Variation in cold tolerance among populations of the invasive Asian Clam *Corbicula fluminea*

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ABSTRACT

The Asian Clam *Corbicula fluminea* is one of the most invasive freshwater molluscs in the world. In north temperate regions, this subtropical species is typically restricted to artificially heated waterbodies. Its distribution has long been believed to be constrained by a lower temperature tolerance limit of 2°C, based on a frequently-cited study of a single southern population. However, overwintering populations have been discovered in some lakes and rivers in northeastern North America in recent years. I explored the hypothesis that cold tolerance of *C. fluminea* varies across its invaded range, by using a series of experiments on clams from six geographically distinct populations along a latitudinal gradient in eastern North America. These experiments revealed that *C. fluminea* can tolerate prolonged exposure to near freezing conditions and that survivorship differs across populations. Each population contained individuals that survived two months at ~0.5-1°C. Survivorship of individuals across all populations was significantly enhanced by prior acclimation to a low temperature (10°C) compared to a higher one (18°C). When acclimated to 10°C, clams from northern populations showed greater survivorship (55.0± 16.1%) than those from southern populations (26.7± 19.2%), but no difference was observed at 18°C. At both acclimation temperatures, larger clams had a higher survivorship than smaller clams. I hypothesize that physiological plasticity and acclimation history accounts for variation in cold tolerance across these populations.

The results highlight the need for risk assessments of invasive species to incorporate physiological data from multiple populations, as predictions derived from single populations could be erroneous. However, a literature search of experimental studies on environmental tolerances of aquatic invasive animals found that only 8% of these studies compared at least two or more distinct populations of a given species. Similarly, a specific search that focused on the 20 aquatic invasive animals considered among the "world's worst" invaders found that only <4% of studies compared thermal tolerances of multiple populations. Although inter-population variation in environmental tolerance may be common, it is generally untested even for geographically widespread invasive species.

RÉSUMÉ

La petite corbeille d'Asie *Corbicula fluminea* est un des mollusques les plus envahissant du monde. Dans les régions tempérées du nord, cette espèce subtropicale est typiquement limitée aux eaux artificiellement chauffées. Sa distribution a toujours été pensée d'être limitée par sa intolérance aux températures moins de 2ºC, basée sur une étude souvent cité qui a étudié une seule population du sud. Cependant, des populations hivernantes ont été découvertes dans des lacs et des rivières dans le nord-est de l'Amérique du Nord au cours des dernières années. J'ai exploré l'hypothèse que la tolérance au froid de *C. fluminea* varie à travers sa distribution envahie, en utilisant une série d'expériences sur des palourdes de six populations géographiquement distinctes au long d'un gradient latitudinal dans l'est de l'Amérique du Nord. Ces expériences ont démontré que *C. fluminea* peut tolérer une exposition prolongée à des conditions quasiment congelées et que la survie diffère à travers les populations. Toutes les populations avaient des individus qui ont survécu deux mois à ~0.5-1ºC. La survie d'individus à travers toutes les populations était renforcée significativement par l'acclimatation à une basse température (10ºC) comparé à une température plus haute (18ºC). Quand l'acclimatation était de 10ºC, les palourdes des populations du nord ont démontré une plus haute survie (55.0 ± 16.1%) que celles des populations du sud (26.7 ± 19.2%), mais aucune différence n'à été notée à 18ºC. Aux deux températures d'acclimatation, les palourdes plus larges avaient une plus haute survie que les palourdes plus petites. J'ai émis l'hypothèse que la plasticité physiologique et l'histoire d'acclimatation comptes pour la variation de tolérance au froid à travers ces populations.

Ces résultats soulignent le besoin d'évaluation des risques d'espèces envahissantes d'incorporer des données physiologiques de plusieurs populations, puisque des prédictions dérivées d'une seule population peuvent être erronées. Toutefois, une recherche de la littérature d'études expérimentales sur la tolérance environnementale des animaux aquatiques envahissants a trouvé que seulement 8% de ces études ont comparé au moins deux ou plus populations distinctes d'une espèce donnée. De même, une recherche spécifique qui a concentré sur 20 animaux

aquatiques envahissants, considérés entre les envahisseurs les « pires du monde », a trouvé que <4% des études ont comparé la tolérance thermique de plusieurs populations. Bien que la variation inter-population dans la tolérance environnementale puisse être commun, elle est généralement non testée, même pour des espèces envahissantes avec une grande répartition géographique.

