^{o}C, finding that clams from cold temperate regions are generally more tolerant of long-term exposure to near-freezing temperatures than those from warm temperate regions. Clams from both Lake George and Lake Cheston were tested in this experiment, which found that Lake George clams were more tolerant than Lake Cheston clams (Cvetanovska 2015).

These examples provide a robust illustration of how *C. fluminea* may respond differently to a stressor across class sizes depending on the experimental protocol, source population, and stressor intensity or concentration. Risk assessments that rely on environmental tolerance tests must account for variation across populations, sizes or treatment protocols.

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Figures

Figure 1.1: Locations of the three populations tested in this study: Lake Cheston, TN, Nolichucky River, TN and Lake George NY.

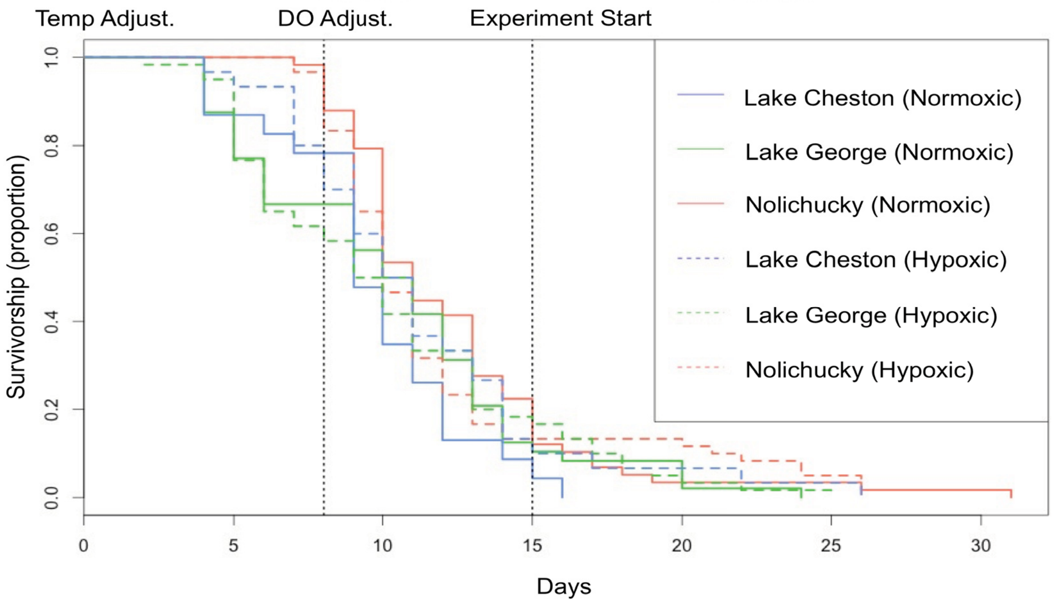

Figure 1.2: Clam survivorship through time under 5% and 90% DO conditions at 25°C, with datelines.

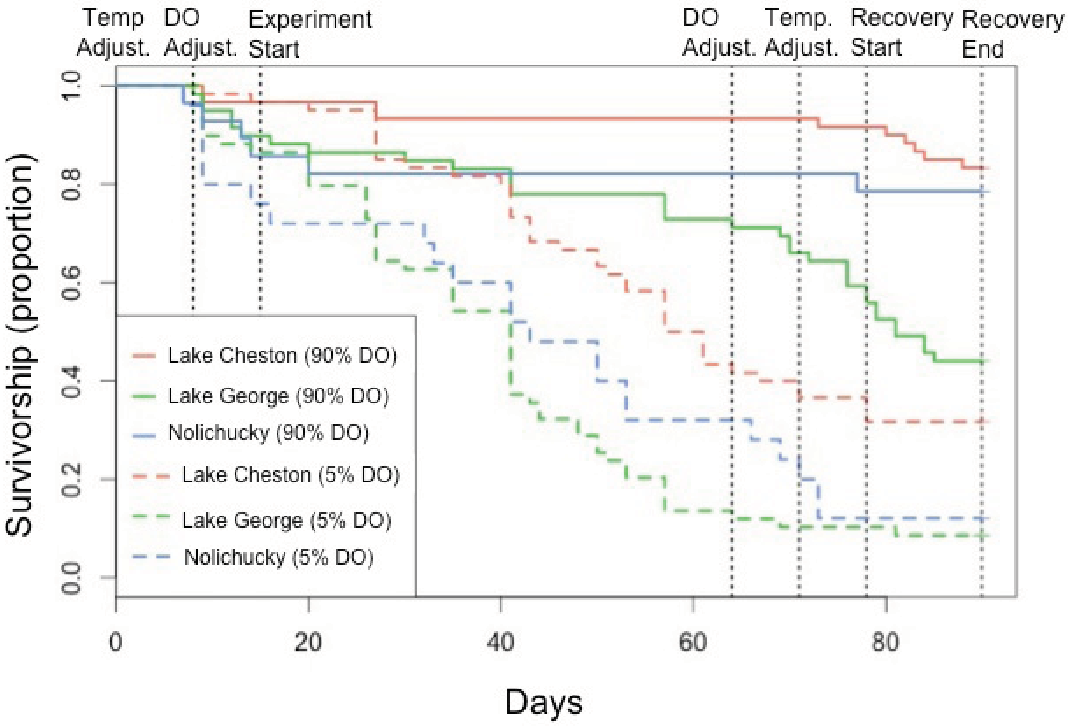

Figure 1.3: Clam survivorship through time under 5% and 90% DO conditions at 5°C, with datelines. Statistical analyses conducted for data collected between ‘Experiment Start’ and ‘Recovery End’.

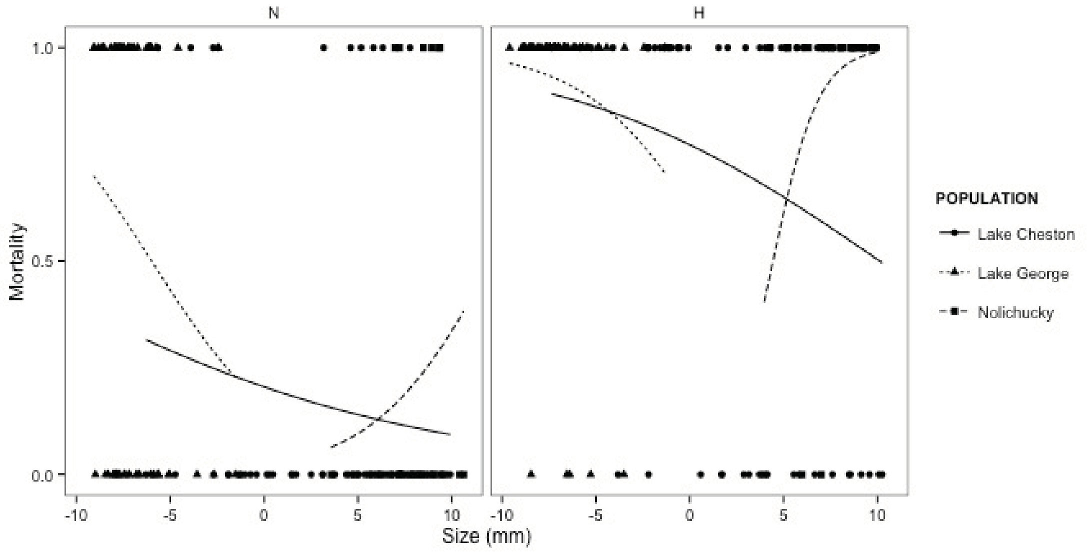

Figure 1.4: Mortality versus size (centred) for populations in each DO treatment (N = normoxic, H = hypoxic). Smoothers were generated using the binomial generalized linear model function.

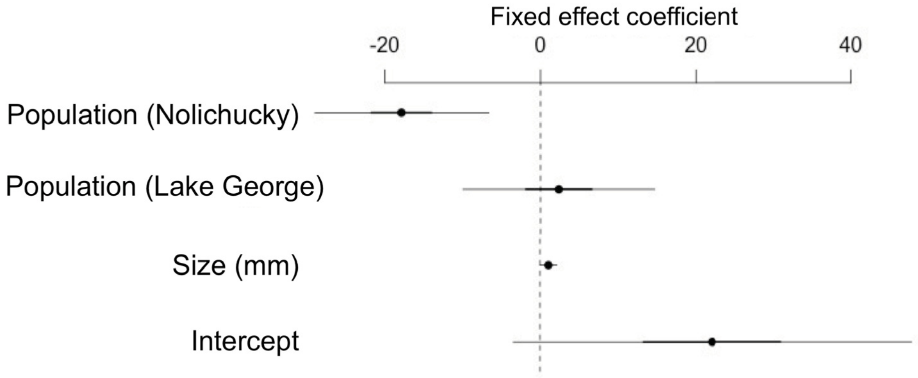

Figure 1.5: Plot of coefficients of fixed effects in linear mixed model of the effects of population and size on survival time in clams that died in the cold treatment experiment. Error bars reflect 95% confidence intervals, and indicate the effect is significant if they do not overlap with 0 (note: size is significant).

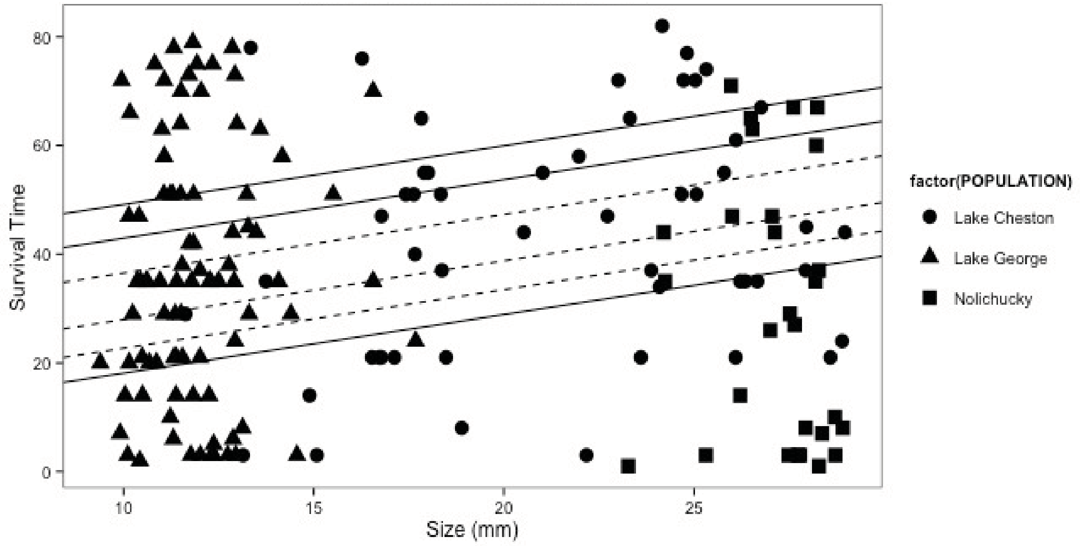

Figure 1.6: Survival time versus size of clams that died, by population. Overlays show regression lines of all six levels of the random effect, Tank. Dashed lines represent hypoxic tanks, bold lines represent normoxic tanks.

Figure 1.7: Hypothetical data showing relationship between body condition and clam size or age for a *C. fluminea* population, fitted with a polynomial overlay.

Tables

Table 1.1: Distinguishing habitat and morphological traits for clam populations from Lake George, Lake Cheston and the Nolichucky River.

	Lake George	**Lake Cheston**	**Nolichucky River**
Location	New York, USA	Tennessee, USA	Tennessee, USA
Watershed and Basin	Lake George Watershed, Mid-Atlantic Basin	Isolated reservoir	Nolichucky Watershed, Tennessee Basin
Habitat	Cold temperate and well-oxygenated	Warm temperate, summer hypoxia events recorded	Warm temperate and well-oxygenated
Collection location (GPS)	N: 43.428275 W: -73.709482	N: 35.210726 W: -85.929648	N: 36.0927018 W: -83.035883
Colour	Gold	Gold mottled with dark brown	Orange-brown
Morphology			

Table 1.2: Summary statistics across 3 replicate tanks, for each DO treatment at 5°C. Mean sample sizes do not equal 20 (Lake Cheston or Lake George) or 10 (Nolichucky River) because some mortality occurred during the 2-week adjustment period to treatment conditions. Survival time includes all clams, to a maximum of 77 days.

Treatment	**Population**	**Mean N per replicate**	**Mean survival time (days) ± SD**	**Mean LT_{50} (days) ± SD**	**Mean Survival (%) ± SD**
90% DO saturation	Lake Cheston	19	73.1 ± 5.0	-	86.7 ± 18.9
	Lake George	18	62.1 ± 4.1	73.0 ± 5.2	48.8 ± 16.7
	Nolichucky River	8	72.2 ± 5.4	-	91.5 ± 7.5
5% DO saturation	Lake Cheston	19	48.3 ± 13.6	50.3 ± 22.3	32.4 ± 22.2
	Lake George	17	31.3 ± 7.7	30.5 ± 4.8	9.2 ± 11.6
	Nolichucky River	6	41.6 ± 12.3	43.7 ± 13.7	14.3 ± 24.7

Table 1.3: Estimated coefficients for fixed effects of the binomial GLMM predicting mortality, with standard error (S.E.), statistical significance (*p*) and odds ratio. The * corresponds to *p*-values that are significant (α = 0.05).

	Estimate	**S.E.**	***p***	**Odds ratio**
Intercept	-1.474	0.554	0.001*	0.229
Population (Lake George)	-0.149	1.102	0.893	0.862
Population (Nolichucky River)	-2.922	1.696	0.085	0.054
Size (mm)	-0.130	0.053	0.014*	0.876
Treatment (Hypoxia)	2.918	0.721	<0.001*	18.505
Population (Lake George):size	-0.133	0.162	0.412	0.876
Population (Nolichucky):size	0.552	0.218	0.012*	1.736

N_{clams} = 259, N_{tank} = 6

Table 1.4: Clam size distributions for each population included in the analysis.

Population	**Mean ± SD (mm)**	**Minimum (mm)**	**Maximum (mm)**	**N ± SD**
Lake Cheston	22.32 ± 4.58	11.63	29.25	19.3 ± 0.8
Lake George	12.12 ± 1.61	9.39	17.68	17.3 ± 1.9
Nolichucky River	26.64 ± 1.72	22.55	29.63	7.2 ± 1.2

Table 1.5: Mean rank (Kruskall-Wallace test) and homogeneity of variance (Levene's test) differences among clam size distributions within populations across replicates, and between populations across replicates, for the cold treatment. The * corresponds to *p*-values that are significant (α = 0.05).

Population	**Kruskall-Wallace χ^2**	**df**	**p-value (α = 0.05)**	**Levene F-value**	**p-value (α = 0.05)**
Lake Cheston	0.5756	5	0.99	0.3950	0.85
Lake George	0.9458	5	0.97	0.4407	0.82
Nolichucky River	3.3027	5	0.65	0.9006	0.49
Between all populations	220.5821	2	<0.001*	74.876	<0.001*

Linking Statement: Chapters 1 and 2

Chapter 1 highlights the limitations of conducting aquatic invasive species risk assessment using environmental tolerance data derived from few populations. *Corbicula fluminea* has established in water bodies that were historically thought to be invulnerable to invasion because their minimum winter temperatures were below the presumed lower thermal limit (2^{o}C) of the species (Mattice and Dye 1975). Yet, some invasive populations have recently been shown to survive and recover from exposure to 0^{o}C, and there is significant interpopulation variation in cold tolerance (Cvetanovska 2015; Müller and Baur 2011). Similarly, I detected interpopulation variation in cold hypoxia tolerance among North American *C. fluminea*, as well as differing size-tolerance relationships.

Given these results, it seems valuable to re-assess the potential large-scale distribution of the species. In Chapter 2, I model its predicted climate envelope in North America, using bioclimatic suitability modeling techniques. I identify multiple uninvaded areas of North America that are suitable to *C. fluminea* (or will be by 2050) that should be prioritized in monitoring and outreach programs. Although both mechanistic environmental tolerance models and empirical climate suitability models have their limitations, they collectively provide lines of evidence that can inform aquatic invasive species risk assessment and management.

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CHAPTER 2

Climate suitability projections for the Asian clam *Corbicula fluminea* in North America

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Abstract

Recent evidence suggests that the Asian clam *Corbicula fluminea* can persist in cold temperate climates and is expanding its range into seasonally ice-covered water bodies previously thought to be invulnerable to invasion; new populations have been detected in thermally unmodified waters in upper New York State, New England and in southwestern Ontario. Climate suitability modeling techniques can be used to identify regions at risk of invasion by projecting the climatic space the species is known to occupy over the landscape of interest. I present a MaxEnt model that projects climatic suitability across North America for *C. fluminea* under current and future climate regimes. The results indicate that this species poses a significant invasion risk to waterways in cold temperate regions. Further spread is likely in parts of upper New York State and southern Michigan and Ontario that are currently climatically suitable to *C. fluminea*, and extensive regions of southern Canada are projected to be suitable by 2050. These results can inform monitoring, control and awareness programs to prevent the continued spread of this invader in North America.

Introduction

Control or eradication strategies for invasive species are more likely to be effective at the early stages of establishment when the population is limited in spatial scale and abundance (Pluess et al. 2012). Consequently, tools are needed to predict where invasive species may establish or spread to inform monitoring efforts and reduce detection and response times. Given the recent establishment of *C. fluminea* populations in cold temperate lakes in North America, it is likely that this species' invasive range will continue to expand, especially since *C. fluminea* is more cold-tolerant than previously thought (Cvetanovska 2015; Müller and Baur 2011). Numerous populations have been found in upper New York State including Lake George and the Finger Lakes, and satellite populations have been detected in southwestern Ontario and British Columbia (pers. obs.; Holm 2011; Kirkendale 2008; U.S. Geological Survey 2015). *C. fluminea* has caused substantial impacts to invaded aquatic ecosystems and industry (reviewed in McMahon 2000), and so should be of concern to aquatic managers in climatically suitable regions of North America that have not yet been invaded.

The probability of an invasive species establishing in a novel habitat is controlled by many factors including recipient community composition, dispersal ability, propagule pressure, and climate and habitat characteristics (Gallardo 2014; Jeschke and Strayer 2008; Leung and Mandrak 2007; Lockwood et al. 2005; Pyšek et al. 2010). Climate in particular limits invasive species' distributions at a regional level, and climate change is likely to alter their ranges by changing environmental conditions and, potentially, biotic interactions (Hellmann et al. 2008; Thomas 2010). Although *C. fluminea* populations can be locally patchy owing to habitat heterogeneity, at a regional level the species is more limited by climate - which better predicts *C. fluminea*'s realized niche than habitat structure (Crespo et al. 2015; McDowell et al. 2014). Given its ubiquity in climatically suitable regions of the continental United States, this invader's establishment seems limited by neither natural barriers to dispersal, nor North

America’s native biological communities (McMahon 2000). The potential range of *C. fluminea* in North America under current and future climate regimes should be determined to develop appropriate monitoring and response protocols.

Bioclimatic modeling of invasive species’ potential ranges

One predictive tool that is being used with increasing frequency is habitat suitability modeling, a category of bioclimatic modeling where environmental conditions at a given location are compared to the conditions in locations occupied by an invasive species. The species’ distribution across environmental space is measured, and then used to project a region of suitability across geographic space (Franklin 2009).

Habitat suitability modeling has traditionally been used to predict species’ presence or describe realized niches, but some key assumptions are violated when projecting the expanding ranges of invasive species (Jeschke and Strayer 2008). Specifically, bioclimatic models assume that 1) biotic interactions are unimportant in determining an invasive species’ range or are constant across the study space and time period; 2) genetic and/or phenotypic traits are constant across populations and time; and 3) species dispersal is unrestricted (Jeschke and Strayer 2008). However, when creating models to inform invasive species risk assessment, the objective is not to accurately predict the invader’s *current* range. Rather, we seek to identify regions whose climates may support an invasive population based on its known climatic envelope, if the other conditions for establishment success (e.g. dispersal opportunity, low biotic resistance) are met (Jimenez-Valverde et al. 2011).

When generating this type of prediction for a species like *C. fluminea* with a very high dispersal capability and no evidence for biotic resistance to establishment in introduced systems (McMahon 2000), concerns about biotic interactions or dispersal probability are minimized. With respect to phenotypic uniformity across the study space, environmental tolerances can vary across *C. fluminea* populations, and, thus, phenotypes related to increased tolerance to specific environmental stressors might arise (Cvetanovska 2015). As such, the prediction is limited to the current distribution of environmental tolerance phenotypes across North America's *C. fluminea* populations. We can further parameterize species distribution models to better achieve the objective of predicting regions that are climatically suitable to species undergoing range expansion (Elith et al. 2010; Jeschke and Strayer 2008; Jimenez-Valverde et al. 2011; Merow et al. 2013).

Existing bioclimatic models for C. fluminea

Several studies have attempted to quantify and project the habitat requirements or bioclimatic niche of *C. fluminea*. By comparing the performance of climate-only, habitat-only and climate-habitat logistic regression models developed using presence-absence survey data, McDowell et al. (2014) concluded that climate variables better predict *C. fluminea*'s presence in the continental USA than habitat variables. Guided by these results, they developed a spatially explicit MaxEnt bioclimatic suitability model and projected *C. fluminea*'s distribution using the CCCMA global circulation model in the years 2050 and 2080 according to several climate change pathways (McDowell et al. 2014). Under multiple scenarios, large portions of the northeastern United States and the Great Lakes region were projected to become climatically suitable for this species by 2080 (McDowell et al. 2014). Their analysis is limited, however, by the exclusion of territory outside the continental United States - such as Canada, where the invasion front seems to be moving - and by not attempting to correct sampling bias inherent in their presence-only dataset beyond spatial rarefaction, which can significantly reduce model quality (Phillips et al. 2009). Gama et al. (*in press*) compared the influence of climate and topographical variables on *C. fluminea*'s distribution at a global scale, using nine algorithms from the BIOMOD2 package and presence-only data from invasive and native *C. fluminea* ranges. They also found that climate variables are better predictors of *C. fluminea*'s distribution than topographical variables, although in the mixed climate-topography model altitude explained a significant amount of variation (Gama et al. *in press*). However, their presence dataset included populations persisting in thermally modified habitats, and combined presence records from the native and invasive ranges - despite an unresolved taxonomy within the genus in the native range, uncertain origin of invasive populations, and large variation in life history traits and tolerances among invasive and native populations (Crespo et al. 2015; Cvetanovska 2015; Morton 1986; Pigneur et al. 2014). Additionally, their projected suitable habitat for *C. fluminea* in North America does not conform to the known distribution, suggesting their model is not highly transferable.

C. fluminea *climate suitability in North America*

I developed a climate suitability model for *C. fluminea* in continental North America to identify regions at risk of invasion under current and future climate conditions, and inform monitoring and mitigation strategies. I elected not to create a global model of *C. fluminea* climate suitability, owing to the unresolved taxonomic forms in the native range that could produce questionable presence records (Morton 1977; Morton 1986). Only two homozygous *Corbicula* lineages have been identified in North America, including the dominant white Form A and rare purple Form B, so the risk of species misidentification is minimal (Hillis and Patton 1982; Lee et al. 2005; Pigneur et al. 2014). I predicted that the northern range limit of *C. fluminea* in North America would expand into novel habitat with climate change, and discuss consequences for monitoring efforts in jurisdictions inexperienced with managing this invader.

Methodology

MaxEnt

To quantify the current distribution and potential range of *Corbicula fluminea* in North America, I developed a climate suitability model using MaxEnt. MaxEnt is a machine-learning modeling program that uses presence locations to define the environmental space occupied by the target species as a function of environmental predictor variables (Phillips et al. 2006). The environmental space is then projected into geographic space, generating an index of bioclimatic suitability across the landscape of interest using local climate conditions. This is done by estimating a target probability distribution of maximum entropy (the least constrained), within the constraints of the species' known occupied environmental space (Phillips et al. 2006). I used MaxEnt's logistic output, which I interpret as an index of relative climate suitability. A more detailed description of the MaxEnt modeling process and output formats can be found in Appendix B.

The MaxEnt climate suitability modeling method is appropriate for modeling the potential range of *C. fluminea* in North America. Only presence data is required to build the model, rather than the presence-absence data that is required for other regression-based bioclimatic models. Species distribution models developed using both presence and absence data, rather than just presence data, generally tend to perform better, particularly for widespread generalist species like *C. fluminea* (Brotons et al. 2004; Franklin 2009). However, obtaining reliable absence data for invasive species can be very challenging, and *C. fluminea* is no exception; available datasets are not based on systematic surveys but rather compilations of reported sightings. Furthermore, absence data for a species whose range is still expanding may build poorly parameterized models, as absences may be due to a lack of dispersal opportunity or low propagule pressure rather than poor climatic suitability; therefore presence-only modeling can be more accurate for invasive species than presence-absence methods even if absence data is available (Cianfrani et al. 2010; Gu and Swihart 2004). MaxEnt, like other presence-only models, has tools available to help reduce the effects of sampling bias in the presence dataset and increase the performance of the model (Elith et al. 2010).

MaxEnt is the most appropriate presence-only data modeling strategy because it is generative rather than discriminative, and so allows us to make no assumptions about the suitability of habitat that has no presence records (Franklin 2009; Phillips et al. 2006). Additionally, comparative studies have shown that MaxEnt consistently outperforms other presence-only species distribution models when predicting habitat suitability (Elith et al. 2006; Jeschke and Strayer 2008; Wisz et al. 2008). When parameters are set appropriately, it can also successfully model scenarios where the target species violates some model assumptions, such as when modeling range-shifting or invasive species (Elith et al. 2010; Ward 2007).

Environmental data collection and preparation

The Bioclim suite of climate data was obtained from WorldClim, a publically available global climate dataset (Hijmans et al. 2005). The Bioclim layers are derived from four WorldClim climate layers: monthly precipitation, and monthly mean, maximum and minimum temperature. These variables are spatially continuous surfaces that are created by interpolating between climate monitoring stations (Figure 2.1). The data is averaged over a fifty-year period (1950-2000) to provide an estimate of climate in the last half of the 20th century. These surfaces are then used to calculate the 19 Bioclim parameters, which are biologically relevant climate surfaces (Table 2.1). This dataset was selected because the methods used to generate the environmental layers are available, it performs well in bioclimatic models where variables are pre-selected to the target species, and station coverage is excellent over the North American study area (Figure 2.1; Beaumont et al. 2008; Hijmans et al. 2005).

I used grids with a 5 arc-minute resolution, which corresponds to ~9.3km grid cells (86.5km^2 resolution), although resolution varies with latitude. This grid size was selected to ensure that the final projection had relatively high resolution, without increasing the files' resolution beyond what was ecologically meaningful or the computer's capacity.

All data preparation and visualization was performed in ArcMap using ArcGIS Version 10.1 (ESRI 2012). Base map features to support data visualization, including geopolitical boundaries and water features, were sourced from the Commission for Environmental Cooperation of North America (INEGI et al. 2009; 2010). Bioclim data layers were clipped to the study extent (continental North America, delineated by geopolitical boundaries). A pairwise correlation matrix was calculated for the Bioclim variables using SDMtoolbox, and highly correlated variables were removed (Pearson coefficient > |0.7|; Brown 2014). MaxEnt can train models using correlated predictor variables, however this makes the relative contribution of the correlated predictors non-interpretable, and including too many predictor variables may cause overfitting (Beaumont et al. 2005; Elith et al. 2011; Merow et al. 2013). Predictor variables were selected based on ecological relevance to *C. fluminea*: they are very sensitive to cold temperatures and desiccation (Byrne et al. 1988; Cvetanovska 2015; McMahon 2000; Müller and Baur 2011). This reduced the nineteen-parameter dataset down to three predictor variables: annual temperature range, mean temperature of the coldest quarter and precipitation in the driest quarter. When choosing between highly correlated variables, I selected those with intermediate temporal resolution (for example mean temperature or precipitation over a quarter, rather than a month or year) to capture seasonality, rather than seasonal extremes that may not be relevant for an aquatic species whose environment is buffered by water's high heat capacity. Altitude was also included, as it explains much variation in *C. fluminea*'s distribution and may correlate with habitat characteristics such as nutrient availability and substrate grain size (Crespo et al. 2015; Gama et al. *in press*).

Initial model runs revealed that with these predictors alone, the upper thermal limit of the species was not adequately captured and the prediction was unreliable. Mean temperature of the warmest quarter was introduced as an additional predictor, which is highly correlated with mean temperature of the coldest quarter. Therefore, relative contributions of the mean temperatures in the warmest versus coldest quarter are not interpretable, but their combined relative contribution can be compared to the other predictor variables.

Future climate scenario and model selection

I projected the output of the final model into both the current climate, and projected climate conditions in the year 2050, to explore how *C. fluminea* climate suitability across North America may change in the future. The most recent 5th edition of the International Panel on Climate Change (IPCC) report described four representative concentration pathways (RCPs), or different scenarios of greenhouse gas (GHG) emissions and resulting atmospheric concentrations, air pollutant emissions and land use change for the 21st century (IPCC 2014). These pathways are RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5, named for the estimated increase in radiative forcing (in W/m^2) they will cause in 2100 (and therefore listed in order from smallest to greatest increase in the Earth's mean temperature). I chose to model *C. fluminea* climate suitability along RCPs 4.5 and 8.5, in order to generate "best case scenario" and "worst case scenario" projections. Consensus today is that RCP 2.6 is not attainable given the current political climate, and current GHG emissions are tracking above the RCP 8.5 projection (Sanford et al. 2014).

Over fifty global circulation models (GCMs) have been developed and refined to describe possible climate conditions at specific time steps along different RCPs; their projections have been made available by the IPCC (Harris et al. 2014; IPCC 2014). Currently, the best practice for species distribution modeling in future climates is to create multi-model ensemble projections with GCMs that perform well (predict current climate conditions accurately) in the study area; a range of outcomes across equally plausible descriptions of future climate is presented (Beaumont et al. 2008; Harris et al. 2014; Porfirio et al. 2014). I chose to use the following GCMs, all of which are being tested in the IPCC's Climate Model Intercomparison Project 5 (CMIP5):

1) HadGEM-2ES: an Earth System model (Bioclim code HE; Collins et al. 2011);
2) MIROC-ESM-CHEM: an Earth System model with an atmospheric chemistry coupling (Bioclim code MI; Watanabe et al. 2011);
3) MRI CGCM3: coupled atmosphere-land, aerosol and ocean-ice models subset from the MRI Earth System model (Bioclim code MG; Yukimoto et al. 2012); and
4) MPI-ESM-LR: an Earth System model (Bioclim code MP; Giorgetta et al. 2013).

These models were selected because their previous generations perform well in comparison to other models when predicting current climate conditions in North America (Radić and Clarke 2011). Furthermore, Bioclim layers were available for all of these models through the WorldClim portal, consistently downscaled and bias-corrected using the current climate Bioclim layers as a baseline (Hijmans et al. 2005). The same Bioclim layers used to train the climate suitability model (mean temperature of the warmest and coldest quarters, annual temperature range and precipitation of the driest quarter, in addition to altitude) were used to project *C. fluminea* climate suitability across North America in 2050.

Presence data

All presence data for *C. fluminea* in North America was obtained by accessing government datasets and through personal observation. The primary data source was the *C. fluminea* presence records from the United States Geological Survey's (USGS) Non-indigenous Aquatic Species (NAS) Database (2014). This dataset is compiled from presence observations in museum archives, scientific literature, monitoring programs, government agencies, expert communication and online/hotline invasive species reports. It has been supplemented with personal observations of northern populations that have not formally been published, such as McGeachy Pond. American states that did not provide their *C. fluminea* presence records to the USGS were directly contacted for additional known presence records to counteract any bias introduced by states' varying participation in the USGS NAS program. Michigan, New York and Indiana provided additional data, as well as British Columbia, which recently observed a new invasion front in the Fraser and Coquitlam rivers (Fisher 2014; Herborg and Philip 2015; Midwest Invasive Species Information Network 2015; New York Natural Heritage Program 2014). Additional data was also obtained from the Rhode Island and Wisconsin state websites (Rhode Island Department of Environmental Management 2013; Wisconsin Department of Natural Resources 2014).

After cleaning, the dataset comprised 6,795 presence locations, and was further reduced to 2,605 records after being spatially rarefied to the resolution of the environmental layers (5 arc-minutes). For more details on how the dataset was cleaned of erroneous presence records, as well as an explanation of spatial rarefaction, please see Appendix B.

Bias layers and background selection

Another potential bias in the dataset is the lower density of presence points at the species' range limit. *C. fluminea* is spreading outward from its initial introduction points on the west coast of North America (1920s) and the southeastern United States (1957; U.S. Geological Survey 2015). Regions that have been invaded for longer are better represented in the datasets, creating a 'time since invasion' temporal bias that skews towards regions where *C. fluminea* invaded earlier (Figure 2.10). This violates the MaxEnt modeling assumption that presence points represent a random sample of the suitable environmental space the species can occupy (Elith et al. 2011; Phillips et al. 2006). To address this concern, I incorporated a Gaussian kernel density bias grid that increases the weight of presence points with fewer neighbours in the model using SDMtoolbox in ArcMap (Brown 2014; Elith et al. 2010; Kramer-Schadt et al. 2013). Moreover, all analyses were conducted in the WGS1984 coordinate system, which is not an equal-area projection. Since the study area covers a large latitudinal range, cell size increases with latitude and introduces a poleward bias when selecting both presence and background points (Elith et al. 2011). To correct this, I incorporated a latitudinal bias grid made available at the SDMtoolbox website (Brown 2014), and multiplied it with the Gaussian kernel density bias grid to obtain a single bias file that was included in the model.

The area from which background points can be selected greatly influences the output of a MaxEnt model, as it is assumed to be the range of conditions available to the target species (Merow et al. 2013). A large background area introduces much environmental space to the model that is certainly unsuitable and provides little useful information when MaxEnt develops response curves

(Merow et al. 2013). However, if the background is too limited, a narrower range of environmental conditions is encountered during training, resulting in a model with low transferability (Elith et al. 2010; Merow et al. 2013).

I selected an intermediate background selection method: background points were limited to a buffer of 1,200km beyond the known range limit of *C. fluminea* in North America. This model will predict the potential range of *C. fluminea* in 2050, so background points are selected from geographic and environmental space into which *C. fluminea* could feasibly disperse, barring a long distance overland dispersion via human facilitation and given the historical North American dispersal rate of ~400km/decade (Crespo et al. 2015; McMahon 2000). Selecting background points from within a buffer determined by dispersal rates has been used successfully to reduce sampling bias and model establishment risk for invasive species (Elith et al. 2010; Fourcade et al. 2014). The bias layer was clipped to the background extent, and used during model selection and in the final model.

Model settings, selection and validation

Although the default settings of MaxEnt have been selected to maximize general predictive strength, these settings may not be appropriate for modeling species undergoing range expansion. Therefore, some of the default settings were adjusted to accommodate species-specific characteristics.

First, only hinge-response curves (threshold responses with linear responses between the levels of the threshold function) were permitted when training the model (Elith et al. 2010; Syfert et al. 2013). For continuous predictor variables, MaxEnt permits linear, quadratic, product, threshold and hinge response types, but including all types can create overfitted response curves that are not ecologically meaningful (Merow et al. 2013; Phillips and Dudik 2008; Warren et al. 2014). Second, extrapolation was not permitted, and clamping was enabled to ensure predictions were not permitted beyond the environmental space encountered during training.

When modeling the regions that are climatically suitable to a species undergoing range expansion, conventional model validation is not possible because independent verification datasets are usually unavailable, the true potential distribution of the species is unknown, and the model that most accurately predicts the species' current distributions may not translate well into different regions or climates due to overfitting (Elith et al. 2010; Jeschke and Strayer 2008; Jimenez-Valverde et al. 2011). Therefore, my model validation criteria were to generate a model that 1) is general and not overfitted to the training data, 2) accurately predicts current presence locations, and 3) identified regions of extrapolation where predictions are unreliable, to ensure a useful projection into future climate space (Elith et al. 2010; Jimenez-Valverde et al. 2011).

I validated my model by randomly selecting 20% of the presence locations using a random seed to test the predictive performance of the model trained on the remaining 80% of the observations. The difference in the area under the curve (AUC) of the receiver-operator curve (ROC) between the training and testing data was inspected for evidence of overfitting (e.g., a training AUC that is much higher than a test AUC). The ROC shows the model's accuracy when distinguishing presence points from the available background, where the AUC can range from 0.5 (random model) to 1 (perfect model).

Response curves were studied for irregular features (such as spikes at specific values) as evidence of overfitting. This was corrected by increasing the regularization parameter λ from 1 to 2.5, which increases the penalty for overly complex fits; this is also recommended when modeling range-shifting species to improve transferability (Elith et al. 2010; Merow et al. 2013).

Finally, the projected suitable habitat for *C. fluminea* in North America was visually inspected in comparison to the presence data to look for irregularities. Specifically, I looked for habitat defined as suitable in environments that differ from the current known range (which triggered my inclusion of the mean temperature in the warmest quarter as a predictor layer) and habitat defined as unsuitable where there are many presence locations. The parameterization choices described above produced a model that passed these validation assessments, and so was used for the final model.

Final model execution and evaluation

The final model run comprised ten replicate runs using the settings, predictor variables, climate projections, bias layers and presence data described above. Jackknife tests were conducted on the predictor variables to evaluate variable contribution. The continuous logistic output was assigned a threshold, which attempts to maximize agreement between the predicted and observed occurrences by assigning a cut-off on the relative climate suitability index (Elith et al. 2010; Merow et al. 2013); this was required to compile current binary and future climate suitability projections. I assigned a maximum sensitivity plus suitability threshold on the training data since it is a robust method that minimizes omission errors; commission errors are not meaningful when modeling species undergoing range expansion (Elith et al. 2010; Jimenez-Valverde et al. 2011; Liu et al. 2005). The model was then projected into current and future climate space.

2,585 presence locations were used to train and test the model. Across the ten replicate model runs, the number of background points ranged from 11,980 to 11,992, with an average of 11,986. The number of iterations ranged from 180 to 260, with an average of 218, indicating rapid convergence since MaxEnt's default convergence threshold is 10^{-5} up to a maximum of 1000 iterations (Phillips et al. 2006). The percent contribution and permutation importance of each predictor variable were used to evaluate their value to the final model, as well as the jackknife test results.

Projections

The binary climate suitability map for current climate conditions was created by taking the average of the ten replicate binary climate suitability projections across the study area, and then reclassifying it into two categories, suitable (1) or not suitable (0) at a 0.5 threshold (or, where more than half of the replicates identified that region as suitable). Future climate ensemble projections were compiled by summing the mean binary suitability projections along each RCP for each GCM; therefore the pixel values of 0 through 4 indicate the number of future climate projections that are in agreement that the location will be climatically suitable to *C. fluminea*. Model agreement was mapped, rather than the average of the models' predictions, because this would produce an implausible projection that is not a product of any of the GCMs (Harris et al. 2014; Porfirio et al. 2014). This is similar to a bounding box approach, but shows the level of agreement between models for specific region's projected suitability as an indicator of consistency across GCMs.

Results

The final model average had a mean AUC of 0.79 with a standard deviation of 0.006, therefore the model is significantly better at predicting climate suitability than a random model (Figure 2.2). The mean omission rate on test data did not strongly deviate from the predicted omission rate, suggesting that the model is not overfitted to the training data (Figure 2.3).

Variable contribution was evaluated for each predictor, although since BIOL11 (mean temperature in the coldest quarter) and BIOL10 (mean temperature in the warmest quarter) are highly correlated, comparing the relative contributions of these layers to each other is not reliable. These temperature variables together explain most of the variation in the model, with both high permutation importance and percent contribution (Table 2.2). Altitude and the annual temperature range also explained a small amount of variation in the model, and mean precipitation in the driest quarter explained no variation. The jack-knife test confirmed that temperature variables BIOL11 and BIOL10 contribute the most gain to the model (Figure 2.4.a-c). Altitude contains the most information important for predicting climate suitability not present in other layers, as the test AUC decreases when it is excluded from the model (Figure 2.4.c).

Response curves show that *C. fluminea* is more likely to be present at low altitudes (Figure 2.5.a-b), and at high mean temperatures of the warmest and coldest quarters (Figure 2.5.e-i). The value of the response curves drops to zero beyond the range of environmental conditions encountered during training because extrapolation was disabled. The relationship between *C. fluminea* climate suitability and mean precipitation in the driest quarter (BIOL17) was slightly negative in the complete model, which was unexpected since this is a drought-sensitive species (Figure 2.5.j). Further examination of the response curve where BIOL17 is the sole predictor variable reveals that across most of the range of potential BIOL17 values the relationship is in fact positive, but probability of occurrence increases significantly when approaching zero (Figure 2.5.k).

The final average continuous logistic output of the ten replicate runs had good performance in predicting which regions are climatically suitable to *C. fluminea* under current climate conditions (Figure 2.6, 2.7). Some known *C. fluminea* populations at the current range limits fall outside the binary suitability map, such as those in Michigan, Wisconsin and the Lake George area of New York State (Figure 2.8). This prediction is reliable because no areas of suitability occur in regions whose climates occupy environmental space outside that which was encountered during training, and therefore no clamping was required (Figure 2.9). Few regions that have not already been invaded were identified as climatically suitable, however some regions, such as Oklahoma, Texas and Nebraska, were deemed to be suitable but have not reported *C. fluminea* populations to date.

In the year 2050 under the RCP 4.5 scenario, some regions projected to be suitable to *C. fluminea* by several GCMs include large areas of the coast of British Columbia including Haida Gwaii, central British Columbia, southern Ontario, southern Wisconsin, Michigan, upper New York State including the Champlain basin, New England, and the Maritimes excluding Newfoundland (Figure 2.10). Under the RCP 8.5 scenario, the results are not substantially different, although the projected climate suitability limit is slightly farther north in some regions (Figure 2.11).

Some regions within *C. fluminea*'s current range - specifically southern Arizona, New Mexico and Florida – are projected to experience climate change that causes them to move into environmental space not encountered during model training. Because extrapolation was disabled, climate suitability at these locations falls to 0 to avoid erroneous prediction. Multivariate environmental similarity surfaces indicate the location of novel climate conditions under various future RCPs and GCMs, and novel climate maps indicate the limiting environmental condition at those locations (Figures 2.12.a-h, 2.13.a-h). Regions identified as unsuitable in areas where an environmental predictor is encountering new environmental space should not be interpreted.

Discussion

Environmental predictors of C. fluminea *climate suitability in North America*

Temperature variables, including mean temperatures in the warmest and coldest quarters, proved to be the most useful for model training and reflect *C. fluminea*'s known tolerances, whose upper and lower thermal limits approach 34°C and 0°C, respectively (Cvetanovska 2015; Mattice and Dye 1975; Müller and Baur 2011). Altitude also contributed significantly to the model, with regions at lower elevation more likely to be climatically suitable, which suggests the value in supplementing *C. fluminea* climate suitability models with topographic or habitat variables (Crespo et al. 2015; McDowell et al. 2014).

Neither precipitation in the driest quarter nor annual temperature range explains much variation in the model. Annual temperature range may be unimportant because the high heat capacity of larger aquatic ecosystems might buffer against substantive changes in air temperature. Alternatively, *C. fluminea* populations may persist in habitats with a large annual temperature range because they display r-selected life history traits, including a high reproductive rate, that allow them to persist in unstable habitats (McMahon 2002).

Precipitation in the driest quarter was hypothesized to be important because of *C. fluminea*'s sensitivity to desiccation (Byrne et al. 1988); however, probability of presence markedly increased as this predictor approached zero (Figure 2.5.k). This possibly reflects the influence of anthropogenic habitat modifications, such as dams, that reduce desiccation risk in arid or semi-arid regions of the North American range (see Aldridge and McMahon 1978). The most impounded watersheds in the United States lie in regions that are both arid and within *C. fluminea*'s known range, such as Texas (Graf 1999).

These results underscore the importance of interpreting climate suitability model outputs in the context of other factors that control establishment success of invading species. *C. fluminea* may establish beyond the suitable range delineated in this study, if local habitat is modified to reduce the impact of the limiting environmental variable. Similar to the effect of impoundment in desert regions, thermal habitat modification in cold regions can facilitate *C. fluminea* establishment. *C. fluminea* populations were detected in thermal plumes of electrical generating stations in the St. Lawrence River and Lake Superior, which are both projected to be climatically unsuitable (Simard et al. 2012; Ward and Hodgson 1997). Therefore, indices of climate suitability are useful at the regional level; but, when assessing the invasion risk of a specific water body, the mediating or amplifying effects of local habitat structure must be considered.

Regions at risk of invasion

The results of this analysis indicate that *C. fluminea* has likely not reached equilibrium in North America, and there are additional regions it may occupy. Updated reports of *C. fluminea* establishments support this assertion: during the summer and fall of 2015 after this dataset was established, additional populations were reported in New England, including the first known population in Maine in the Piscataqua River (U.S. Geological Survey 2015). Regions that are identified as climatically suitable to *C. fluminea* but contain few reported populations include the coastal region of southern British Columbia, Washington State, and Oregon, where this species may continue to spread. Much of the southwestern United States such as eastern California, Nevada, Arizona and

New Mexico has been identified as suitable, where the species will likely be limited to impounded waterways or reservoirs.

All GCMs and climate scenarios used in this analysis suggest that certain regions in the northern United States and southern Canada will become climatically suitable to *C. fluminea* within the next 35 years (Figures 2.10, 2.11). This encompasses significant new territory whose ecosystems may already be affected by stressors including other aquatic invasive species, habitat modification and climate change. Regions in RCP 4.5 and 8.5 projections whose climate conditions are outside the environmental space the model was trained on include southern Florida, Arizona and New Mexico (Figures 2.12.b,d,f,h; Figure 2.13.b,d,f,h). These areas should therefore be interpreted as regions of no prediction rather than truly not suitable. They are in fact likely to remain suitable, since numerous populations are known to persist in equatorial regions with similar climates (Crespo et al. 2015).

One unexpected result was the relative consistency in predictions between the various GCMs and RCPs. Although some regions are only identified as suitable by one or two models, in general most regions that are projected to become suitable to *C. fluminea* were identified in at least three GCM projections, particularly in the RPC 4.5 scenario. Furthermore, the RCP 8.5 did not result in substantially more area becoming suitable to *C. fluminea* than the RPC 4.5, although there was more variation between GCMs. This suggests that regardless of the aggression with which society responds to climate change, certain regions are likely to become climatically suitable to *C. fluminea*. Even if carbon emissions are reduced after 2020 according to the RCP 4.5 scenario, a certain amount of climate change is no longer avoidable, ensuring that regions to the north of *C. fluminea*’s current North American invasive range will continue to become more suitable (Sanford et al. 2014).

Limitations to prediction and uncertainty

These results capture and project the current environmental space *C. fluminea* is known to occupy in North America according to specific climate narratives. However, climate change scenarios are themselves subject to limitations, and there is a great deal of uncertainty in how carbon emissions will change over the next several decades (Beaumont et al. 2008). Therefore, the projected climate suitabilities for *C. fluminea* in North America across the various RCPs and GCMs should be thought of as a range of valid potential outcomes, and not considered probabilistically (Porfirio et al. 2014). These models assume that the recipient biological community composition is unaffected by the changing climate, which is may not hold true (Beaumont et al. 2008; Jeschke and Strayer 2008). Some communities may become more vulnerable to *C. fluminea* as climate change reduces resilience, or its composition may change (such as with the establishment of a new invasive species) and alter biotic resistance (Rahel et al. 2008). However, *C. fluminea* is a generalist with a global distribution, and its ubiquity in North American waterways suggests that biotic resistance may not limit its spread (McMahon 2000).