PREFACE

Contributions of Authors

The research described in this thesis reflects my own independent work, supervised by Dr. Anthony Ricciardi of McGill University and conducted in consultation with Dr. Andrew Hendry at McGill University. Dr. Ricciardi provided guidance with the experimental design as well as editorial comments, while Dr. Hendry provided guidance with the statistical analysis. I planned and carried out the experimental design, conducted the field sampling, laboratory work, and data analysis for the two chapters. I wrote all sections of this thesis, and Dr. Ricciardi revised drafts of this document. As such, both Dr. Ricciardi and Dr. Hendry will be co-authors for the manuscript when it is submitted for publication. Dr. Bruce Conn (Berry College, Georgia) provided specimens and environmental data for some southern populations of clams that were used in the experiment; therefore, he will also be an author of the resulting manuscript.

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Coming to a new city without knowing a single person seemed like it would be really difficult, but I was so lucky to come into such an incredible lab. From day one they all treated me as a friend rather than the new lab member. I am sincerely grateful to all of my friends in the lab (in order of proximity): my office mates Dustin Raab, and Kayla Hamelin, who made every day a little more fun having someone to chat with, and to my other lab members Andrea Morden, Josie Iacarella, Katie Pagnucco, Ahdia Hassan, Jordan Oulette-Plante and Suncica Avlijas for always being available to provide useful advice. An extra special thanks to Rowshyra Castañeda, who turned out to not only be my best mentor, but also one of my close friends. I could not have completed this thesis without all of her valuable input and support – science and life based. As well as to my friends at McGill, who have made my time here such a blast. I am so fortunate that I will be leaving here having gained some very valuable friends. A special thanks to my roommate and amazing friend Leanne Elchyshyn, whom I met during my very first days at McGill; she has always been there for a laugh at the end of even the toughest days. I don't think I would have gotten through this without her friendship, daily chats and encouragement.

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And finally to Alan, thank you for your patience, but most importantly for your love and friendship.

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GENERAL INTRODUCTION

Aquatic invasions worldwide

Through the last century, the rate of spread of invasive species has accelerated as a result of human activities such as agriculture, aquaculture, global trade, recreation, and transportation (Karatayev et al. 2007, Ricciardi 2007). Recent evidence from genetic studies combined with historical introduction records has shown that most modern invasions are the result of multiple introductions, from multiple sources, to multiple locations, in contrast to slow, diffusive range expansion through natural processes (Wilson et al. 2009b). In many large freshwater systems, hundreds of non-native species have been established (Table 1) and the rates of accumulation are apparently increasing (Ricciardi 2006).

Table 1. Major global waterbodies and the number of invasions

Location	Number of non-native species	Reference
Great Lakes	182	(Ricciardi 2006)
St. Lawrence River	85	(De Lafontaine and Costan 2002)
River Thames	96	(Jackson and Grey 2013)
River Rhine	45	(Leuven et al. 2009)
Hudson River	113	(Mills et al. 1996)

With the continuously increasing types of vectors, freshwater systems are extremely vulnerable to invasions. Human vectors are transporting more non-native species than ever before. Large scale transoceanic spread can be a result of large ships which exchange ballast water, carrying thousands of propagules between coasts (Ricciardi 2006). On the more small scale, vectors such as local boating between lakes can result in spread due to improper cleaning of boats. Other methods include bait bucket dumping by fishermen, live trade of exotic aquatic pets, and dumping of unwanted pets.

In addition to human vectors, natural range expansion can be accelerated by anthropogenic climate warming, which opens novel pathways for invasion through such processes as the creation of land bridges or opening of water passages, exemplified by the latitudinal shift of marine species due to warming of the North Sea (Perry et al. 2005).

Variation in invasion success

Much research has been devoted to understanding why some species are more successful than others and why some regions are more readily invaded, with the ultimate goals of predicting when and where future invasions might happen so that their consequences may be mitigated (Mack et al. 2000). Most introduced species fail to establish or don't spread post establishment, because translocated populations are often incapable of surviving in the receiving environment (Williamson 1996). One phenomenon responsible for failed invasions is known the "boom-and-bust" cycle, where the population size of an invading species declines after an initial peak density, often due to a lack of resources to support the booming population (Simberloff and Gibbons 2004). Low propagule pressure can result in invading species that are too dispersed, becoming reproductively isolated, and at a higher risk for predation (Lockwood et al. 2005). Invasion success can be influenced by several main factors:

1) Propagule pressure – the total number of propagules entering the new environment: individuals introduced in greater numbers and more frequently increases the probability that a species will establish (Lockwood et al. 2005).

2) The characteristics of the invader (Kolar and Lodge 2001). Species that are able to survive in unfavorable conditions, quickly adapt to new environments, disperse rapidly, and possess a high reproductive potential will have an advantage (Mills et al. 1993).

3) The physico-chemical characteristics of the recipient environment (Lonsdale 1999). It is often believed that invasions are more frequent in disturbed sites because non-native species are more likely to be transported there by human activity (Williamson and Fitter 1996). In addition, invasive species are more tolerant to a wide range of

conditions, and more tolerant to disturbance than the native species in that habitat (Dukes and Mooney 1999).