Furthermore, the recent revision of *C. fluminea*'s lower thermal limit from 2^{o}C to 0^{o}C, and interpopulation variation in tolerance to environmental stressors, suggest *C. fluminea* could persist in environmental conditions previously thought to be intolerable in the future (Cvetanovska 2015; Evans et al. 1977; Müller and Baur 2011). Therefore, these predictions should be updated if information on this invader's environmental tolerances continues to change. A global model of climate suitability for *C. fluminea* should only be attempted once the genus' native taxonomy has been resolved and conclusively linked to specific invasive populations.

Finally, this model was purposefully parameterized to be general, so that it could be projected into new climate conditions. Therefore it may not be accurate at a local scale, as complex response curves were not permitted. However, this model provides useful projections of *C. fluminea* climate suitability at the regional level across a variety of potential climate outcomes. If a particular water body of interest is near the suitable/unsuitable climate cut-off, local habitat characteristics and how they might amplify or mediate environmental stressors should be considered. These results are best put to use to identify regions of high climatic suitability outside the current known distribution of *C. fluminea* that should be prioritized for monitoring efforts.

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Figures

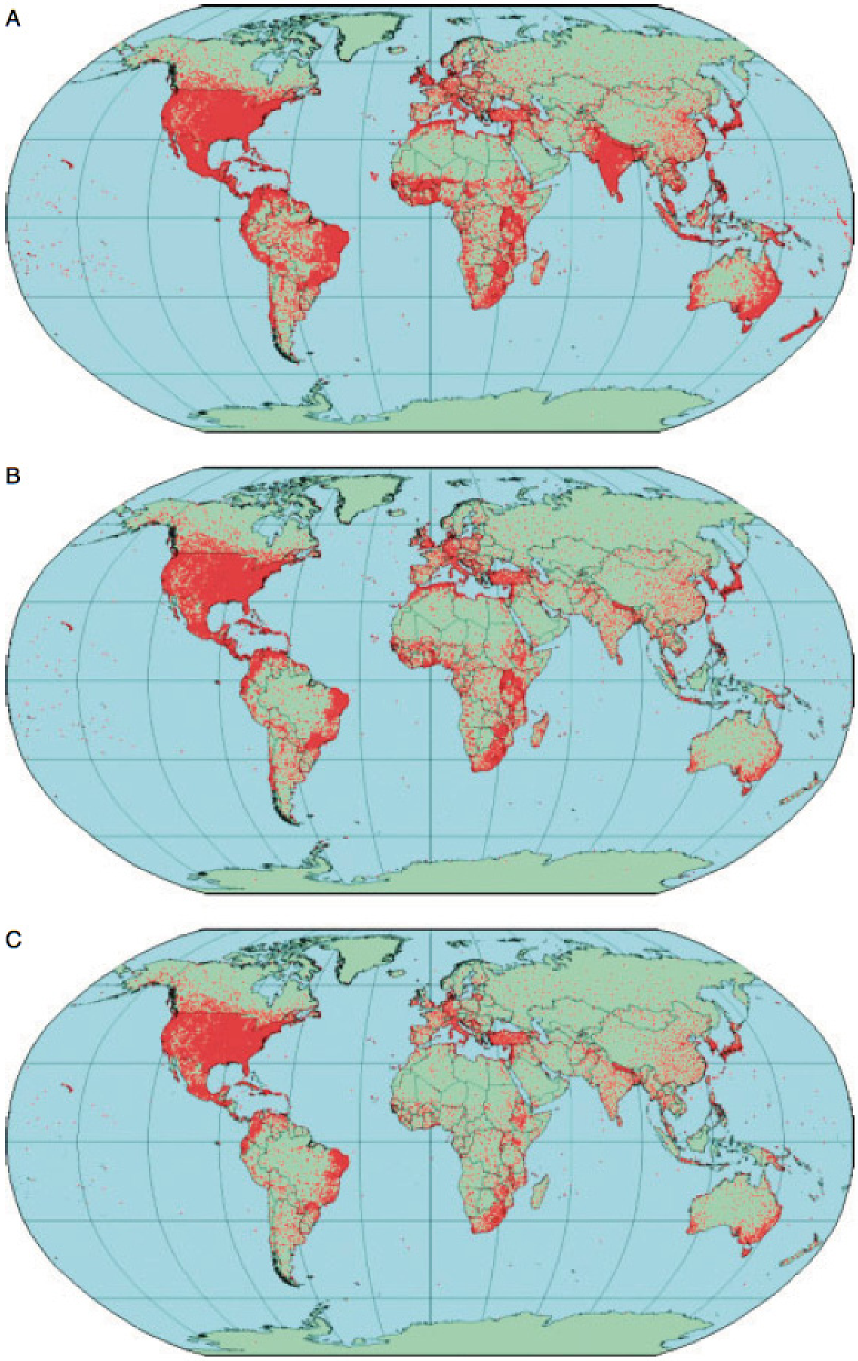


Figure 2.1: Weather stations used to generate WorldClim climate data layers, including A) stations used to generate precipitation data, B) stations used to generate mean temperature data, C) stations used to generate maximum and minimum temperature data (Hijmans 2005).

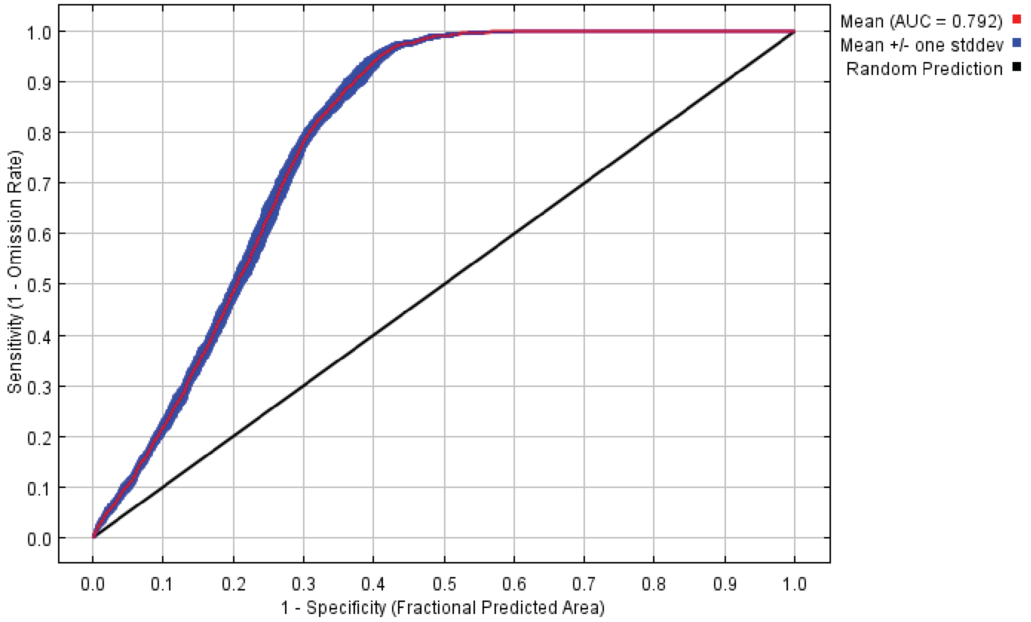


Figure 2.2: Receiver-operator curve of final climate suitability model averaged over ten replicate model runs. Sensitivity (1-omission rate) is a measure of the number of presence locations correctly identified, and 1-Specificity (fractional predicted area) is the proportion of all map pixels predicted to be suitable.

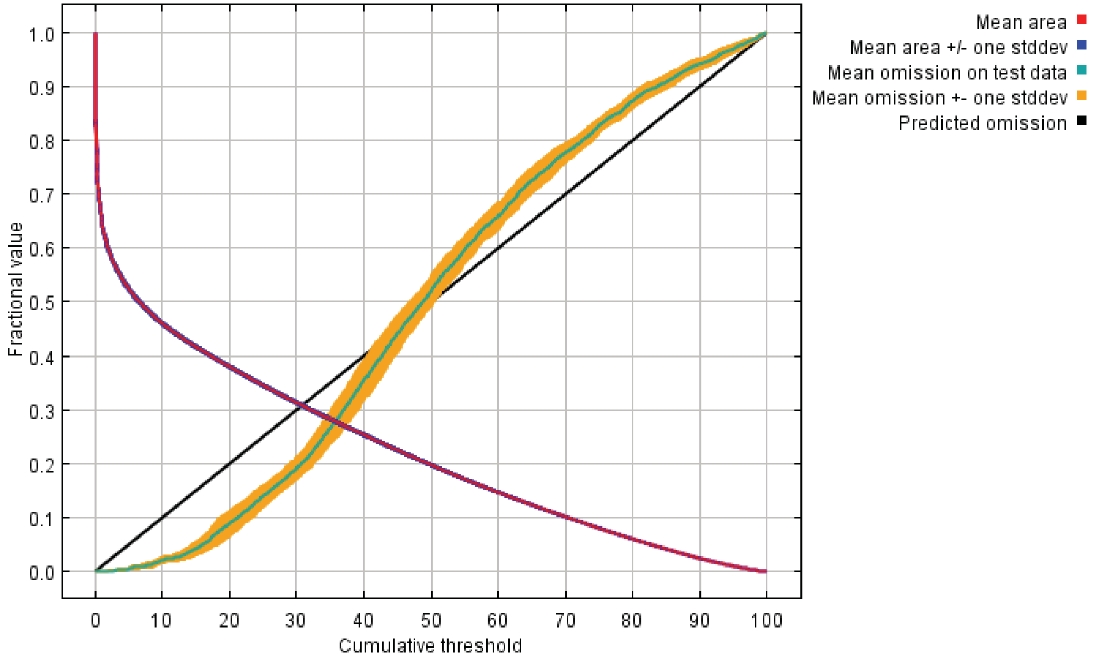


Figure 2.3: Average omission rate and predicted omission rate, versus cumulative threshold of the final model averaged over ten replicate runs.

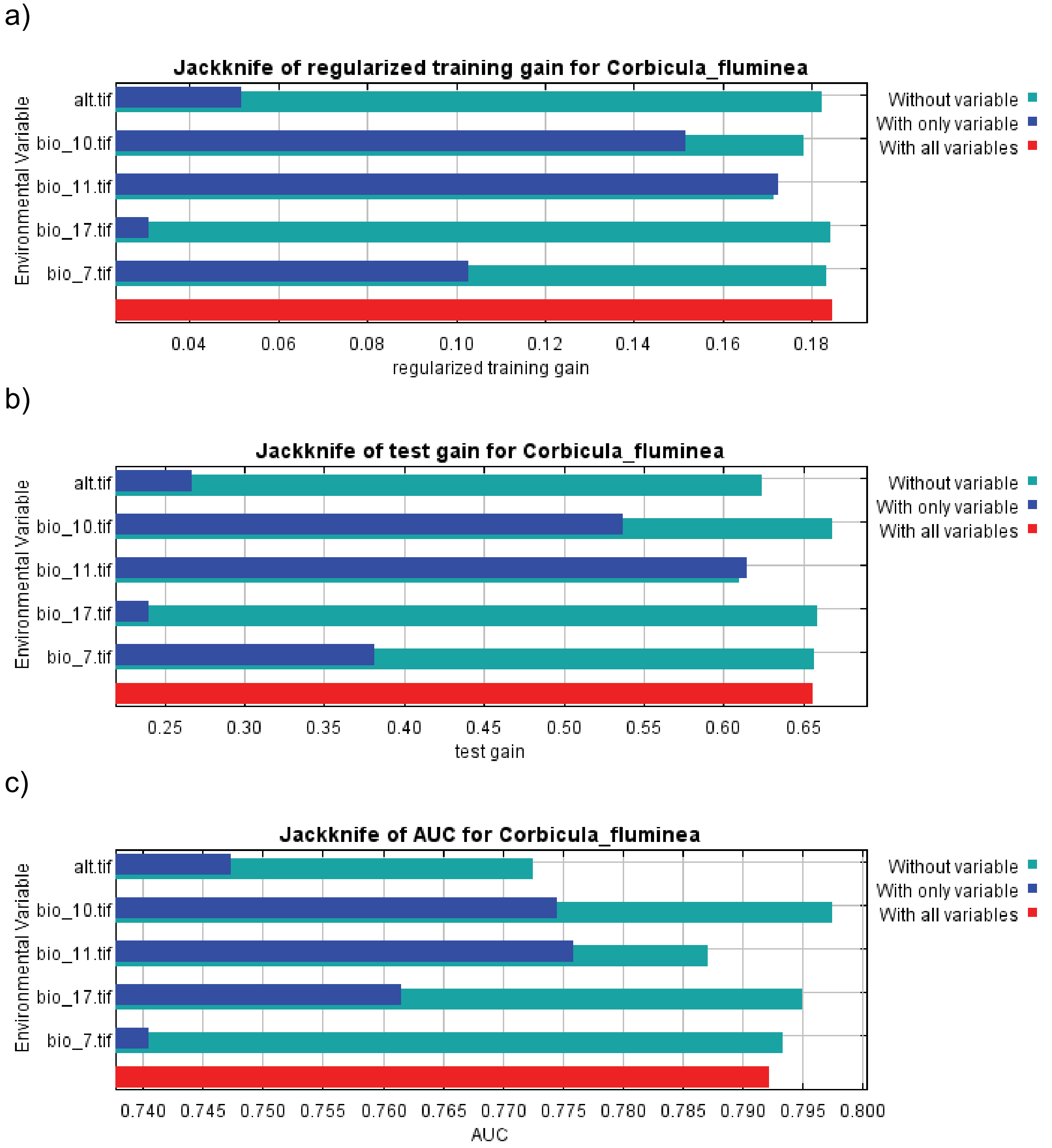
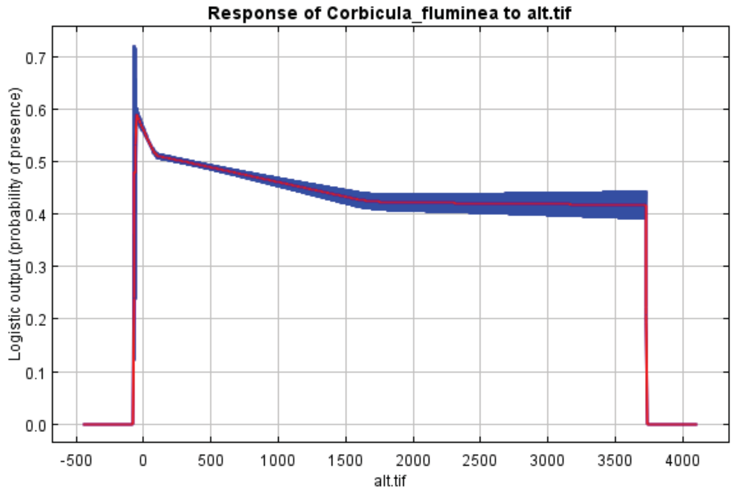


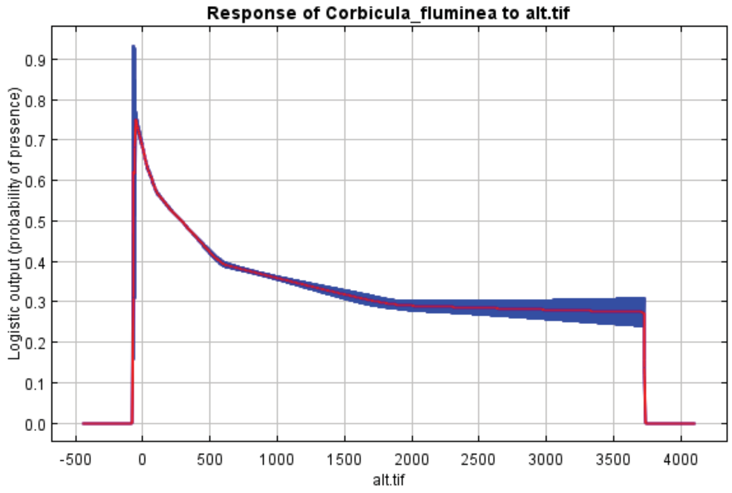
Figure 2.4: Predictor variables' jackknife test results for a) regularized training gain, b) regularized test gain, and c) the AUC for the test data, from the final climate suitability model averaged over ten replicate runs.

Variable response: complete model | **Variable response: sole predictor**

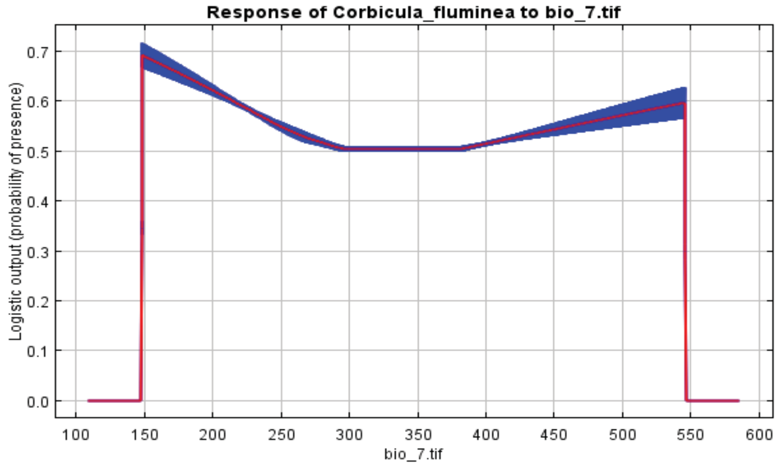
a.



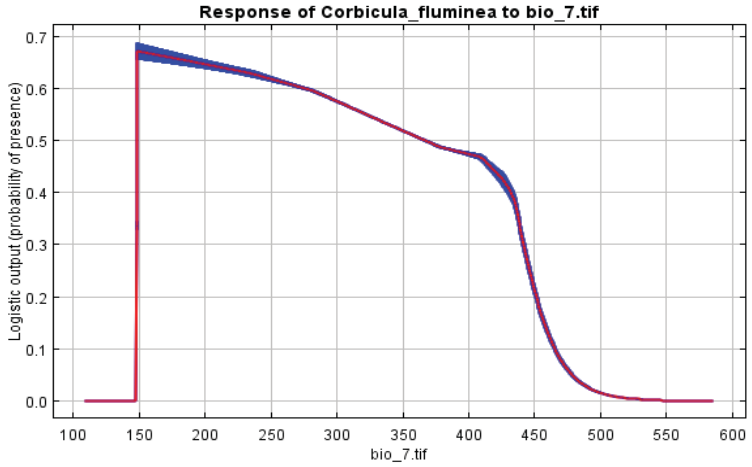
b.



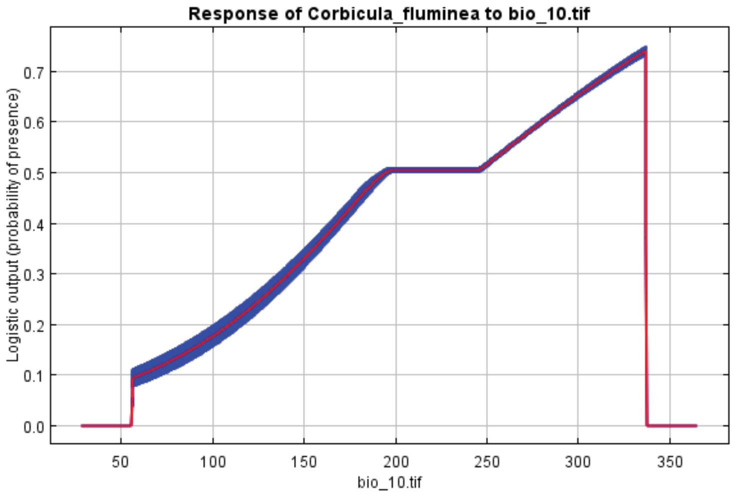
c.



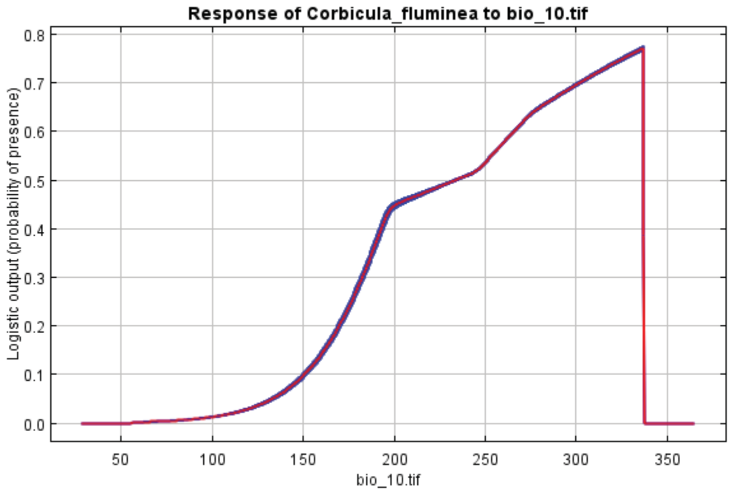
d.



e.


Response of Corbicula_fluminea to bio_10.tif
Logistic output (probability of presence)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
50
100
150
200
250
300
350
bio_10.tif

f.


Response of Corbicula_fluminea to bio_10.tif
Logistic output (probability of presence)
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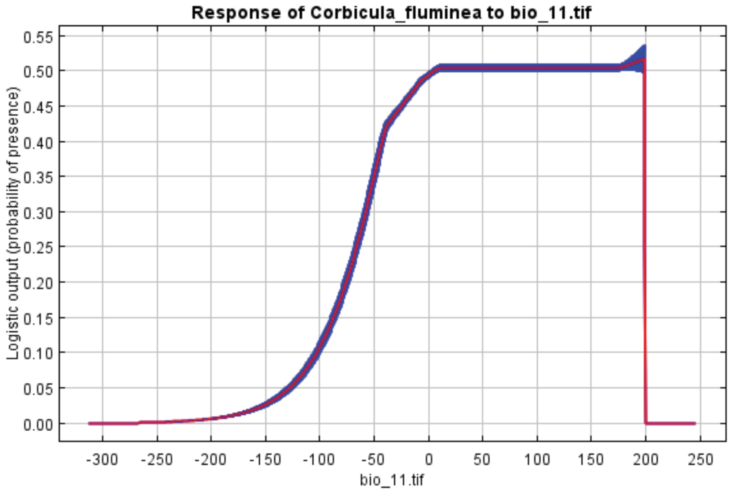
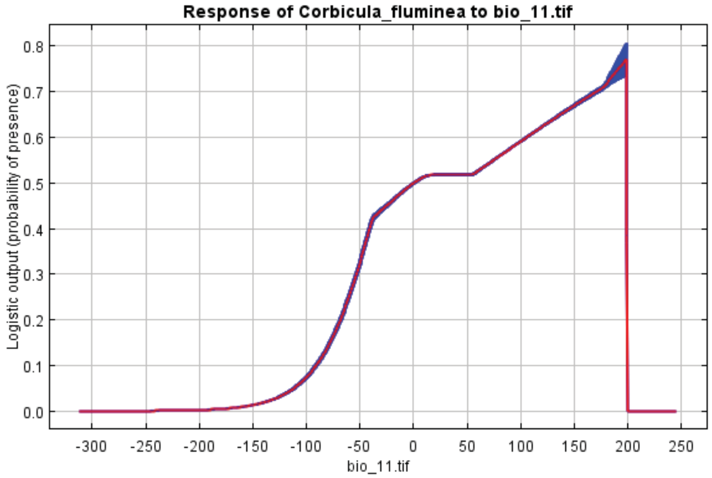
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
50
100
150
200
250
300
350
bio_10.tif

g.


Response of Corbicula_fluminea to bio_11.tif
Logistic output (probability of presence)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
-300
-250
-200
-150
-100
-50
0
50
100
150
200
250
bio_11.tif

i.


Response of Corbicula_fluminea to bio_11.tif
Logistic output (probability of presence)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
-300
-250
-200
-150
-100
-50
0
50
100
150
200
250
bio_11.tif

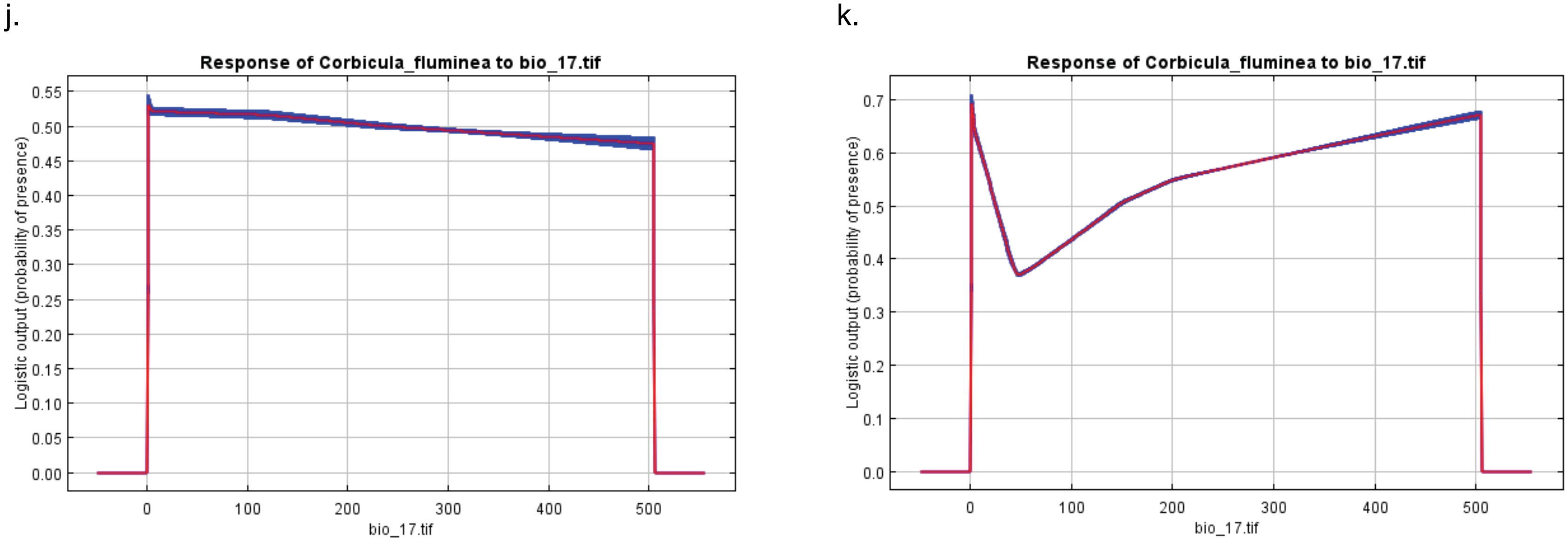


Figure 2.5: Response curves for predictor variables averaged over final model's ten replicate runs. Temperature variables are in degrees Celsius multiplied by 10, precipitation is in millimetres. Left column response curves reflect the variables' responses when part of a complete model with all predictor variables, while the right column response curves show the variables' responses when they are the only predictor variable in the model.

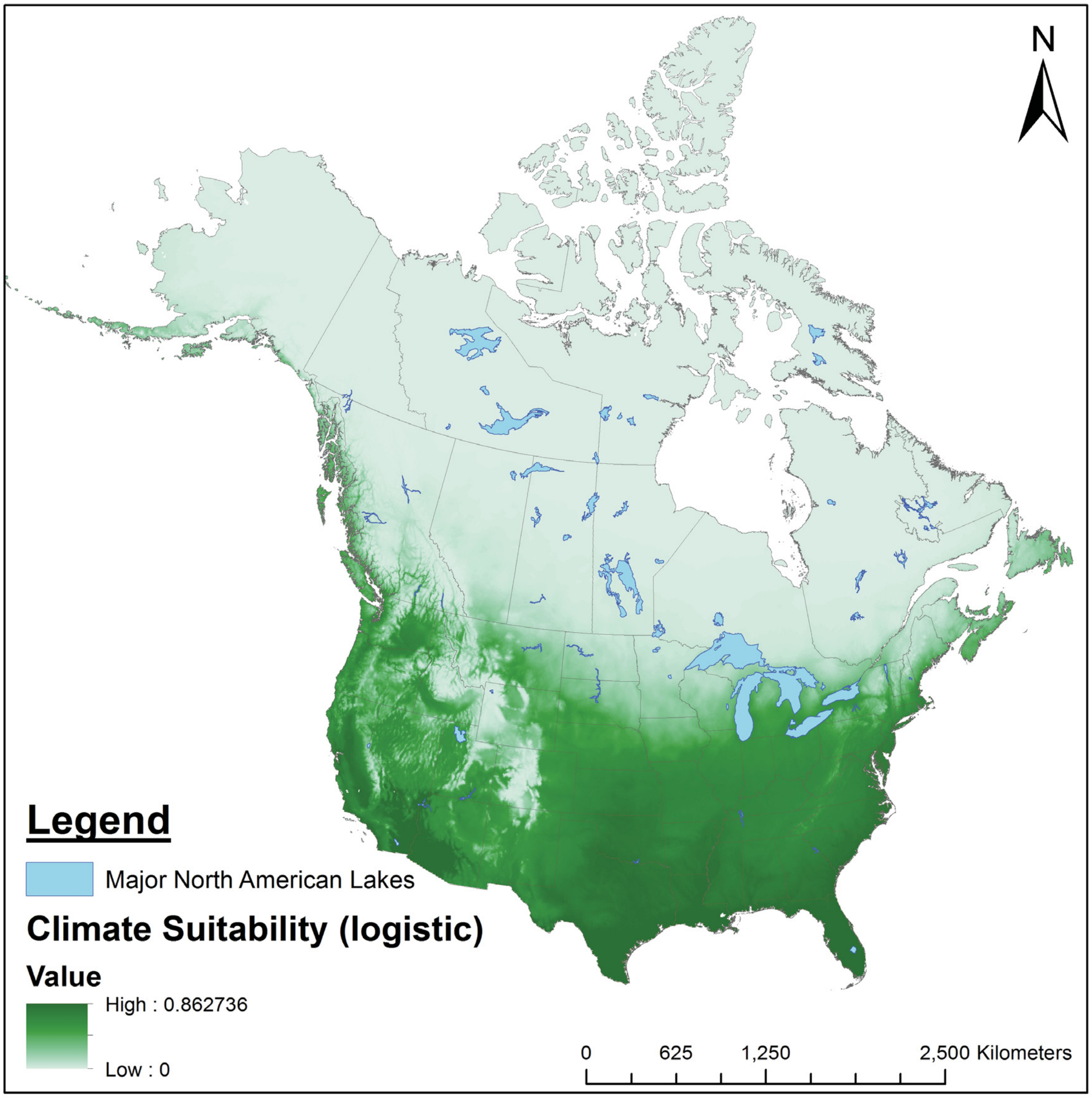


Figure 2.6: The final model's average continuous output of *C. fluminea* climate suitability under current climate conditions.

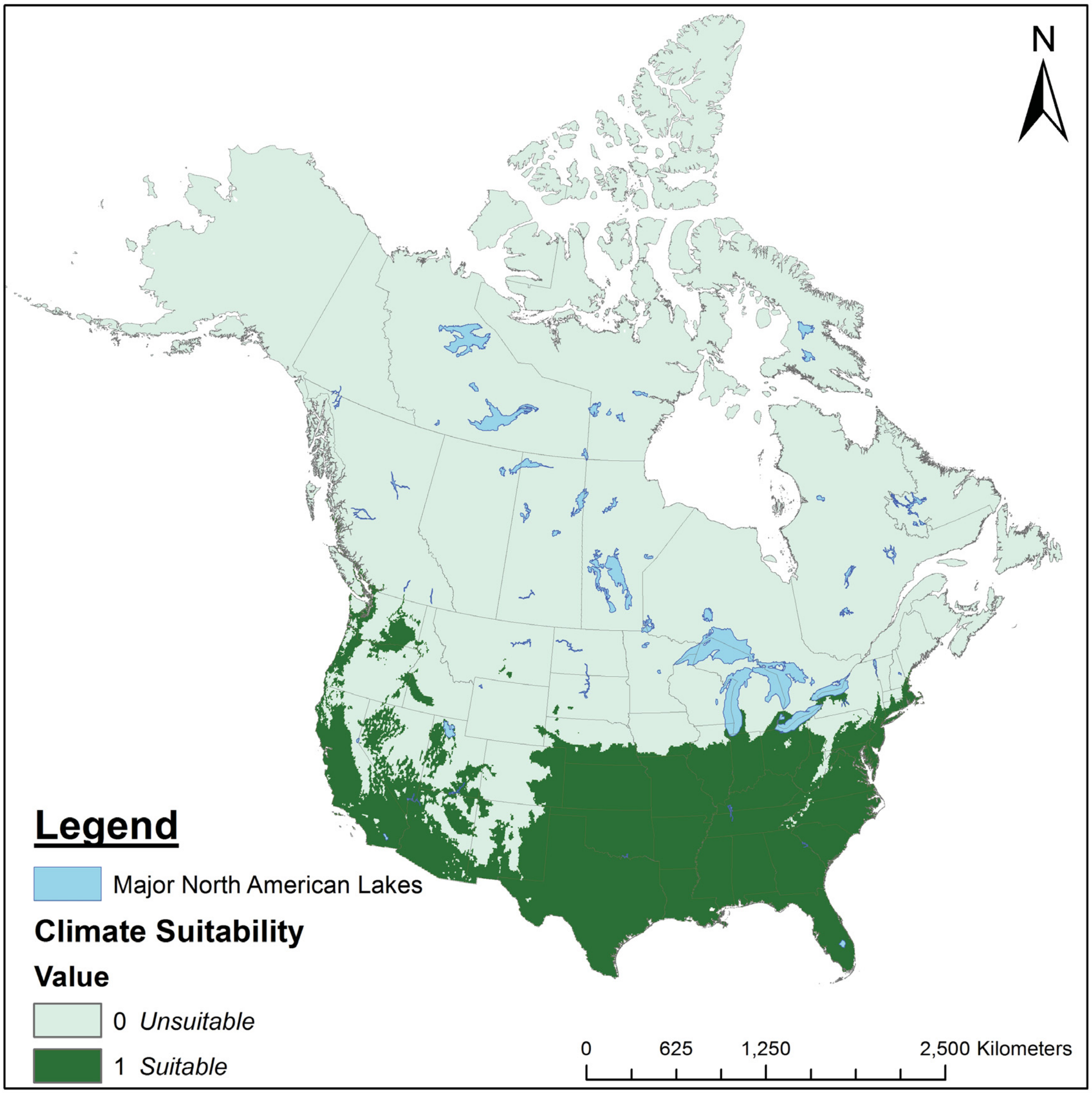


Figure 2.7: The final model’s binary logistic output (maximum sensitivity plus specificity threshold) of *C. fluminea* climate suitability under current climate conditions.

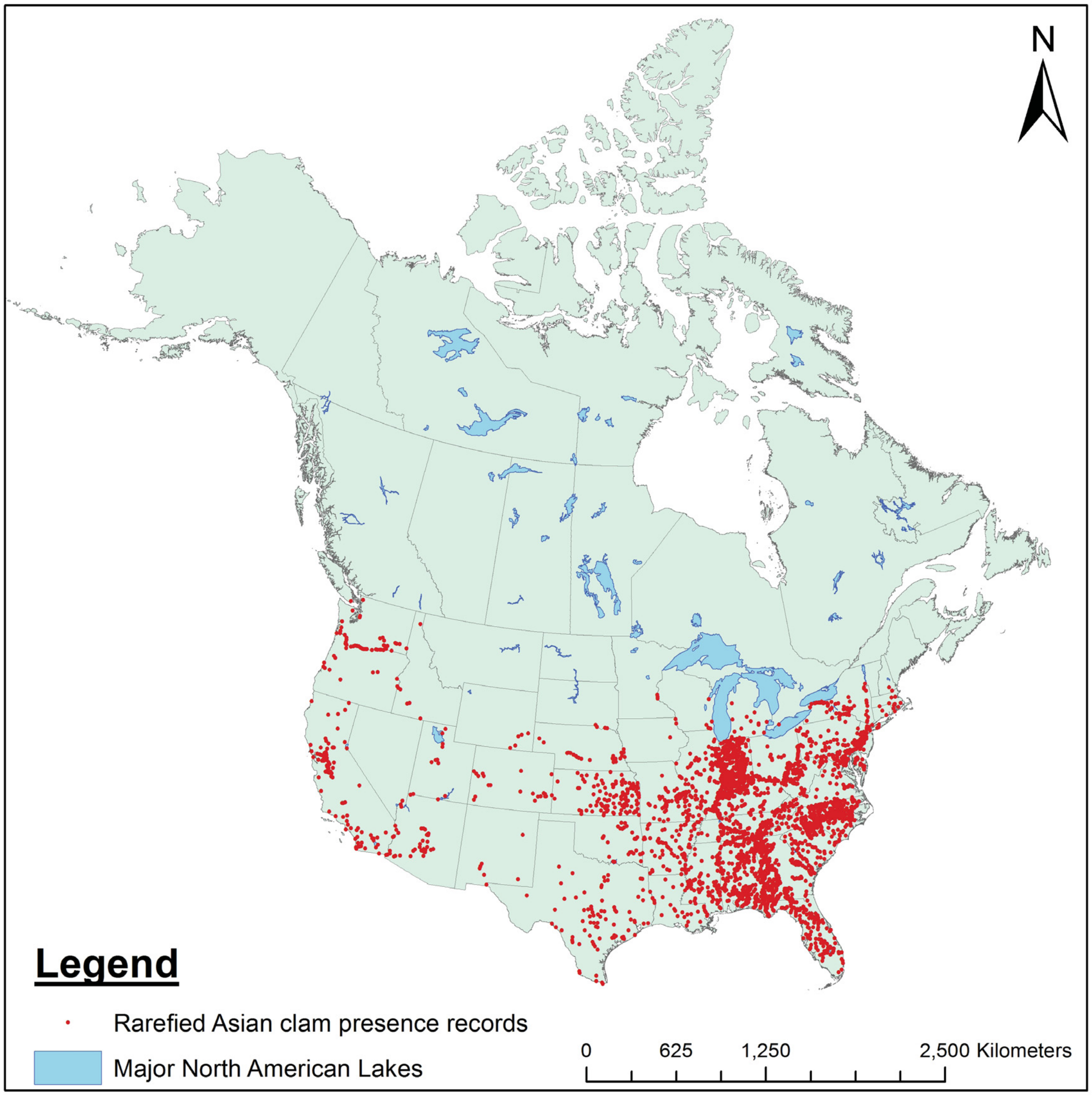


Figure 2.8: The spatially-rarefied presence observations used to create the MaxEnt model of *C. fluminea* climate suitability.

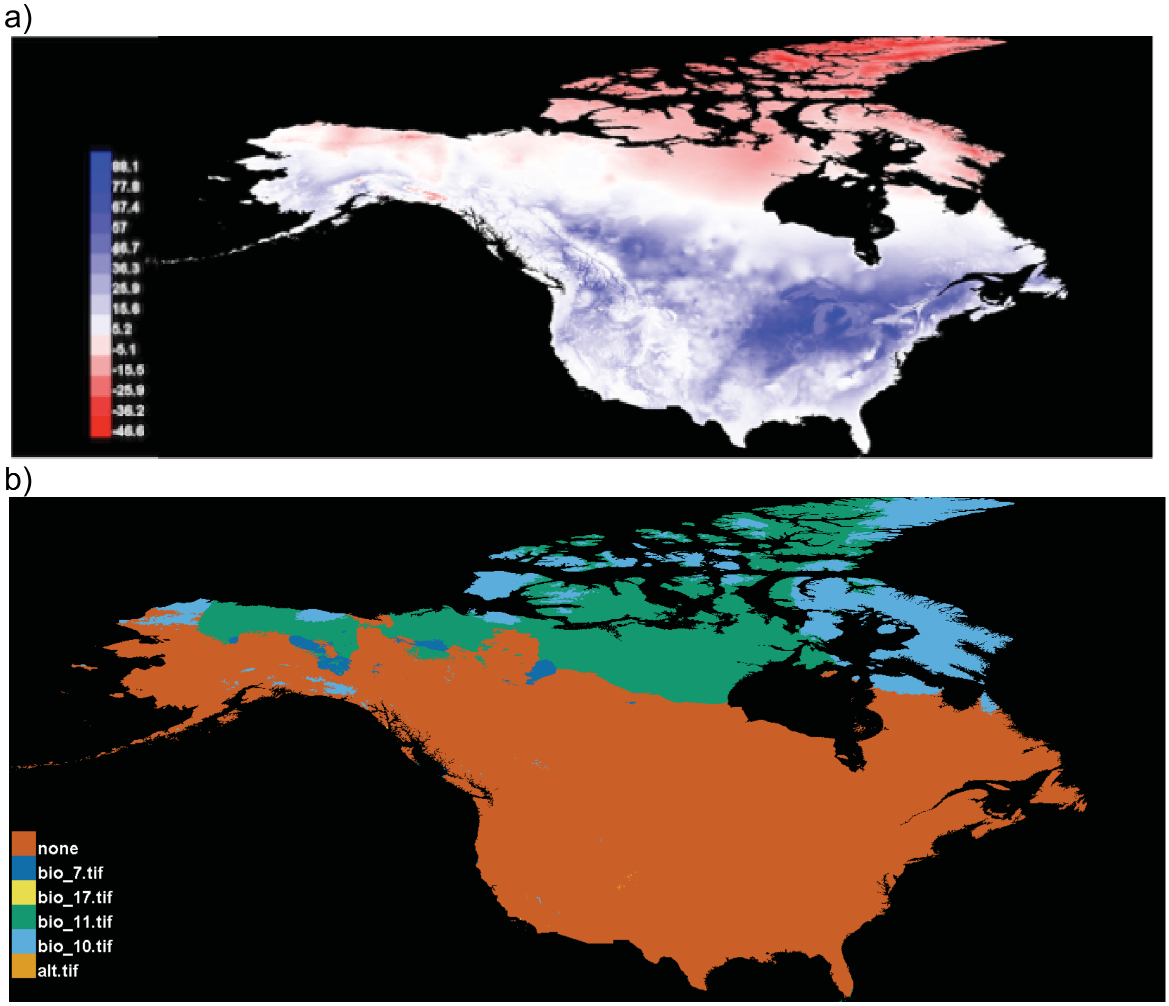


Figure 2.9: Multivariate environmental similarity surface (a) and limiting environmental predictor surface (b) for current climate conditions in North America. Red regions in map (a) have climates outside the range of environmental conditions encountered during model training, and the limiting environmental predictor is indicated in map (b).

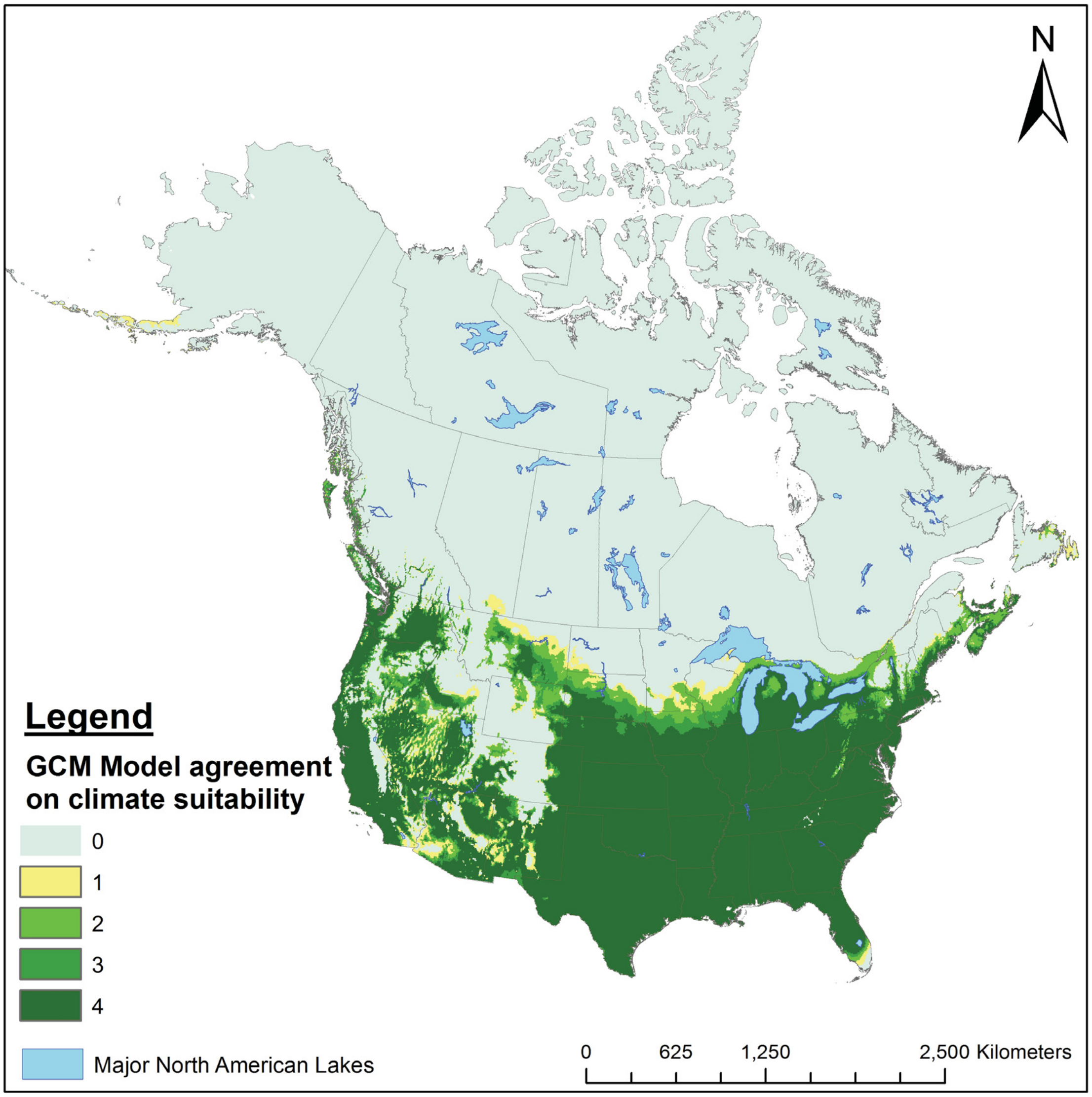


Figure 2.10: Agreement among the *C. fluminea* climate suitability model's binary logistic outputs (maximum training sensitivity plus specificity threshold) for all four GCMs projected to 2050 along the 4.5 RCP.