4) The biological characteristics of the recipient environment. The enemy release hypothesis (ERH) contends that when an invasive species is introduced to a new environment it is likely that it will not have the same natural enemies it had in its native range, thus allowing for a quick increase in population size (Keane and Crawley 2002, Colautti et al. 2004).

5) The availability of required resources (both physical and biological) in the recipient environment (Davis et al. 2000).

Even though the effects of invasions are large and can completely alter what were previously stable systems, making them unbalanced and unpredictable, it is important to note that these effects are often the cumulative effects of only a few successful invasions. A basic measure of how many invasive species establish is known as the "tens rule": 10% of attempted invasions establish, and 10% of those established become pests (Williamson and Fitter 1996). Although there are some notable exceptions such as British edible crop plants, Hawaiian birds, insects for biological control, mammals on continents etc. (Williamson 1996).

Invasions many succeed after multiple attempts

Invading populations derived from single introductions tend to be less diverse than those subjected to multiple introductions (Dlugosch and Parker 2008). Multiple introductions can add genotypes that diversify behavior, morphology and physiological tolerances, thus altering the likelihood an invader will be suited to the receiving habitat. Invasion opportunity fluctuates spatially and temporally; logically, so "windows" of invasion opportunity will more likely coincide with an introduction if multiple introduction events occur at sufficient frequency (Johnstone 1986). The highest probability of success is probably during a time when conditions are most favourable for reproduction and growth. Davis and colleagues (2000) suggest that fluctuations in environmental conditions explain invasion success because of open windows during which conditions produce competitive release among plants when competing for scarce resources.

Range expansion

A species range is governed by how well its niche requirements match the varying environmental factors in its occupied area (Holt 2003). Limits or extensions of species ranges are governed by the ability of a species to disperse, and physical or physiological barriers to that dispersal. Species can shift or expand their range by: 1) An increase in vectors: ballast water release from ships is the vector for 65% of all of the invasions in the St. Lawrence Seaway since its opening in 1959 (Ricciardi 2006); similarly shipping activity in the Great lakes brought in 29% of invasive species (Mills et al. 1993). 2) Removal of physical barriers to dispersal by human activity (Mills et al. 1993, Karatayev et al. 2006, Ricciardi 2007); for example, with the opening of the Suez Canal, hundreds of Red Sea species have been recorded in the Mediterranean Sea (Galil et al. 2007). 3) The removal of physiological barriers associated with anthropogenic climate change (Parmesan and Yohe 2003, Perry et al. 2005).

Over the past 100 years, climate conditions have changed rapidly (IPCC 2001). Many species have already started responding to the changing environment; 434 out of a pool of 893 species have shifted their distribution or abundance in response to changes in the environment (Parmesan and Yohe 2003). Under rapidly changing climatic conditions (outside the range of natural variation) it is expected that only those species able to adapt quickly will thrive (Hellmann et al. 2008). It is widely agreed that species' distributions are primarily reflective of climatic regimes, since all species have physiological temperature and precipitation thresholds (Parsons 1997). With the observed warming trends, ecological niches based on climate are expected to move towards higher latitudes and upward in elevation (Walther et al. 2002).

How do species respond to new environments?

In new environments, introduced organisms often encounter physicochemical conditions that fall outside of the range of those typically experienced in their native environment. This event may result in a sharp reduction in fitness (Hoffmann and Hercus 2000), specifically when the intensity of the stress reaches levels that could limit survival or reproduction, thereby potentially endangering populations (Bijlsma and

Loeschcke 2005). In order to survive, reproduce and persist, populations may respond phenotypically or genetically and evolve adaptive mechanisms to reduce the detrimental impact of the stress.

Certain organisms can respond quickly to new environments by reversible, non-genetic changes in the expression of various phenotypic traits (Bradshaw 1965), called phenotypic plasticity (West-Eberhard 2003, Pigliucci 2005). Studies have shown that organisms can undergo a relatively quick and continuous transformation in ecologically important traits such as behavior, physiology or morphology, within one generation, in response to environmentally induced stress through changes in temperature, DO, salinity, etc. (Kingsolver and Huey 1998, Lee 2002). Generally plasticity will be beneficial to an organism if it confers individuals' higher fitness in a new environment (Ghalambor et al. 2007). It has been suggested that plasticity that is initially induced in a population by environmental conditions can later become genetically assimilated, where the trait can be produced in the absence of the initial environmental stimulus (Waddington 1953), thus reducing the costs of plasticity (DeWitt et al. 1998, Pigliucci and Murren 2003).