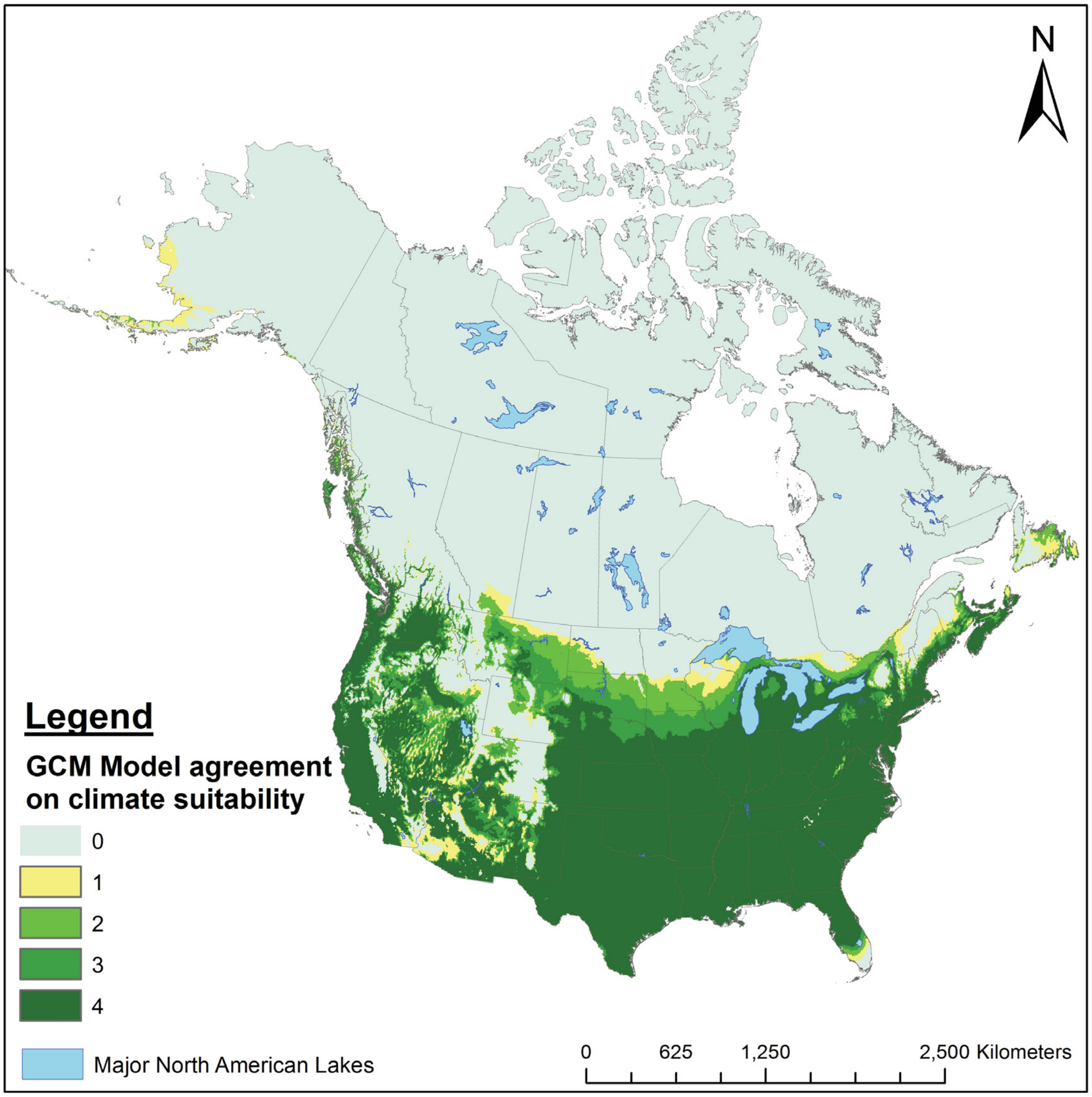
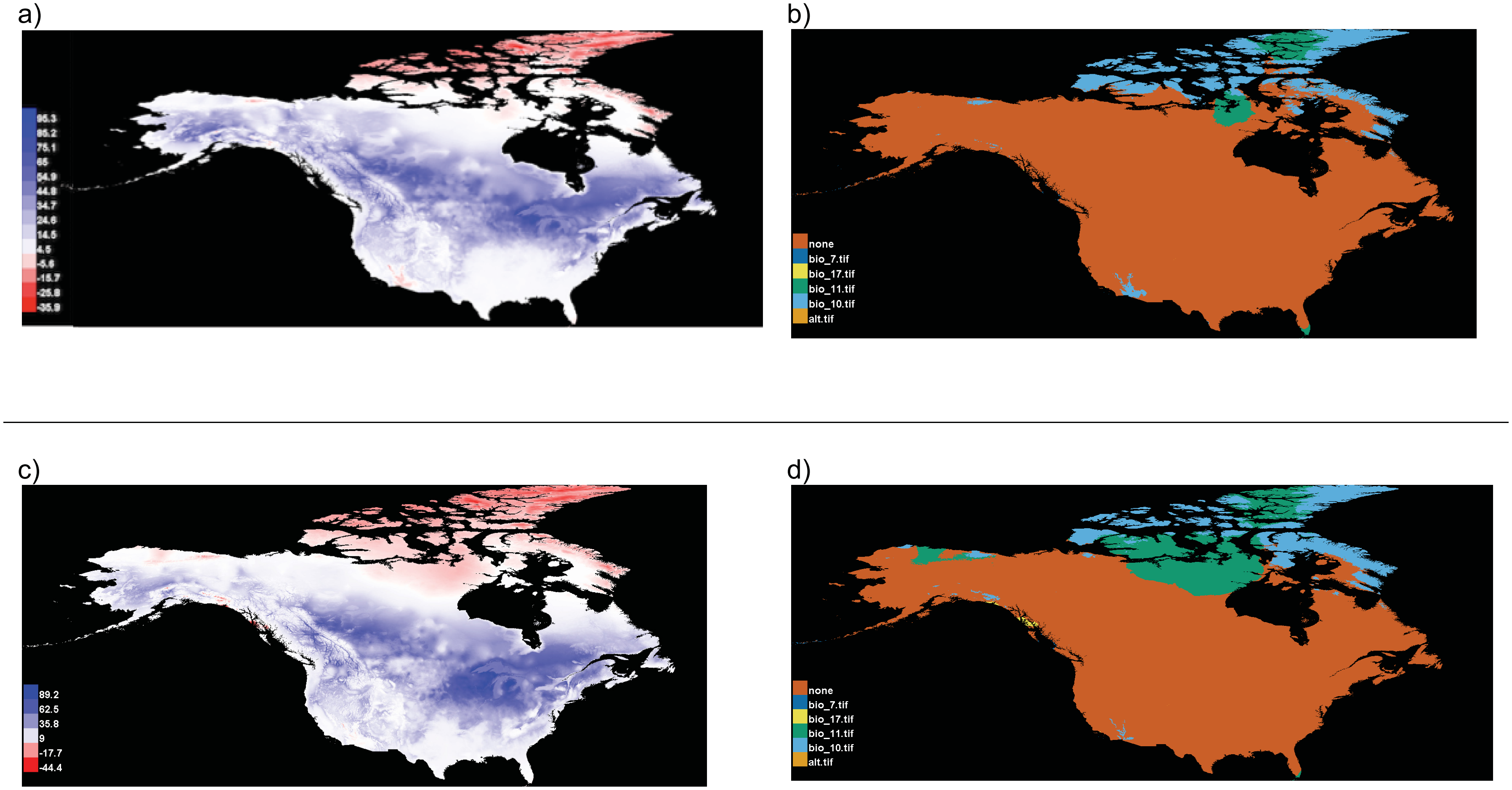


Figure 2.11: Agreement among the *C. fluminea* climate suitability model's binary logistic outputs (maximum training sensitivity plus specificity threshold) for all four GCM projected to 2050 along the 8.5 RCP.

Multivariate Environmental Similarity Surfaces | **Novel climate maps (limiting environmental predictor)**



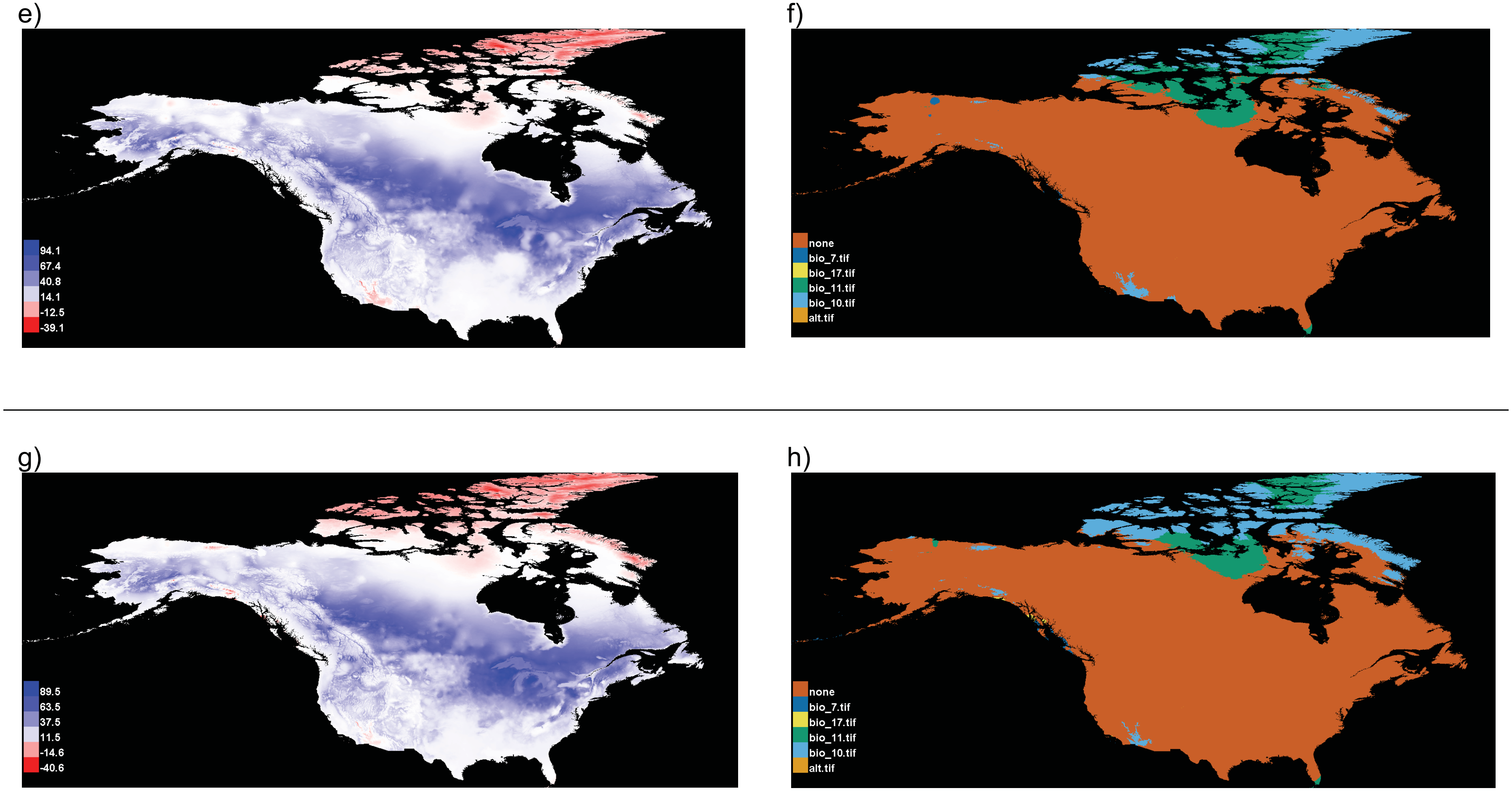
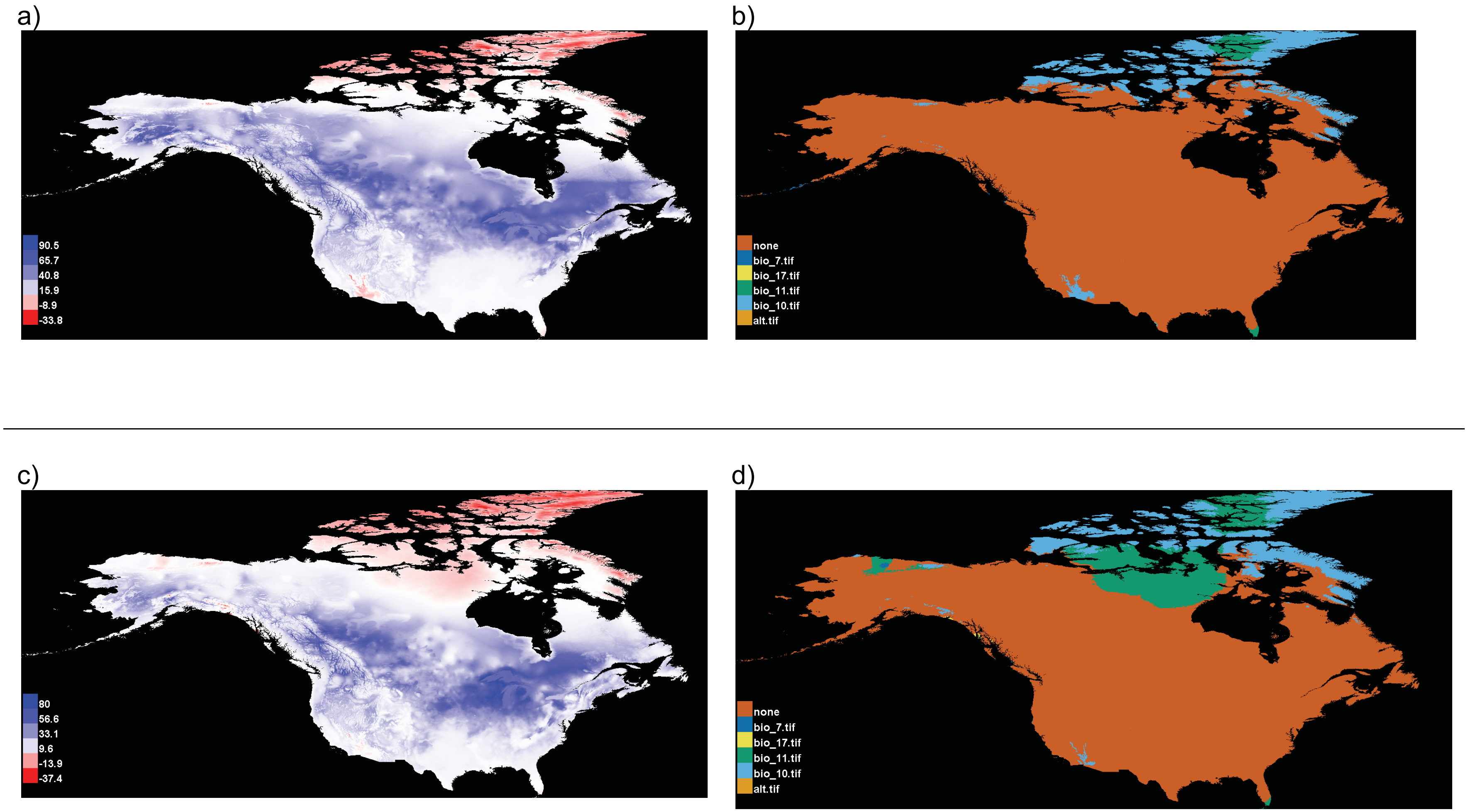


Figure 2.12.a-h: Multivariate environmental similarity surfaces and limiting environmental predictor surfaces for future climate conditions along RCP 4.5 for each GCM: HE45 (a-b), MG45 (c-d), MI45 (e-f), and MP45 (g-h).

Multivariate Environmental Similarity Surfaces | **Novel climate maps (limiting environmental predictor)**



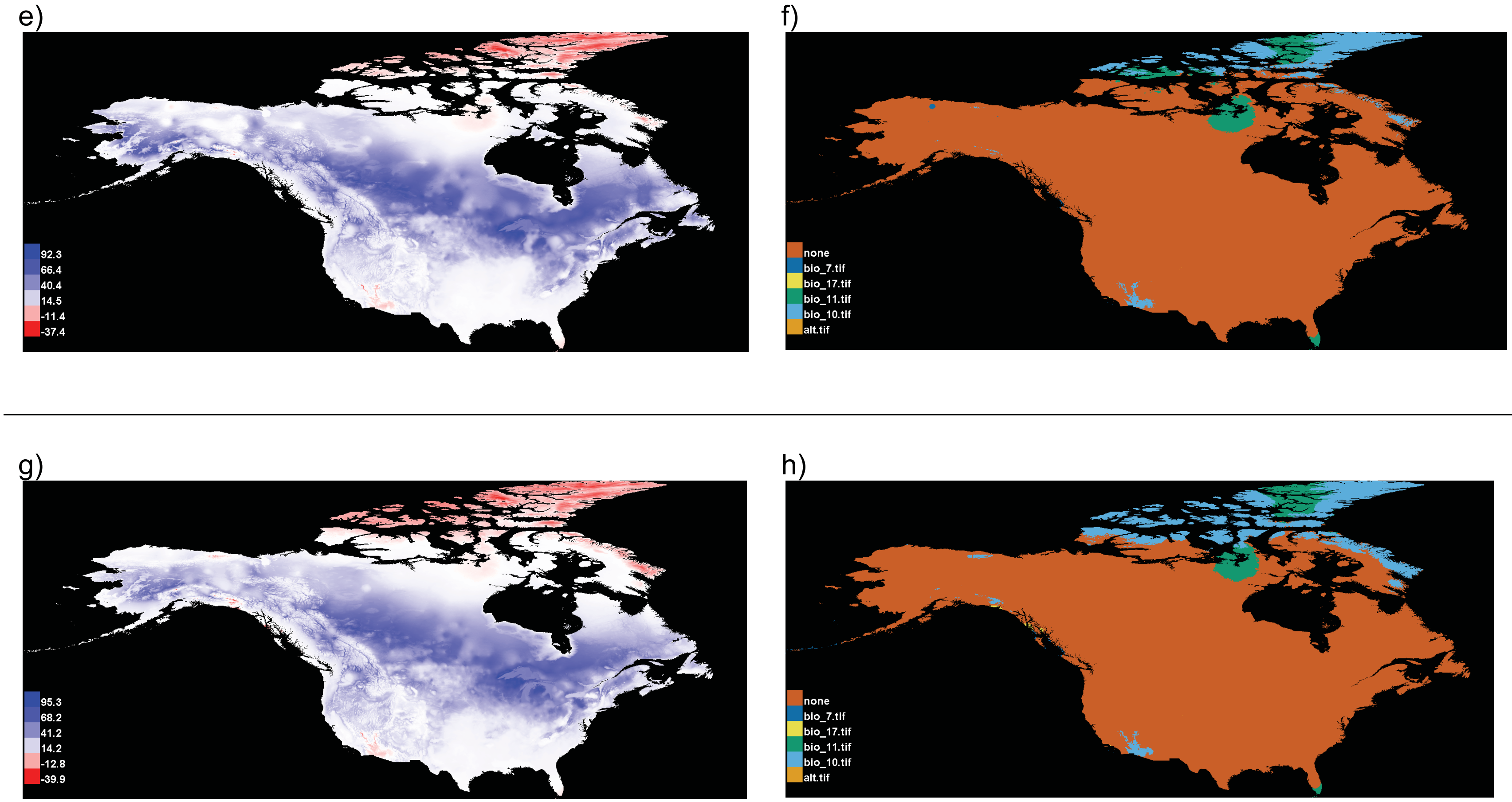


Figure 2.13.a-h: Multivariate environmental similarity surfaces and limiting environmental predictor surfaces for future climate conditions along RCP 8.5 for each GCM: HE45 (a-b), MG45 (c-d), MI45 (e-f), and MP45 (g-h).

Tables

Table 2.1: Description of Bioclim layers derived from WorldClim layers. Asterisk (*) indicates variables that were measured directly; all others are derived from these layers. Layers in italics were used in final model.

Layer	Definition
BIO1*	Annual Mean Temperature
BIO2*	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	*Temperature Annual Range (BIO5-BIO6)*
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	*Mean Temperature of Warmest Quarter*
BIO11	*Mean Temperature of Coldest Quarter*
BIO12*	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	*Precipitation of Driest Quarter*
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter
*ALT**	*Altitude (m)*

Table 2.2: Percent contribution and permutation importance of each predictor variable in the final ensemble model.

Predictor variable	Percent contribution (%)	Permutation importance (%)
BIOL11 (mean temperature in the coldest quarter)	82.1	71.8
BIOL10 (mean temperature in the warmest quarter)	14.8	16.4
Altitude	2.6	11.6
BIOL7 (annual temperature range)	0.4	0.2
BIOL17 (mean precipitation in the driest quarter)	0.1	0

Linking Statement: Chapters 2 and 3

In Chapter 2, I illustrate the utility of using climate suitability modeling techniques to identify regions at risk of invasion by *Corbicula fluminea* in North America. The quality of species distribution models' projections is limited by the accuracy of the presence data used to develop the model. Aquatic invasive species presence datasets are often compiled from public records that may contain omission and/or commission errors, such as species misidentification or the inclusion of an introduced population that failed to persist. McGeachy Pond contains a newly identified satellite population of *Corbicula fluminea*, but it is persisting in low densities that reduce the likelihood of detection.

Tools are needed to support decision-making on the invasion status of low-density populations, both to develop species distribution datasets and models, and to inform monitoring and control efforts. Here, I explore the feasibility of using *C. fluminea* shells to infer a population's establishment success, as well as test rates of shell degradation across a variety of environmental conditions. I apply the results of this experiment to the population in McGeachy Pond, and justify its inclusion in the climate suitability model presented in Chapter 2.

CHAPTER 3

Rates and stages of shell degradation of the Asian clam *Corbicula fluminea*

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Montreal, Quebec, Canada
H3A 0C4

Abstract

Determining accurate presence records for invasive species can be impeded by low population density and detection probability, at both newly invaded sites and in marginal habitats. Invasive bivalves, including the Asian clam *Corbicula fluminea*, are often first discovered by their spent shells. Over time, they can accumulate dense shell beds that are easier to detect. I test variation in *C. fluminea* shell degradation rates and stages across a variety of environmental conditions and shell origins, so as to evaluate the feasibility of using spent shells to infer time since establishment. Shell degradation rates vary across sites in relation to water chemistry (conductivity), and can be used to infer time since mortality in locations where degradation stages have been described, although the results may not be transferable to other locations. I use these findings to estimate the duration in which a *C. fluminea* population has persisted in a satellite pond of Lake Erie (McGeachy Pond). This novel technique can inform post-establishment or post-eradication monitoring for invasive bivalves.

Introduction

Inherent challenges in detecting aquatic invasive species can limit evaluations of their distribution. First, aquatic invasive species with low population density can avoid detection, even under high sampling intensity (Harvey et al. 2009). Second, the detection of an exotic species in a novel location does not necessarily imply that an invasion has occurred, since many introductions fail to persist (Simberloff and Gibbons 2004; Williamson and Fitter 1996). Repeated detections of an aquatic invasive species over several years may be required before a site can be considered part of that species' invasive range. Many water bodies with low human traffic are unlikely to be sampled repeatedly through time, given constraints on monitoring resources. Additionally, low-density exotic populations may require many man-hours of monitoring before a positive detection occurs, increasing the risk of a false negative. Some new methods, such as eDNA detection, may help resolve the logistical limitations to aquatic invasive species sampling and detection, but are in early stages of development and may not be accessible to all water body managers (Rees et al. 2014). Therefore, we must find alternative or supplemental strategies to infer the persistence of exotic species at newly established sites.

Freshwater invasive bivalves provide useful evidence of their presence: spent shells. Managers are often first alerted to a new *C. fluminea* establishment by their presence, and they may be more easily detected than live animals that can bury in the substrate. Additionally, *C. fluminea* are known to establish fluctuating populations in marginal, unstable habitats due to their rapid growth and high reproductive capacity, and therefore spent shells may be significantly denser than the live population (McMahon 2000).

Spent *C. fluminea* shells may prove useful as a tool to infer the time since establishment of a newly detected population, in order to evaluate its persistence. Spent shell assemblages may have one of the following characteristics:

1. The presence of only old, spent shells with no live individuals, suggesting a *C. fluminea* population was introduced, but either a) failed to establish or persist, or b) is persisting at low densities with low detection probability;
2. The presence of only live adults and recently spent shells, suggesting a recent establishment that may or may not persist;
3. The presence of live adults and spent shells of various ages, suggesting a population that has persisted for several seasons and is either self-sustaining, or is a sink population. In either case, it provides the strongest evidence that a population is persisting at the site.

In order to assess the reliability of these inferences, we must determine the rates and stages of *C. fluminea* shell degradation and evaluate how much they vary among habitats.

Shell degradation rates for invasive clams have primarily been studied to understand their impact as ecosystem engineers (as defined in Jones et al. 1994). Many invasive bivalves achieve high densities and alter their invaded habitat through shell accumulation, which has largely positive effects on benthic invertebrate composition and abundance (Bodis et al. 2014; Gutierrez et al. 2003; Sousa et al. 2009; Werner and Rothhaupt 2007). Shells augment substrate complexity, provide attachment surfaces for sessile organisms and refugia for cryptic animals, and promote interstitial sediment trapping that creates habitat for infaunal organisms (Gutierrez et al. 2003; Sousa et al. 2009). Shell deposition can foul human infrastructure, with significant economic costs (McMahon 2000; Sousa et al. 2009).

Strayer and Malcom (2007) defined the dynamics of the spent shell pool in invaded habitats as a function of the quantity of spent shell material entering the pool (M), the standing stock of shells (S) and the rate of shell loss (l):

$$\frac{dS}{dt} = M - lS$$

Investigating variability in shell degradation rates (l) across habitats is required to better predict context-dependent impacts in newly invaded habitats driven by the shell standing stock's rate of change. While M and S are limited by factors that control *C. fluminea* population growth, including environmental tolerance limits and biotic interactions, *I* may be controlled by a different set of abiotic predictors such as water velocity or calcium concentration. As such, the rate of shell material loss (or accumulation) at a site may not always be correlated with population density.

Several studies have explored *C. fluminea* shell decay rates in North American and European invaded waterways. Strayer and Malcom (2007) compared shell decay rates among invasive bivalve species including *C. fluminea* under a variety of water velocity and chemistry regimes. They found that shell loss rates were higher in lotic sites, and related to calcium concentration, reflecting the calcium requirements for shell growth (Fritz et al. 1990; Strayer and Malcom 2007). Ilarri et al (2015) expanded on this research by comparing the rates of invasive bivalve shell decay among species and between terrestrial and aquatic habitats; shells degraded much faster in aquatic habitats. These results suggest that much variation between sites is driven by water chemistry and other environmental factors. However, how degraded shells can be used as evidence of population persistence remains to be explored.

The goals of this study were to describe the rates and stages of *C. fluminea* shell degradation across a range of lentic habitats (in order to evaluate the feasibility of using shells to infer time since establishment), and to quantify the variation in shell decay rate across environmental gradients. I hypothesized that the shell decay process would be consistent across sites, and the rate of decay would be controlled by water chemistry, specifically conductivity (as a proxy for calcium concentration).

Methodology

To evaluate spatial and temporal variation in *C. fluminea* shell decay, I conducted two separate experimental tests. Test 1 measured the rates of shell degradation, and evaluated the feasibility of using shell characteristics (umbo, hinge, base or nacre) to infer time since mortality, across a range of environmental conditions. Test 2 measured rates of degradation among shells of different origins and along a calcium gradient; these shells were also used to evaluate the transferability of the index of shell degradation developed from the previous test.

I deployed freshly spent *C. fluminea* shells in the benthos of six sites in Ontario and Quebec (labeled A through D, and X through Z; Figure 3.1). These sites have similar slow water velocity and substrate, but vary in conductivity and pH. Test 1 was deployed from August 2013 to August 2015, whereas Test 2 was deployed from August 2014 to August 2015 for one year. With the exception of McGeachy Pond, all deployment sites were located within the waters of active marinas or yacht clubs, with the support of the property owners.

Site selection

Test 1 was conducted at sites A through D (Figure 3.1). Site A at McGeachy Pond is located near Rondeau Provincial Park in southwestern Ontario, and was selected so that the results could help determine the establishment success of a local *C. fluminea* population. Three sites were also selected around the confluence of the St. Lawrence and Ottawa Rivers, in order to take advantage of strong pH and conductivity/calcium gradients (Table 3.1). Test 2 involved shells deployed along the south shore of the Island of Montreal to limit environmental variation among sites to the strong local conductivity and pH gradient (Table 3.2).

Shell preparation

Live clams were obtained from Lake George (New York), Lake Cheston, (Tennessee) and the Upper Clinch River (Virginia). Lake George clams were used in Test 1 and transported to Montreal via vehicle during the summer of 2013, and Lake Cheston and Upper Clinch River clams were used in Test 2 and were shipped to Montreal in the summer of 2014. The clams were cleaned, euthanized by freezing in distilled water for 4 to 6 days, and then thawed for 48 hours in a laboratory refrigerator prior to dissection. I recorded the rinsed shells' air-dry wet weight and shell length (distance from the posterior to anterior margins), and described the quality of each shell's umbo, edge, hinge and nacre (Figure 3.2) according to the categorical ranks of wear (Table 3.3). Shells were photographed and stored in distilled water for no more than 8 days prior to deployment.

Shell deployment and retrieval

Shells were deployed in the benthos in fishing tackle boxes modified by the replacement of the top and bottom sides of each cell with plastic mesh (4mm; Figure 3.3). The box was then secured shut using plastic zip ties. This modification permitted the movement of sediment, water and small macroinvertebrates through the cells without risk of losing shells. Each shell was placed into a labeled cell, and the boxes were anchored on the substrate.

For Test 1, three tackle boxes, each containing nine shells, were deployed in August 2013 at each of sites A through D. One box was collected from each site at 9 months, 12 months and 24 months after deployment. At each collection, shells that were removed from the field were stored in distilled water and all shell parameters were recorded. During each site visit, environmental data including temperature, dissolved oxygen concentration, sediment quality, turbidity, depth, specific conductivity and pH were measured. In this system, conductivity and calcium are highly correlated (Jones and Ricciardi 2005) and long term conductivity data exists for these sites (Ricciardi, unpubl. data), so conductivity was used here as a proxy measure for calcium concentration. Environmental chemistry was measured using a YSI Pro2030 (temperature, dissolved oxygen and specific conductivity) and an Oakton portable water meter (pH).

In Test 2, the same tackle boxes described in Test 1 were used as experimental units to hold the shells (Figure 3.3). Two tackle boxes containing 15 shells each from the two collection locales (Lake Cheston and the Upper Clinch River) were deployed at sites X through Z. Each box contained shells from a single source population to prevent the misidentification of shell origin. The tackle boxes were anchored on the substrate in August 2014 and collected one year later in August 2015. Upon retrieval, all shell size and quality parameters were recorded after the shells were cleaned in distilled water, and environmental data were collected during each site visit.

Analysis

All analyses were completed using R Studio Version 0.98.953 (RStudio 2013). The response variable of interest was the instantaneous rate of shell loss (*k*):

$$k = (1/t) \times \left[\ln\left(\frac{mass_{final}}{mass_{initial}} \right) \right]$$

where t is the exposure time, and $mass_{initial}$ and $mass_{final}$ are the shell's mass before and after exposure (Strayer and Malcom 2007). This equation allows for shell loss rates to be compared among sites, exposure periods, populations and studies (Strayer and Malcom 2007). Additionally, I calculated percent shell loss ($mass_{final}$ / $mass_{initial}$) for each shell, as well as the mean and standard deviation of percent shell loss and k for each exposure period at each site for Test 1, and for each population at each site for Test 2.

Test 1 analysis

To test the effects of exposure time and site on the instantaneous rate of *C. fluminea* shell decay, I conducted an analysis of covariance (ANCOVA) of k with shell length as a covariate. Six shells had positive k values, likely due to adsorbed particulate matter; all of these were collected at the 9-month time step. All shells from this time step were therefore excluded to avoid introducing a size bias into this group. $|k|$ was ln-transformed to achieve normality in the residuals. The most parsimonious model was identified via a backward elimination protocol, and post-hoc testing was conducted with a contrast matrix using the glht function in the multcomp package (Hothorn et al. 2015).

Test 2 analysis

To test the effects of site and shell origin on the instantaneous rate of shell decay, I conducted an ANCOVA of *k* with shell length as a covariate. $|k|$ was square-rooted transformed in order to achieve a normal distribution of the residuals. The most parsimonious model was identified via a backward elimination protocol, and post-hoc testing was conducted with a contrast matrix using the glht function in the multcomp package (Hothorn et al. 2015).

Stages of shell degradation

I assessed the feasibility of using a shell's hinge, edge, nacre and umbo to infer time since mortality by testing the effects of site and exposure time on each characteristic using the shells from Test 1. The goal was to detect specific stages of degradation and, if variation within time steps and sites was minimal, to create an index of shell degradation through time and across a range of environmental conditions. The transferability of the index was evaluated by comparison to the shells from Test 2. I fitted an ordinal logistic regression model to each response variable using the ordinal package (Christensen 2015). The most parsimonious model was identified using backward elimination, and re-leveling the model allowed for post-hoc testing for differences between the levels of a predicting factor.

In the event that the ordinal regression model could not converge due to complete separation in the data, I instead used Firth's penalized likelihood to fit a binomial generalized linear model with a logit link function between each rank of the response variable in question (no analogous method for ordinal regression was identified; Heinze and Schemper 2002). This method is appropriate for modeling small binomial data sets with complete separation (when parameter estimates reach $\pm\infty$), and was implemented using the brglm package (Kosmidis 2013).

Results

During the winter of 2013-2014, the experimental unit at Hudson was lost due to ice scouring, thus was not included. All other units were retrieved.

Test 1 shell degradation rates

The instantaneous rate of shell loss was highly variable across sites. Percent shell loss and *k* were highest at site D at all time steps, while sites A and B had similar rates of loss (Table 3.4). Shell length, site and their interaction were significant predictors of ln|*k*|, while exposure time was not significant (Table 3.5). The relationships between shell length and *k* differed significantly among sites A and B, and post-hoc testing also revealed a significant interaction between site and shell length among sites B and D ($p=0.0266$). Simple linear regression revealed that the rate of shell decay decreased with increasing shell length at sites A ($p=0.0357$) and D ($p=1.11\times10^{-6}$), while at Site B there was no significant relationship (Figure 3.4). The significant interaction term in the ANCOVA violates the model's parallel slopes assumption, so I performed an ANOVA testing the effect of site on ln|*k*|, not taking into account the effect of shell length since there was no significant difference in shell length distributions between sites (Table 3.4). Site D had significantly higher rates of shell decay than site A ($p<0.0001$) and site B ($p<0.0001$; Table 3.6).

Test 2 shell degradation rates

Shell loss rates were highest at site Y and lowest at site Z (Table 3.7). The most parsimonious model included the effects of site and shell length with a significant interaction term (Table 3.8). The relationships between shell length and *k* differed significantly among sites X and Z, and post-hoc testing also revealed a significant interaction between shell length and site among sites Y and Z ($p=0.00352$). Simple linear regression revealed that *k* decreased with shell length at site Z ($p<0.0001$), while the relationship was neutral at sites X and Y (Figure 3.5). The significant interaction term in the prior model violates the parallel slopes assumption of the ANCOVA, so I conducted an ANOVA testing the effect of site on square root-transformed *k*, not taking into account shell length since there was no significant difference in shell length distributions between sites (Table 3.7). Compared to site X, shells degrade more slowly *k* at site Z ($p<0.001$), and more quickly at site Y ($p=0.002$; Table 3.9).

ANOVAs revealed no significant difference in shell length distributions among sites within Test 1 and within Test 2, and mean shell length was significantly larger for shells from Lake Cheston than the Upper Clinch River in Test 2 ($p<0.001$; Tables 3.4, 3.7).

Index of shell decay derived from Test 1 shells

Hinge intactness was found to be a useful indicator of shell degradation that varied across sites and through time, with no significant interaction effect (Table 3.10, Figure 3.6). With all other factors being equal, each additional year of exposure reduces a shell's likelihood of having an intact hinge, versus a compromised or broken hinge, by 96%. Shells at site B and site D are, respectively, 94% and 99% less likely to have an intact hinge than a partially or completely broken hinge, than a shell from site A. Post-hoc testing revealed that hinge decay was marginally more likely at site D than B ($p=0.0629$). Four shells whose hinges were compromised at the start of the experiment were excluded from the analysis.

The quality of the shell's nacre was a useful indicator significantly affected by site and exposure time (Table 3.11, 3.12; Figure 3.7). Post-hoc testing revealed that nacre quality at sites B and D did not differ significantly; shells at both sites were >99% less likely to have a pearly nacre (rank of 3) than shells at site A. Additionally, there was a significant interaction between the effects of site and exposure time on nacre quality among nacre ranks 3 and 2 (Table 3.11). The likelihood of a shell losing its pearly nacre through time was much higher at sites B and D, than site A. Shells at site D were more likely than shells at both sites A and B to have an entirely chalky nacre after two years.

Neither the exterior of the umbo nor the quality of the shell edge were found to be useful indicators of shell degradation, owing to the large variation in initial quality of a shell's umbo before deployment (Figure 3.8) and the lack of variation in edge quality (Figure 3.9).

Discussion

Variation in shell degradation rates by site, exposure time and population

Local environmental conditions and shell size both control rates of *C. fluminea* shell decay. In Test 1, shell decay rates were highest at site D, which had the lowest conductivity, and in Test 2 rates were lowest at site Z where conductivity was maximal. This finding agrees with a previous study that described a negative correlation between shell decay rates and calcium concentration (Strayer and Malcom 2007). In Test 1, *k* at sites A and B were not significantly different from each other despite site A having higher conductivity, and in Test 2 sites X and Y had similar *k* even though site X had lower conductivity. This indicates that water chemistry (conductivity or calcium concentration) generally controls rates of shell loss, but that other factors contribute spatial variation.

Interaction effects with shell length complicated detection of differences in shell decay rates between sites. Strayer and Malcom (2007) reported a negative relationship between shell mass and shell decay rates, likely due to smaller shells having a higher surface area to mass ratio. At sites A and D in Test 1, and site Z in Test 2, this relationship was also detected. However, at sites B, X and Y, there was no significant relationship between *k* and shell length. This may be due to artificially elevated shell mass readings for smaller shell sizes at certain sites, the same factor that required the 9-month time step shells to be excluded from Test 1 analyses. In this study I observed that nacre did not seem to degrade evenly across a shell, but rather small pits would initially form (~9 months) that would then expand over the shell surface (after 1-2 years). Small shells degrade faster, and therefore may develop pitted nacre more quickly, causing relatively more particulate matter to adhere to these pockets compared to larger shells and obscuring the negative *k*-length relationship. This may occur at sites with sediments that have a higher silt or clay content than other sites, but further investigation would be required to verify this hypothesis.

Finally, there were no significant differences in *k* among shells from different origins, although since Lake Cheston and Upper Clinch River clams have different size distributions, variation explained by origin may have been attributed to length in the final model. More populations of similar size distributions should be tested before any assertions can be made regarding the existence of interpopulation variation in shell degradation rates. However, given the morphological variation between *C. fluminea* populations (Britton and Morton 1986), it is plausible that differences in environment drive differences in shell structure that affect the rate of degradation.

Stages of shell degradation across sites, exposure times and populations

The results of this experiment do not support the creation of an index of shell degradation, as there was inadequate differentiation between sites and time steps with respect to shell nacre and hinge characteristics. Several metrics of shell quality were ruled out as useful for determining time since mortality: the wornness of the umbo, and the intactness of the shell edge and mucous membrane. The shells' edges did not significantly change through the course of this experiment, and may be more influenced by the manner in which the clam died, or physical weathering. The umbo's wornness is already highly variable before mortality, and may be influenced by environmental chemistry of the habitat whence the clams originate or the roughness of the sediments where the clams burrowed.

Based on strong significant relationships in the hinge and nacre quality data I formulated several rules of thumb for low-energy sites to evaluate with Test 2 shells:

1. Degradation of the shell's nacre and hinge occurs more quickly at ion-poor sites, e.g. those of [Ca^{2+}] < 13mg/L – the concentration measured at site D (Jones and Ricciardi 2005);
2. Hinges may remain entirely or partially intact for at least two years across environmental conditions, and so may not indicate recent mortality;
3. Completely intact hinges at low-calcium (<13mg/L) sites may indicate recent mortality (<2 years);
4. Pearly shell nacres begin to develop chalky patches after one year across environmental conditions, and so completely pearly nacre may indicate recent mortality (<1 year);
5. Shells retaining any pearly nacre at low-calcium sites may indicate that they died recently (<2 years).

Applicability to Test 2 shells and McGeachy Pond shells

When comparing these rules of thumb created from Test 1 shells to the qualities of shells collected after one year in Test 2, it is clear that their transferability is low. The Test 2 shells' hinges degrade most quickly at site Z, which has the highest conductivity/calcium levels (Figure 3.10), and nacre quality degrade most quickly at site Y (Figures 3.11). Secondly, there seems to be some differentiation between populations' shell decay stages, as Lake Cheston clams lose their nacre more quickly than Upper Clinch River clams while retaining their hinges for longer, despite having a larger shell size.

However, these rules of thumb can be used to evaluate shells at sites tested in this experiment. We last obtained data at McGeachy Pond in August of 2014; that year we did not find live animals, but found two spent, mature shells (~15mm) with intact hinges and pearly nacres (Figure 3.12). Given the rules of thumb and photographic comparison to the experimental shells deployed at McGeachy Pond (Figure 3.13), it seems likely that these shells are from clams that died less than a year ago. Live animals were detected at McGeachy Pond in 2010 and again in 2013. Although this population may yet be extirpated, these results support the population's inclusion in the climate suitability model of

Chapter 2, since it has persisted through multiple winters when environmental conditions are at their most restrictive.

Our inability to transfer these rules of thumb to other sites and shell morphologies indicates that other factors, both environmental and morphological, may be affecting shell degradation rates. The hinges of Upper Clinch River clams were most degraded at site Z, even though this site had the lowest rates of shell decay, indicating the visible stages of degradation may not always align with rates of shell loss when comparing populations. Presumably the quality and integrity of the shells were also influenced by the environmental conditions of the habitats where the clams were collected, as are other *C. fluminea* traits. Finally, the average health of the different clam populations may play a role in determining the quality of the nacre and other shell integrity characteristics, and this factor was not accounted for in the experiment.

The utility of an index of shell degradation may be limited by variation in shell morphology among *C. fluminea* populations, as well as the shell structure's response to environmental conditions (Britton and Morton 1986; Prezant et al. 1988). *C. fluminea* from the white Form A had shells with crossed lamellar microstructures with internal purple highlights when grown under optimal environmental conditions, however when stressed they developed crossed acicular microstructures with white internal pigmentation (Prezant et al. 1988); it is unknown if this change would affect shell decay rates. However, it confirms that shell colour is affected by environmental conditions, and the process of shell bleaching may commence even before death (Prezant et al. 1988). No shells in our experiment became entirely bleached (rank of 0 for nacre quality) after the two-year exposure period; however, if shells are evaluated after a mass-mortality event they may show more bleaching due to changes in shell structure incurred before death.

Physical weathering in high-energy environments

The interpretation of these experiments is limited to lentic or low-flow habitats. This is because water energy, the most significant driver of variation in shell degradation rates, was not tested in this experiment. Prior studies measured values of *k* in high-velocity aquatic systems that were an order of magnitude higher than those measured at both their low-velocity sites, and the low-velocity sites in this study (Ilarri et al. 2015; Strayer and Malcom 2007). This is because high velocities increase dissolution rates, and may also increase physical weathering. The rates and stages of shell degradation described here are intentionally limited to low-velocity sites to isolate the variation explained by environmental chemistry and shell characteristics. Certain shell characteristics may be more affected by physical erosion, such as the intactness of the shell edge and its mucous seal; most shells in Test 1 retained their membrane at the end of the two-year experiment. Additionally, mechanical forces in high-energy environments likely accelerate declines in all other metrics of shell quality including percent shell loss, nacre quality, umbo wornness, and hinge integrity.

Corbicula fluminea *management using spent shells*

This experiment illustrates a low-cost, accessible way of evaluating the time since mortality of spent shells at a newly invaded site. As long as the study shells are similar in morphology to those found at the newly invaded habitat, fresh spent shells could be deployed and periodically retrieved over several years. A photographic index could be used to evaluate the time since mortality of spent shells found at a later date. This protocol could also be useful to evaluate the efficacy of eradication attempts; since many eradication attempts have reduced clam densities by up to 98%, it may be difficult to detect remaining live animals after the extermination (Wittmann et al. 2012). After eradication, fresh spent shells could be deployed, retrieved periodically, and compared to spent shells to provide evidence that reproduction is not occurring.

Invasive bivalves such as *C. fluminea* can pose unique challenges to recipient communities by restructuring the habitat through accumulation of shell beds that may persist for some time, even following an eradication or failed establishment (Gonzalez et al. 2008; Sousa et al. 2014). However, spent shells also create unique opportunities to evaluate invasion success and impact, by gaining a predictive knowledge of the results of physical processes acting upon those shells.

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Figures

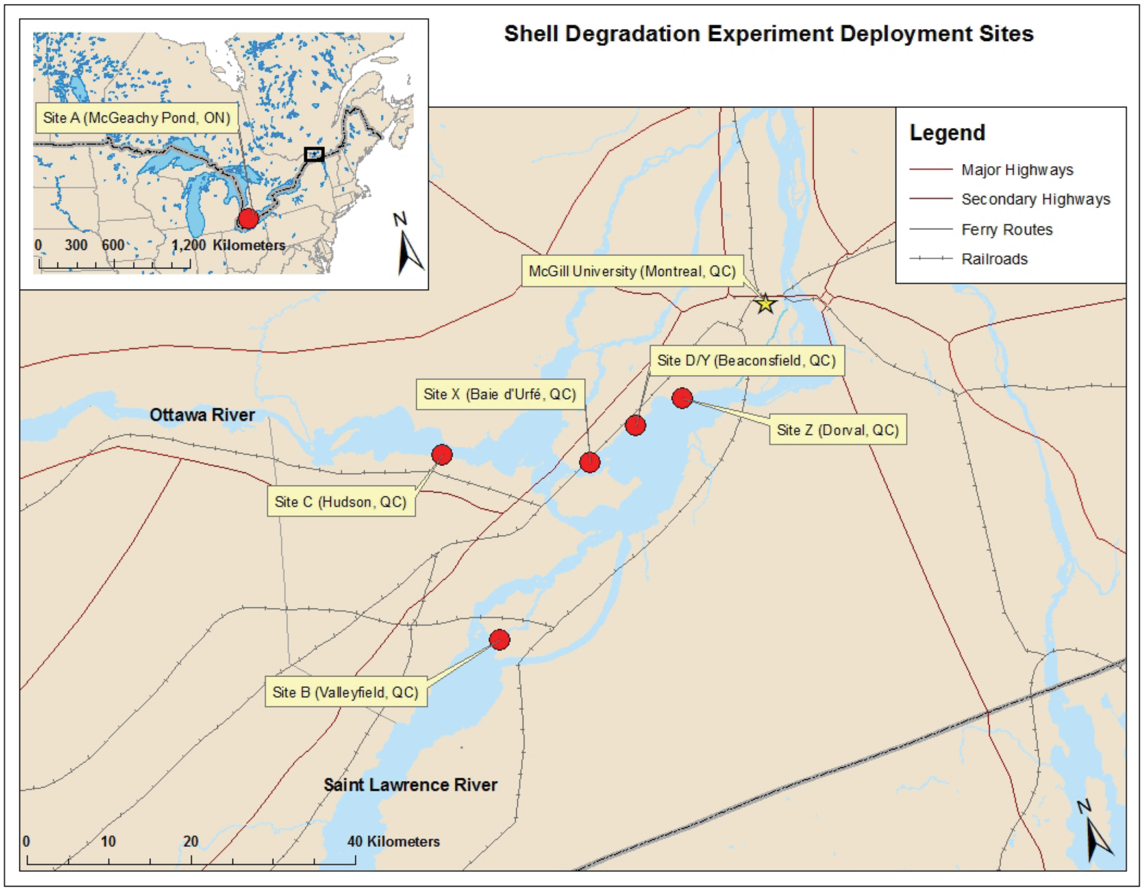


Figure 3.1: Locations of sites used in Test 1 (A-D) and Test 2 (X-Z). Site D in Test 1 and Site Y in Test 2 are both at the same location (Beaconsfield).

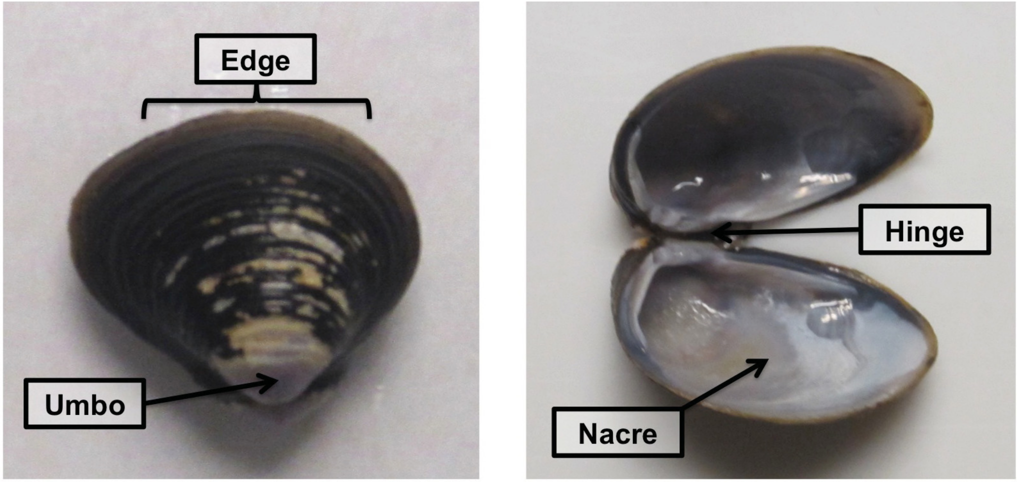


Figure 3.2: Diagram illustrating the shell quality measures recorded for each shell in Tests 1 and 2.

Figure 3.3: Experimental units within which shells were stored during deployment periods. Pictured here is a unit containing Upper Clinch River clam shells deployed at Site Y in Test 2, after collection in August 2015.

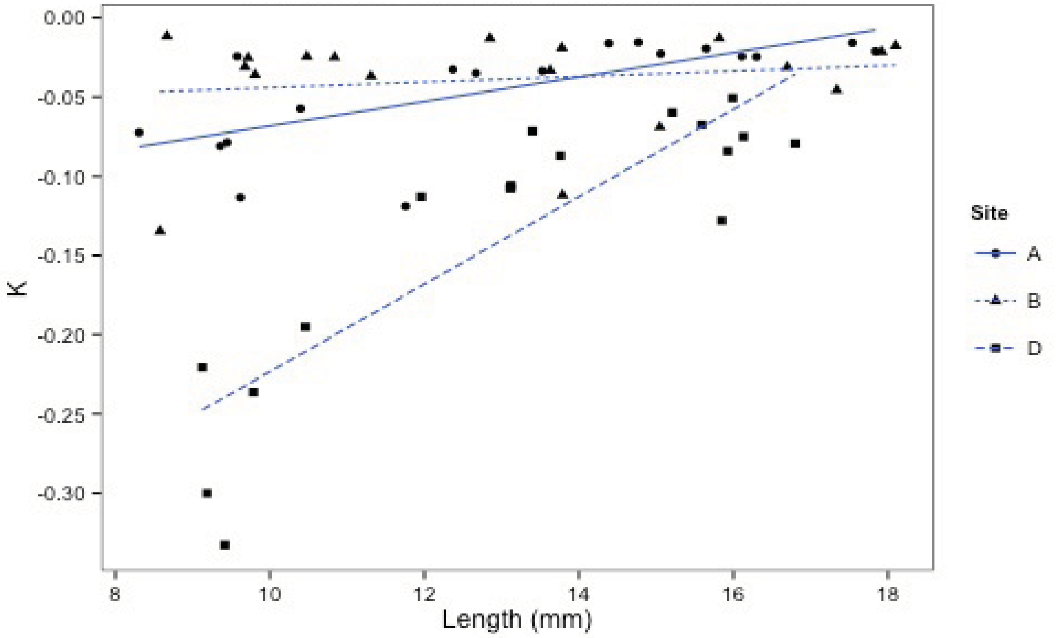


Figure 3.4: Instantaneous rate of shell loss versus shell length, across three sites, from Test 1 data. Note that *k* in this graph is not ln-transformed (unlike in the model), to aid chart interpretation.

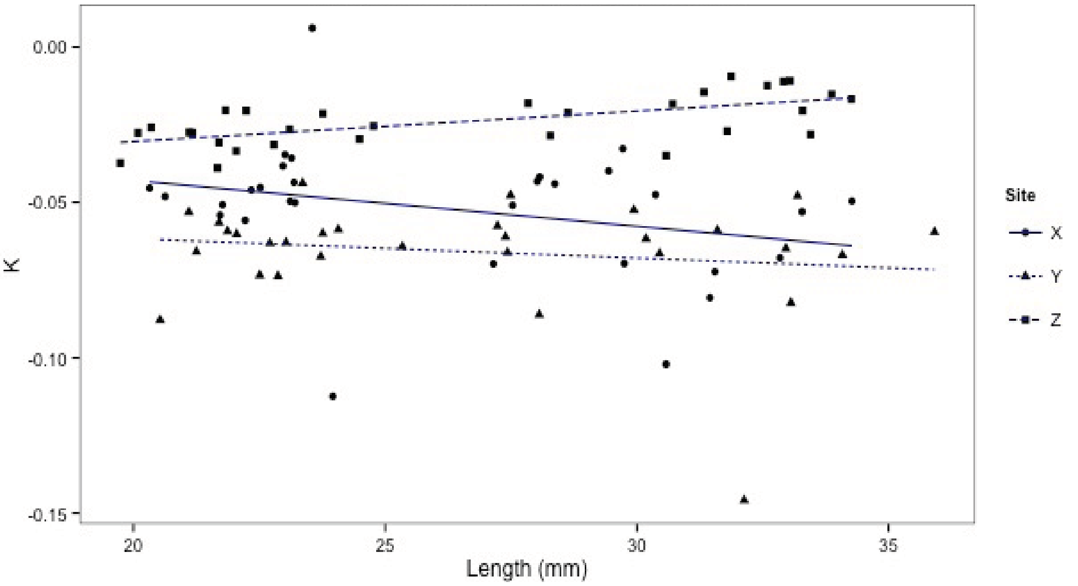


Figure 3.5: Instantaneous rate of shell loss (*k*) versus shell length, across three sites, from Test 2 data. Note that *k* is not square-root transformed in this chart (unlike in the model) to aid chart interpretation.

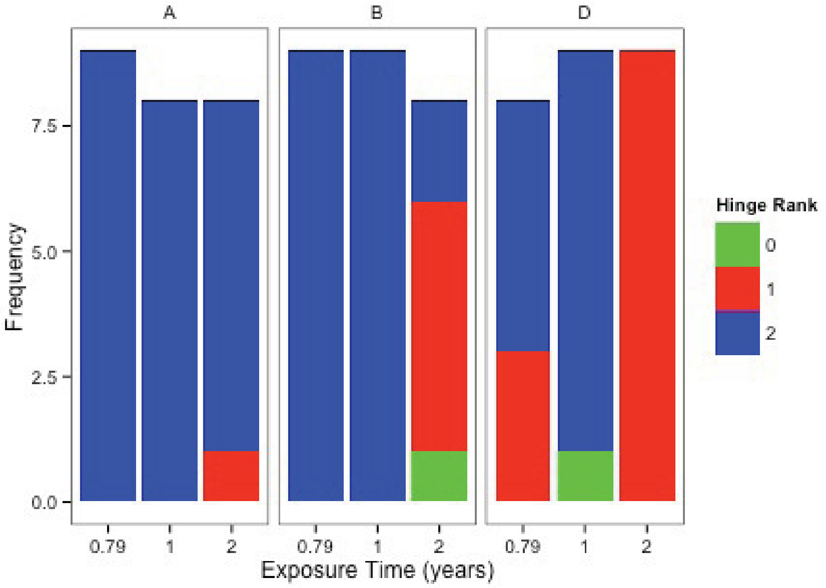


Figure 3.6: Test 1 shells' final hinge quality through time, across three sites. Not all bars total 9 due to exclusion of four data points who had compromised hinges at the start of the experiment.

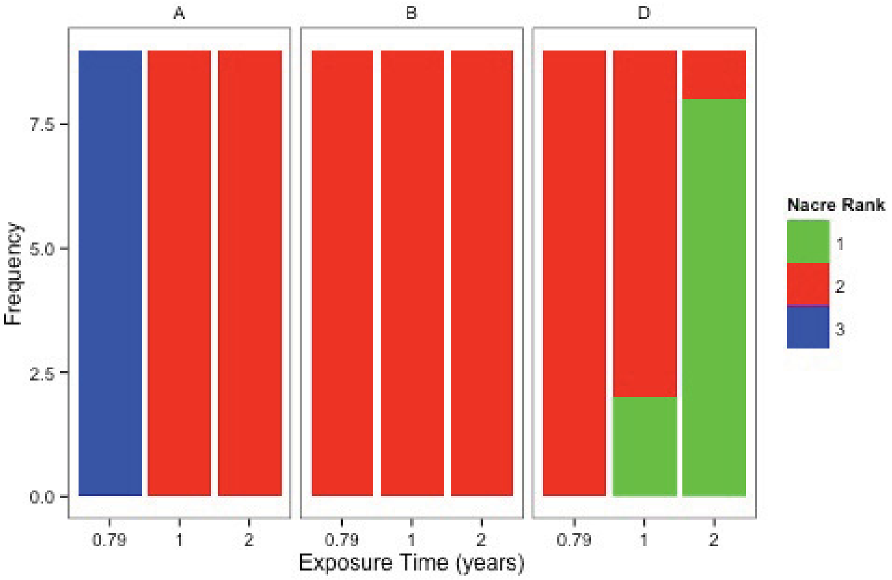


Figure 3.7: Test 1 shells' final nacre quality ranks across sites and through time.

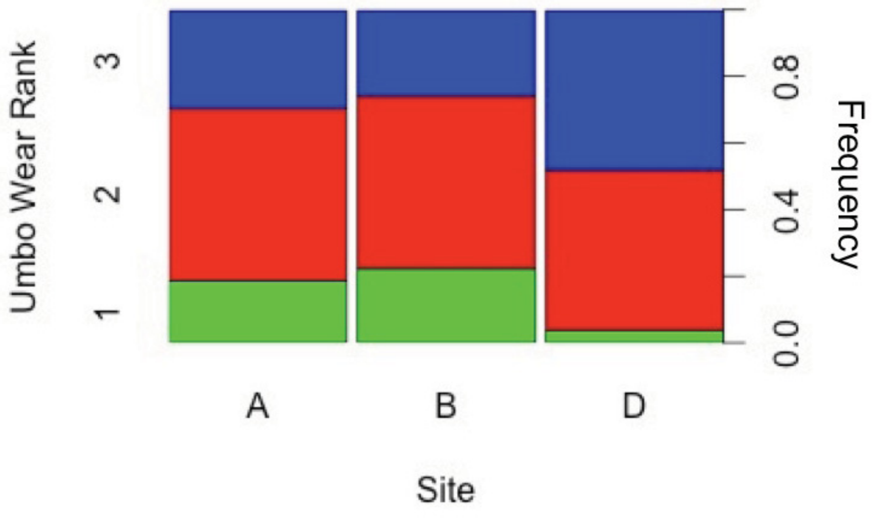


Figure 3.8: The initial quality of Test 1 shells' umbos, across sites.

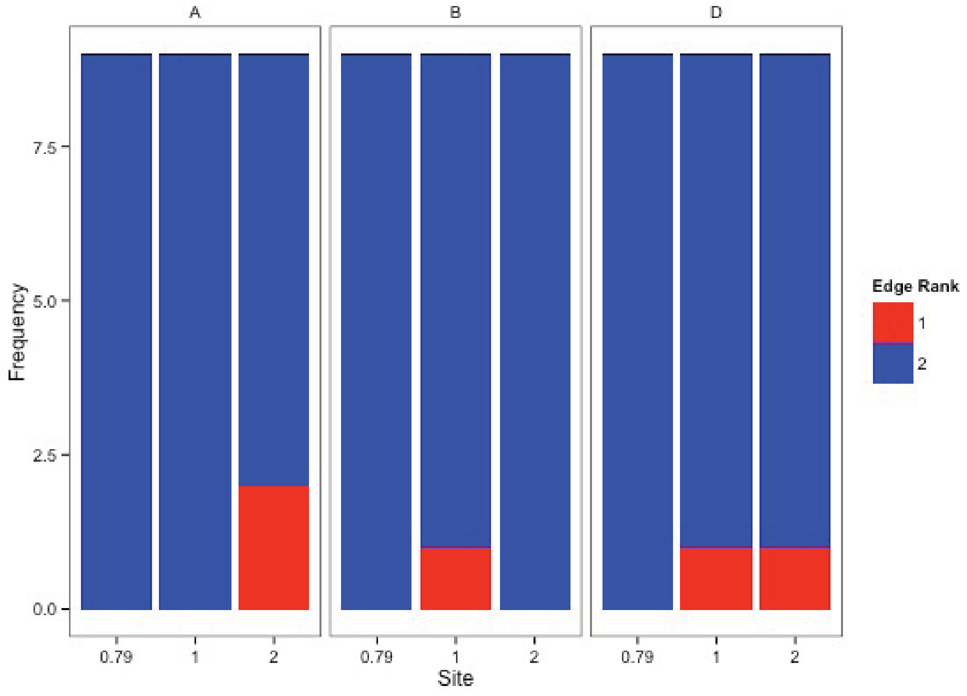


Figure 3.9: Test 1 shells' final shell edge quality across sites.

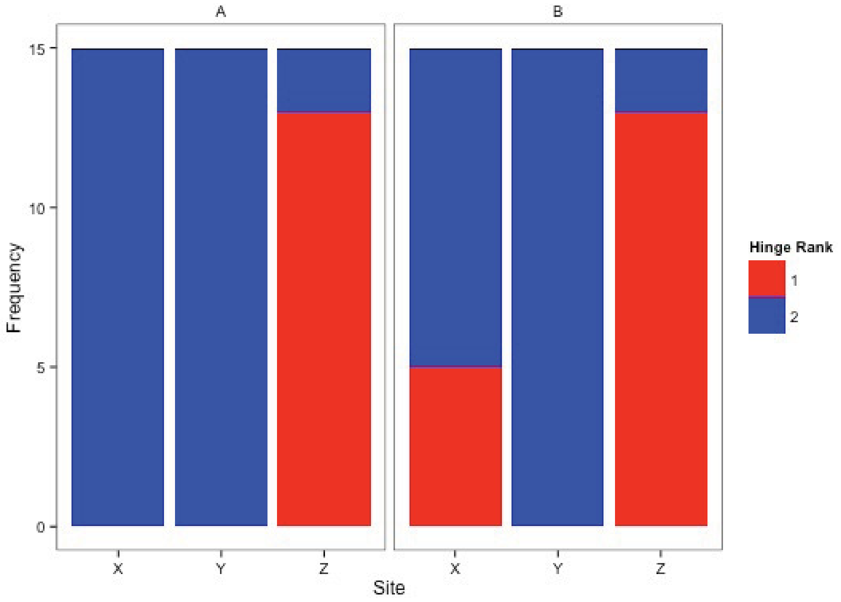


Figure 3.10: Final hinge quality of Test 2 shells after one year, across sites and originating populations. Population A = Lake Cheston, Population B = Upper Clinch River.

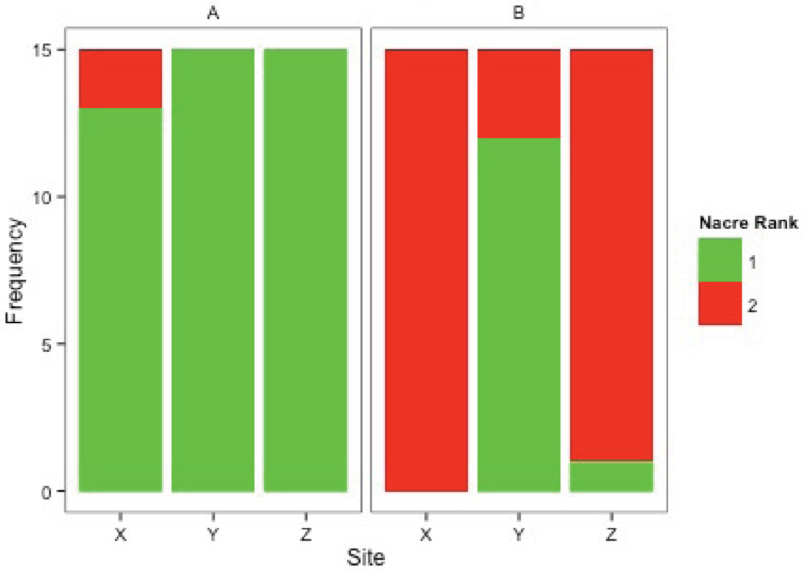


Figure 3.11: Final nacre quality of Test 2 shells after one year, across sites and populations. Population A = Lake Cheston, Population B = Upper Clinch River.

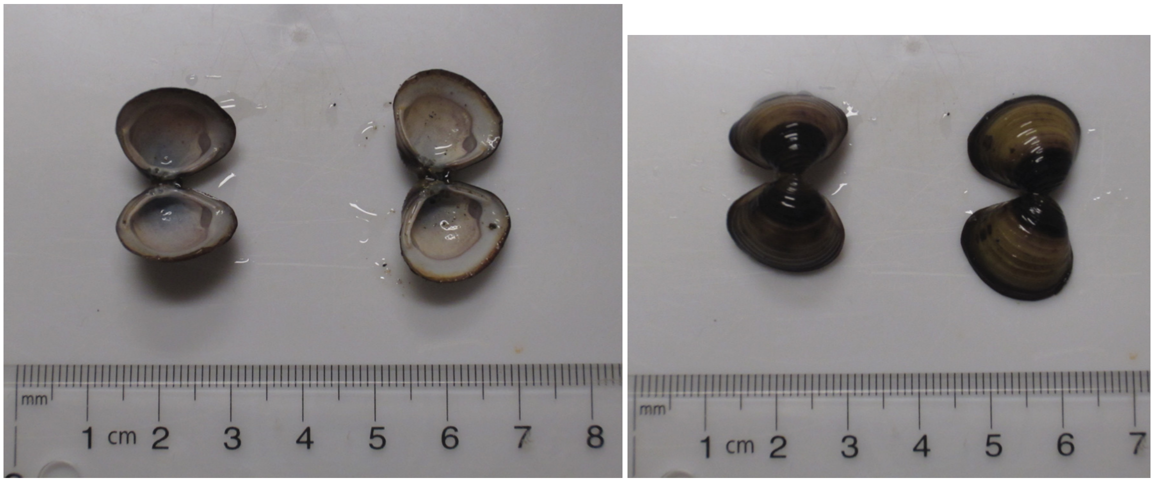


Figure 3.12: Spent shells collected from McGeachy Pond in August, 2014.

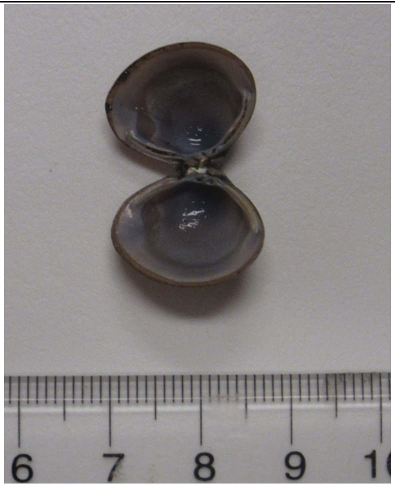
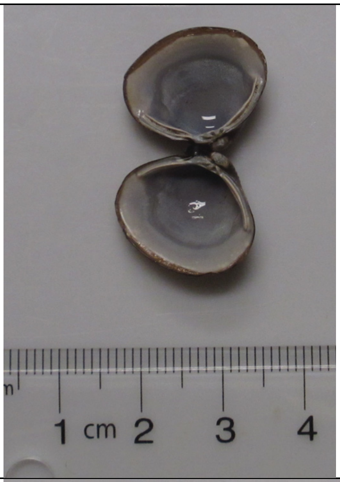
Time	Interior	Exterior
9 Months		
1 Year		
2 Years		

Figure 3.13: Examples of shells deployed at McGeachy Pond collected after 9 months, 1 year and 2 years.

Tables

Table 3.1: Environmental chemistry (mean ± standard deviation) measured in August 2013, June 2014, August 2014 and August 2015 at sites where Test 1 shells were deployed. Hudson readings were not taken after June 2014 due to loss of units. pH data collection started in June of 2014.

Site	**Location**	**DO (mg/L)**	**Specific conductance (mS/cm)**	**pH**	**Turb. (JTU)**	**Depth (m)**	**Sediment**
A	McGeachy Pond	5.1±3.5 (n=4)	0.3921±0.0342 (n=4)	7.39±0.29 (n=3)	<5 (n=4)	0.60	Sandy silt with organic matter
B	Valleyfield Marina	9.8±0.6 (n=4)	0.2869±0.0117 (n=4)	8.06±0.21 (n=3)	<5 (n=4)	2.05	Silty with organic matter
C	Hudson Yacht Club	9.7±0.6 (n=2)	0.0891±0.0003 (n=2)	7.09 (n=1)	9 (n=2)	0.90	Silty with organic matter
D	Beaconsfield Yacht Club	10.1±1.5 (n=4)	0.1854±0.0232 (n=4)	7.86±0.31 (n=3)	<5 (n=4)	1.15	Silty with organic matter

Table 3.2: Environmental chemistry (mean ± standard deviation) at sites where Test 2 shells were deployed. Values calculated over two site visits in August 2014 and August 2015 (n = 2 for all values).

Site	**Location**	**DO (mg/L)**	**Specific conductance (mS/cm)**	**pH**	**Turb. (JTU)**	**Depth (m)**	**Sediment**
X	Baie d’Urfé Yacht Club	8.9±0.4	0.1036±0.0001	8.00±0.66	<5	1.45	Silty with organic matter
Y	Beaconsfield Yacht Club	8.8±0.3	0.1864±0.0348	8.03±0.13	<5	0.65	Silty with organic matter
Z	Royal St. Lawrence Yacht Club	9.7±0.3	0.2232±0.0422	8.24±0.59	<5	1.75	Silty with clay and organic matter

Table 3.3: Shell quality traits' categories of wear.

	Rank			
Trait	**3**	**2**	**1**	**0**
Umbo	Not worn	Slightly worn	Well worn	Worn through (holes in shell)
Edge		Intact with mucous seal present	One small chip **or** mucous seal not present	Large chip or multiple chips **and** mucous seal not present
Hinge		Intact	Partially intact	Broken
Nacre	Pearly	Some nacre intact, some chalky areas	Entirely chalky but colour retained	Chalky and bleached

Table 3.4: Summary statistics (mean ± standard deviation) for Test 1 shells, calculated for each exposure period and site.

Exposure Time	**Site**	**% loss shell mass**	***k***	**Initial Length (mm)**	**Initial Mass (g)**	**n**
9 months	A	1.1 ± 4.8	-0.0155 ± 0.0612	12.9 ± 2.8	0.5317± 0.3566	9
	B	2.3 ± 6.6	-0.0315 ± 0.0825	12.6 ± 3.5	0.5758 ± 0.4163	9
	D	10.1 ± 6.6	-0.1382 ± 0.0964	13.0 ± 3.4	0.6107 ± 0.4261	9
1 year	A	4.2 ± 3.4	-0.0430 ± 0.0357	13.0 ± 2.9	0.5418 ± 0.3566	9
	B	3.9 ± 3.0	-0.0400 ± 0.0320	13.1 ± 3.3	0.5788 ± 0.4559	9
	D	11.8 ± 7.7	-0.1300 ± 0.0908	13.3 ± 2.9	0.6128 ± 0.3533	9
2 years	A	8.8 ± 6.0	-0.0468 ± 0.0337	13.1 ± 3.4	0.5512 ± 0.3927	9
	B	7.1 ± 6.4	-0.0380 ± 0.0375	12.9 ± 3.4	0.5412 ± 0.4149	9
	D	23.5 ± 11.4	-0.1395 ± 0.0842	13.2 ± 2.6	0.5465 ± 0.3002	9

Table 3.5: Estimated coefficients for the effects of the ANCOVA of ln-transformed *k* from Test 1 shells, with standard error (S.E.) and statistical significance (*p*). The * corresponds to *p*-values that are significant (α = 0.05).

Effect	**Estimate**	**S.E.**	**p**
Intercept	-1.07048	0.52968	0.04888*
Initial length (mm)	-0.17349	0.03959	6.14×10^{-5}*
Site (B)	-2.21234	0.72853	0.00544*
Site (D)	1.36568	0.81083	0.09862
Length : Site (B)	0.15058	0.05445	0.00804*
Length : Site (D)	-0.01229	0.06036	0.83952

n = 54, R^2 = 0.6053, adjusted-R^2 = 0.5962

Table 3.6: Estimated coefficients for the effects of site on ln|*k*| from Test 1 shells. Significant effects are indicated with a * (α = 0.05).

Effect	**Estimate**	**S.E.**	**p**
Intercept	-3.3385	0.1523	<0.0001*
Site B	-0.1570	0.2154	0.496
Site D	1.1718	0.2154	<0.0001*

n = 54, R^2 = 0.4713, adjusted R^2 = 0.4505

Table 3.7: Summary statistics (mean ± standard deviation) for Test 2 shells calculated by for each population and site.

Population	**Site**	**shell mass % loss**	***k***	**Length (mm)**	**Mass (g)**	**n**
Lake Cheston	X	5.5882 ±1.7585	-0.0577 ± 0.0188	30.16 ± 2.18	5.2852 ± 1.3941	15
	Y	6.5837 ± 2.1747	-0.0684 ± 0.0239	30.74 ± 2.80	5.8878 ± 1.2902	15
	Z	1.8927 ± 0.7413	-0.0191 ± 0.0076	31.63 ± 2.06	5.8477 ± 1.5600	15
Upper Clinch River	X	4.5581 ± 2.2099	-0.0469 ± 0.0233	22.51 ± 1.03	2.6364 ± 0.4157	15
	Y	6.1319 ± 0.9445	-0.0633 ± 0.0101	22.66 ± 1.28	2.7594 ± 0.4273	15
	Z	2.7870 ± 0.5368	-0.0283 ± 0.0055	22.06 ± 1.51	2.5705 ± 0.5517	15

Table 3.8: Estimated coefficients for the effects of the ANCOVA of square root-transformed |*k*| from Test 2 shells, with standard error (S.E.) and statistical significance (*p*). The * corresponds to *p*-values that are significant (α = 0.05).

Coefficient	**Estimate**	**S.E.**	**p**
Intercept	0.141233	0.039406	<0.001*
Length	0.003185	0.001478	0.034*
Site Y	0.087766	0.053796	0.106
Site Z	0.101227	0.051408	0.052*
Length:Site Y	-0.002220	0.002002	0.271
Length:Site Z	-0.006568	0.001909	0.001*

n = 90, R^2 = 0.6582, adjusted R^2 = 0.6378, df = 87

Table 3.9: Estimated coefficients for the effects of site on square root-transformed |*k*| from Test 2 shells. Significant effects are indicated with a * (α = 0.05).

Effect	**Estimate**	**S.E.**	**p**
Intercept	0.225106	0.006499	<0.001*
Site Y	0.029653	0.009192	0.002*
Site Z	-0.073459	0.009192	<0.001*

n = 90, $R^2 = 0.6053$, adjusted-$R^2 = 0.5962$

Table 3.10: Estimated coefficients and odds ratios for the effects of site and exposure time on the quality of Test 1 shells' hinges. Significant effects (according to confidence intervals) are indicated with a * (α = 0.05). Site effect estimates and odds ratios reflect changes in *k* in relation to the reference level, while the exposure time effect estimate and odds ratio reflect changes in *k* in relation to a one-unit increase (one year).

Effect or Covariate	**Estimate**	**S.E**	**Conf. Int. (2.5%)**	**Conf. Int. (97.5%)**	**Odds Ratio**
Site (B)	-2.8200	1.2620	-5.9721	-0.6418	5.9606×10^{-2} *
Site (D)	-4.3999	1.3141	-7.6470	-2.1857	1.2278×10^{-2} *
Exposure Time (y)	-3.4126	0.7988	-5.2218	-2.0089	3.2955×10^{-2} *

n = 77, $R^2 = 0.4713$, adjusted-$R^2 = 0.4505$, df = 51

Table 3.11: Effects of binomial logistic regression with Firth's penalized likelihood estimation between levels 3 and 2 of nacre quality for Test 1 shells. Significant effects are indicated with a * (α = 0.05).

Covariate	**Estimate**	**S.E.**	**p**	**Odds Ratio**
Intercept	25.098	9.282	0.007*	7.4958×10^{10}
Site B	-29.178	9.727	0.003*	2.1290×10^{-13}
Site D	-30.421	9.827	0.002*	6.1429×10^{-14}
Exposure time (years)	-28.042	10.300	0.006*	6.6282×10^{-13}
Site B : Exposure time	28.596	10.476	0.006*	2.6245×10^{12}
Site D : Exposure time	30.134	10.591	0.004*	1.2217×10^{13}

n = 71, df = 70

Table 3.12: Effects of binomial logistic regression with Firth's penalized likelihood estimation between levels 2 and 1 of nacre quality for Test 1 shells. Significant effects are indicated with a * (α = 0.05).

Covariate	**Estimate**	**S.E.**	**p**	**Odds Ratio**
Intercept	9.85209	2.59995	<0.001*	1.8998×10^{4}
Site B	0.00172	2.12839	0.999	1.002
Site D	-4.78213	1.76587	0.007*	8.3781×10^{-3}
Exposure time (years)	-3.44854	1.07633	0.001*	3.1792×10^{-2}

n = 72, df = 71

Conclusions

The recent northern range extension of *C. fluminea* in North America contradicts conventional views of the species' environmental requirements and invasion risk. This thesis addresses two core questions raised by this range extension: 1) why *C. fluminea* has established in habitats previously believed to be uninhabitable to it, and 2) what regions are at risk of invasion under current and future climate conditions.

My results show that *C. fluminea* populations vary in their tolerance to hypoxia at low temperatures, which aligns with previous research that revealed interpopulation variation in cold and salinity tolerance (Cvetanovska 2015; Evans et al. 1977). This variability calls into question the accuracy of risk assessments that are based on environmental tolerance data derived from a single population (Cvetanovska 2015). Second, I developed a climate suitability model using MaxEnt to identify regions that are climatically suitable to *C. fluminea*, based on their current known distribution. The model projects that substantial regions of the northern United States and southern Canada are presently, or will become, climatically suitable to *C. fluminea* by 2050. Finally, my shell degradation experiments reveal a simple, low-cost method of evaluating establishment or eradication success for any bivalve, with the caveat that results from one system should not be extrapolated to other systems.

Implications for ecosystem management – McGeachy Pond

C. fluminea's persistence in McGeachy Pond should be monitored, since it is the only non-thermally modified establishment site detected in eastern Canada. The shell degradation experiment results suggest that fresh spent shells collected at McGeachy Pond in 2014 were approximately one year old, and support the assertion that clams persisted at this site from at least 2010 to 2013. The results of this experiment could be used to further track *C. fluminea* persistence at McGeachy Pond, given the site's significance as a leading edge of the invasion front in southern Canada.

C. fluminea's successful establishment at this site could be attributed to environmental conditions that allow a critical number of individuals sequestering sufficient energy stores to persist through the year's stressful hypoxia events (Spann et al. 2011). While McGeachy Pond is characterized by regular summer and winter hypoxia events and seasonal ice cover, the pond's location and bathymetry also create warm temperatures throughout the spring, likely causing higher *C. fluminea* growth rates (see, for example, Abbott 1977) . In contrast, Lake George clams are unlikely to experience long warm periods, owing to the high heat capacity of this lake. This illustrates how *C. fluminea* populations' size distributions, and therefore hypoxia tolerance, may be typified by local habitat characteristics.

Implications for ecosystem management – C. fluminea *control*

The results of the hypoxia tolerance experiment have significant implications for *C. fluminea* control and eradication. Benthic barrier mats, which create hypoxic or anoxic conditions in the substrate, have become a popular method for controlling *C. fluminea* despite having mixed results. Two eradication plans implemented at Lake George, New York, and Lake Tahoe, Nevada, significantly reduced clam densities (Lake George Association 2012; Nierzwicki-Bauer et al. 2013; Wittmann et al. 2012), but failed to eradicate the invader; when costs reached $900,000 USD at Lake Tahoe and $1.5 million USD at Lake George the plans were abandoned (Nearing 2012; Plaskon 2014). After treatment, the surviving clams in Lake Tahoe skewed toward smaller size classes, which supports prior research that showed larger size classes are in fact more sensitive to hypoxia (Johnson and McMahon 1998; Matthews and McMahon 1999; Wittmann et al. 2012).

Both case studies illustrate the financial and ecological risks incurred when developing invasive species management plans based on incomplete or misleading environmental tolerance data. The majority of Lake George eradication attempts that exceeded two months occurred during the winter when water temperatures are lowest (Lake George Association 2012), facilitating the survival of some hardy individuals. Eradication effectiveness would likely have been higher during the summer months in warmer conditions (Johnson and McMahon 1998; Matthews and McMahon 1999). Benthic barrier eradication plans for *C. fluminea* should be informed by local population size distributions and environmental conditions, as well as seasonality, the length of exposure and the availability of funds to support multi-year treatments.

Bioclimatic models: mechanistic vs. empirical methods

My results illustrate some of the strengths and weaknesses posed by both mechanistic and empirical models of invasive species' environmental requirements. Mechanistic models based on experimentally-derived tolerance data can provide valuable information when predicting invasive species' distribution (see, for example, Strasser et al. 2011). Environmental tolerances are essential for predicting establishment risk and species' distributions at a local scale where climate model performance is weaker (Pearson and Dawson 2003). However, mechanistic models can be inaccurate when predicting the distributions of species undergoing range expansion (Sutherst and Bourne 2009) or those that exhibit high phenotypic plasticity, as shown in this study and results presented by Cvetanvoska (2015). Furthermore, they do not account for biotic interactions (Jeschke and Strayer 2008).

In contrast, empirical bioclimatic models inherently include all environmental tolerance phenotypes represented among the populations used to train the model, and make the softer assumption that relevant biotic interactions correlate with environmental predictors (Jeschke and Strayer 2008). Additionally, climate is a significant constraint on species boundaries (particularly of invaders), and therefore captures much variation in their distribution at a regional level (Hellmann et al. 2008; Thomas 2010), as has been shown for *C. fluminea* (Gama et al. *in press;* McDowell et al. 2014). However, empirical climate models cannot account for physical habitat features that moderate local climate conditions and thus establishment success.

One weakness of the empirical model generated in Chapter 2 was that MaxEnt excluded precipitation in the driest quarter during training, in spite of *C. fluminea*’s low desiccation tolerance (Byrne et al. 1988). This was explained by *C. fluminea*’s high probability of occurrence in desert regions, likely reflective of the numerous populations established in impounded waterways in the southwestern United States (Graf 1999). Climate suitability models for invasive species are also hindered by a lack of independent data with which to validate suitability projections in uninvaded regions or future climates, and their outputs are correlative (Elith et al. 2010; Jeschke and Strayer 2008; Jimenez-Valverde et al. 2011).

Therefore, I suggest that empirical bioclimatic models should not necessarily be preferred over mechanistic environmental tolerance models when predicting invasive species’ distributions or regions at risk of invasion. Rather, it is important to understand the strengths and limitations of each model type and target their application according to invader characteristics and study scale. Integrated mechanistic-empirical bioclimatic models are also being explored (Gallien et al. 2010), which may be a fruitful avenue for future research.

Risk assessment

My climate suitability model projects that many jurisdictions inexperienced with managing *C. fluminea* will become climatically suitable to this high impact invader by 2050. Although there is much publicly available information on *C. fluminea* in the United States, the same cannot be said for Canadian jurisdictions. The species has been identified in federal and provincial regulations, and some provinces control *C. fluminea* in some way by banning their possession, release and/or transport (Table 1). However, only Ontario, Quebec and British Columbia have online public outreach or accessible information materials related to *C. fluminea* (Table 1). Adult *C. fluminea* are quite distinctive in appearance compared to native molluscan fauna, and increased public awareness of this invader could improve early detection and response time as its range extends northward.

Genetic diversity and invasion success

Higher genetic diversity has been proposed as a predictor of invasibility for exotic species since it correlates with a broader variety of phenotypes, increasing the probability that some individuals may tolerate novel environmental stressors in the invaded range (Ehrlich 1984). However, *C. fluminea*'s range expansion in North America demonstrates how invasive species with low genetic diversity (Smith et al. 1977) can succeed in novel environmental conditions. This invader persists across a broad range of environmental conditions, owing to its high reproductive and growth rates, and remarkable phenotypic plasticity (McMahon 2002). In fact, it may be that this species' high reproductive potential allows for rapid re-colonization of marginal or unstable habitats after a mass mortality event, thereby promoting both phenotypic plasticity and potentially rapid adaptation (McMahon 2000; 2002; von Rintelen and Glaubrecht 2006). This adds to a body of evidence that classical *r*-selected life history traits may facilitate some invasions, as suggested for the zebra mussel (McMahon 2002), marine crustaceans (Gothland et al. 2014), and plants (Davis 2005).

Potential future directions

Future research should re-visit interpopulation variation in *C. fluminea* hypoxia tolerance at high temperatures. This will shed further light on how some *C. fluminea* populations persist in habitats that experience summer hypoxia or anoxia despite many reported mass mortality events under these conditions (Ilarri et al. 2011; Oliveira et al. 2015; Vohmann et al. 2010; Weitere et al. 2009). It may also provide further evidence that *C. fluminea* populations' tolerance to environmental stressors vary depending on the conditions experienced in their invaded habitat.

Additional genetic analyses may reveal whether phenotypic differentiation among invasive *C. fluminea* populations, driven by local habitat conditions, leads to genotypic divergence. *Corbicula* species may evolve new physical traits quickly, as shown in Lake Poso in Indonesia, where a sessile corbiculid

Posostrea anomioides was found to be of the same genetic clade as the non-sessile *Corbicula possoenis*, suggesting rapid evolution (von Rintelen and Glaubrecht 2006). Finally, further genetic comparison with populations in the native range may resolve apparent contradictions in described environmental tolerances by more definitely linking invasive *C. fluminea* populations to a specific source region (and therefore environmental space) in the native range.

Finally, continued monitoring of *C. fluminea*'s North American range expansion is needed to improve both mechanistic and empirical models of its environmental requirements. Novel environmental tolerance phenotypes and new populations in marginal habitats should be incorporated into existing models to ensure risk assessment and monitoring protocols are well-informed, given the significant impacts this invader can cause to recipient communities.

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Tables

Table 1: Publicly accessible information on *C. fluminea* from Canadian jurisdictions within the projected suitable regions under RCP 4.5 and 8.5 (presented in Chapter 2).

Ministry/Department	Jurisdiction	Resource
British Columbia Inter-Ministry Invasive Species Working Group	Provincial government	Species Alert factsheet on *C. fluminea* published in January 2015 (https://www.for.gov.bc.ca/hra/invasive-species/Publications/SpeciesAlerts/Asian_clam_alert.pdf)
Alberta Environment and Parks	Provincial government	No *C. fluminea* resources found. Information on AIS is available. *C. fluminea* is listed as an invasive species under the Schedule of the *Fisheries (Alberta) Act* (http://www.qp.alberta.ca/1266.cfm?page=F16.cfm&leg_type=Acts&isbncln=9780779786121&display=html)
Ontario Invading Species Awareness Program (Ontario Ministry of Natural Resources)	Provincial government	Identified *C. fluminea* as an invasive species of concern in Ontario; published factsheets on identification and included it in the aquatic invasive species field guide (3rd Edition) (http://www.invadingspecies.com/invaders/invertebrates/asian-clam/).
Ministère des forêts, de la faune et des parcs du Québec	Provincial government	Detailed factsheet available on *C. fluminea* invasion and life history, identification, control, and impacts (http://www.mffp.gouv.qc.ca/faune/especes/envahissantes/petite-corbeille.jsp).
New Brunswick Department of Natural Resources	Provincial government	No aquatic invasive species resources available.
Nova Scotia Department of Fisheries and Aquaculture	Provincial government	No *C. fluminea* resources found. Provides information on AIS and links to Ontario's Invading Species Awareness Program (where *C. fluminea* information is available).
Prince Edward Island Departments of Communities, Land and Environment, and Agriculture and Fisheries	Provincial government	No *C. fluminea* resources found. Neither department provides information on freshwater AIS, only invasive plants and marine species.
Newfoundland Department of Environment and Conservation	Provincial government	No *C. fluminea* resources found. Some information on aquatic invasive species available.
Ministry of Justice	Federal	Aquatic Invasive Species Regulations (http://laws-lois.justice.gc.ca/PDF/SOR-2015-121.pdf). Possession, transport and release in the province of Manitoba are conditional on the clams being dead.
Fisheries and Oceans Canada	Federal	No *C. fluminea* resources found. Provides information on aquatic invasive species including the Canada Action Plan to Address the Threat of Aquatic Invasive Species.
Environment Canada	Federal	No *C. fluminea* resources found. Provides information on aquatic invasive species.

Appendix A: Chapter 1 supplemental information

Environmental conditions in cold aquaria

In the cold treatment, temperature and DO levels fluctuated throughout the experiment, particularly during water changes. DO levels increased to approximately 7% DO saturation in hypoxia treatment tanks and decreased to 80% DO saturation in normoxia treatment tanks over a 24 hour period (Table A.1) before being re-established using compressed N_2 gas (for hypoxic tanks) or aerators (for normoxic tanks). DO would further increase to approximately 10% for a brief period of time (approximately 20 minutes) in hypoxia treatment tanks during water changes, before being re-established with compressed N_2 gas. Some fluctuation in DO was desirous as this better mimics real world conditions, and the amplitudes measured were consistent between replicates and remained well below those of natural environments (Suncica Avlijas, unpub. data; Grieshaber et al. 1994). Normoxic treatments were kept between 80% and 90% DO. Once in recovery, temperature and DO level fluctuation were significantly reduced.

The tank temperature also increased by approximately 2.5^{o}C during water changes, likely causing minor changes in the clams' O_2 consumption rates (McMahon 1979). Temperature loggers removed from the two cold treatment tanks indicate that these temperature increases were short-lived. Therefore, mean temperatures in the tanks were closer to 5^{o}C than what the measurements in Table A.1 suggest (Table A.2).

Tables

Table A.1: Environmental conditions in cold treatment aquaria, averaged over 3 replicates.

Treatment group	**Tank**	**Temperature (^oC) mean ± SD**	**DO (% saturation) mean ± SD**
Hypoxic treatment	1	6.2 ± 1.2	7.2 ± 2.9
	2	6.3 ± 1.0	6.1 ± 2.5
	3	6.8 ± 0.9	6.9 ± 3.0
	Mean	6.4 ± 1.0	6.7 ± 2.8
Normoxic treatment	1	6.1 ± 1.2	88.5 ± 4.7
	2	6.2 ± 1.1	87.4 ± 5.2
	3	6.4 ± 1.0	88.6 ± 5.1
	Mean	6.2 ± 1.1	88.2 ± 5.0

Table A.2: Temperature data collected by loggers during exposure period in two cold treatment tanks.

Tank	**Mean ± SD (^oC)**
Top shelf left (normoxic 1)	5.6 ± 0.8
Bottom shelf right (normoxic 3)	6.0 ± 0.7

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Appendix B: Chapter 2 supplemental information

An explanation of MaxEnt's output

I interpret the output of my MaxEnt model as a probability distribution (π) of maximum entropy over a finite number of pixels (x) in a study area (X) (Phillips et al. 2006). π assigns a non-negative probability $\pi(x)$ to each point x, and these probabilities sum to 1 across X (Phillips et al. 2006). A distribution of maximum entropy is desired in species distribution modeling, because it is subject to no constraints beyond the known environmental constraints of the study species. For a given feature f (for example, mean annual temperature), MaxEnt constrains the characteristics (mean and variance) of the feature's distribution over the study extent (X) to match the distribution over the presence locations (M) (Merow et al. 2013). For example, the average of feature f, z_f, over the study area's predicted probability distribution (the left side of the equation) is constrained to equal the average of the feature over the presence locations (the right side of the equation; Merow et al. 2013).

$$\sum_{i=1}^{N} z_{if} P^*\left(z\left(x_1\right)\right) = \frac{1}{M} \sum_{m=1}^{M} z_{mf}$$

In solving for $P^*(z(x_1))$ there are multiple distributions that may balance the equation, so the principle of maximum entropy is used to select the distribution that is most similar to a uniform distribution (Merow et al. 2013). A regularization parameter λ prevents these feature characteristics from matching too closely, to prevent overfitting and improve transferability (Elith et al. 2011).

Finally, there are several format choices available for the output distribution, including:

1. the raw probability ($\pi(x)$) assigned to each pixel in X that sum to 1;
2. cumulative, where a pixel is assigned a probability that is the sum of all raw probabilities of pixels with equal or lower value, multiplied by 100;

3. logistic, which is the cumulative output ln-transformed to improve interpretability and includes the constant τ = 0.5, the probability of occurrence at sites with typical conditions (Elith et al. 2011).

The logistic output is designed to predict the probability of presence across the landscape according to the probability of occurrence of 0.5, which does not hold true for many species. However, my objective is not to predict species presence but rather climate suitability, so the output can alternatively be interpreted as a relative index of climate suitability regardless of the value of τ (Merow et al. 2013). It is monotonically related to the raw and cumulative outputs so relative climate suitability is consistent across outputs; furthermore it is more easily interpreted and produces better-calibrated models (Elith et al. 2010; Merow et al. 2013; Phillips and Dudik 2008).

Presence data cleaning and spatial rarefaction

Generally, climate suitability models perform best when the presence data is collected systematically across the study area (Hirzel and Guisan 2002). Presence locations compiled from public datasets such as online databases or museum records are usually biased, and can contain both omission errors (observation not reported to the authorities) and commission errors (species misidentification, or historical populations that failed to persist; Newbold 2010). Furthermore, a population may have established at a modified site where habitat conditions do not reflect the local climate, such as in a thermal plume.

Therefore, the presence dataset was cleaned of these erroneous records as much as possible. First, all presence points recorded before 1950 were eliminated from the dataset, in order to meet the assumption that the climate data’s time window matches that of presence record compilation, and to eliminate historical records with no follow-up verification. Second, since environmental data was clipped to the political boundaries of Canada and the continental United States, all presence points from Puerto Rico and Hawaii were removed. Third, all populations known to persist in thermally modified habitats were removed from the dataset. The following keywords were searched in the presence dataset: “nuclear”, “generat(e)/(ing)/(ion)/(or)”, “power”, “thermal”, “discharge”, “plume”, “sewage”, and “station”. Any site that was described as near or in a thermal or sewage treatment discharge plume was excluded unless it was explicitly upstream. Any populations that failed to persist, those found in water bodies as they were drained, or detections that were explicitly ‘shells only’ were also removed.

One of the potential sources of bias in presence datasets is that observations may be biased toward specific geographic locations or landscape features, such as those with a higher human traffic volume (Newbold 2010; Phillips et al. 2009). If presence locations are biased, the climatic suitability model is over-fitted to the bias of the presence dataset, and not representing the true realized niche of the species (Elith et al. 2011; Syfert et al. 2013). Therefore, the presence dataset was spatially rarefied to the grid cell size of the environmental data layers (5 arc-minutes) to remove duplicates and to avoid skewing the dataset to highly studied water bodies with multiple presence records (Boria et al. 2014; Fourcade et al. 2014; Kramer-Schadt et al. 2013).

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