Phenotypic plasticity can account for some of the variation that is observed between conspecific populations inhabiting different environments. For example temperature tolerance limits of a species are dependent on its plasticity (Chown et al. 2007, Ghalambor et al. 2007) and on climatic conditions in their native region (Chown et al. 2004). Variation between populations can contribute to their survival, by adjusting their tolerance limits based on environmental cues (Terblanche and Chown 2006). In recent decades, habitat changes and disturbances have created new environmental conditions that favour more phenotypically plastic species capable of responding quickly to change (Chown et al. 2007). Invasive species in particular show greater phenotypic plasticity than non-invasive ones, and are known to take advantage of disturbances or environmental fluctuations (Cano et al. 2008, Davidson et al. 2011).

Traditional evolutionary biology suggests that evolution occurs only when natural selection acts on heritable genetic variation (West-Eberhard 2003). In this sense, non-heritable phenotypic variation that is induced by environmental cues within an

organism's lifetime has been thought to limit the rate of adaptation (Via and Lande 1985). However environmentally induced phenotypic variation can be an important precursor to adaptation (West-Eberhard 2003, Bijlsma and Loeschcke 2005). Populations in different environments can display different phenotypes based on specific environmental cues, and survival and reproductive success among the populations can vary based on a population's response to selective pressures exerted by the environment (West-Eberhard 1989). Environmental cues can drive the direction of evolution, since these cues influence which phenotypes are expressed; only the expressed phenotypes can be genetically modified under selection (West-Eberhard 1989). This phenotypic variation could be acted on by natural selection and lead to long-term adaptation (De Jong 2005).

Biological invasions offer insight into evolution and the rate of natural selection (Hendry and Kinnison 1999). When a population spreads into a new region, it is possible that it will be exposed to environmental conditions that fall outside of the range typically be experienced in its native environment. In such situations, the most successful genotype would be one that is able to produce a variety of phenotypes in response to different environmental conditions (such as a general purpose genotype) (Richards et al. 2006). In the initial phases of invasion, adaptive plasticity can play an important role in the fitness of organisms, especially when faced with new environmental stressors (Pigliucci 2005), because it can prompt directional selection to act on extreme phenotypes towards the optimum (Price et al. 2003). In recent years, there has been growing evidence in support of the theory of rapid evolution in invasive populations, especially those responding to environmental stress (Huey et al. 2000, Sexton et al. 2002, Maron et al. 2004). It has therefore been suggested that plasticity can be considered as an important trait itself when assessing evolutionary change in an invading population, because it can differ from its native range and be adapted over time (West-Eberhard 1989, Cano et al. 2008).

Among the species of concern to aquatic resource managers are invasive bivalves, which are particularly disruptive because they possess the ability to alter their physical and chemical environment, sometimes displacing native species (Strayer et al.

1999, Sousa et al. 2009). Perhaps the most invasive of these is the Asian Clam *Corbicula fluminea*, native to southeast Asia, Australia and Africa (Morton 1986, McMahon 1999). Since the 1940s, *C. fluminea* has substantively expanded its range in North America, South America and Europe. Being a subtropical species, it was believed that *C. fluminea* could not invade natural (unheated) water bodies in cold temperate regions (Mattice and Dye 1975, French and Schloesser 1996); however, in recent years clams has been found to overwinter farther north than previously predicted (E. Cvetanovska, personal observations).

Invasion history of the Asian Clam

The first record of *C. fluminea* in North America was of spent shells found on Vancouver Island, British Columbia, circa 1924. Living specimens of *C. fluminea* in the North American wild were confirmed in the Columbia River basin in 1938 (McMahon 1999). The species is believed to have been brought to the continent as a food source by Asian immigrants (McMahon 1999). It has spread to at least 38 American states. It expanded its range in South America in the 1970s (McMahon 1999) and was discovered in Europe in 1980 – first recorded in the Dordogne and Tagus Rivers of western France and Portugal, respectively (Mouthon 1981). In recent years, *C. fluminea* has undergone a northern range expansion, both in Europe (reaching Ireland in 2010; (Lucy et al. 2012)) and in North America (Vancouver Island, New York State, and in the Quebec waters of the St. Lawrence River; (Simard et al. 2012)).

Since its first introduction to North America, *C. fluminea* has dispersed and adapted to many freshwater habitats. Dispersal of *C. fluminea* occurs through various human activities such as ballast water transport, discarded bait, and aquarium release (Sousa et al. 2009). Although most introductions of *C. fluminea* to its invaded range are human mediated, it possesses natural dispersal mechanisms that allow it to spread locally and regionally once introduced. During the juvenile stage, *C. fluminea* produces a byssal thread that can attach to floating debris, enabling it to be carried downstream (McMahon 1999). Larval clams can also attach directly to boats, or to vegetation snagged on boat trailers, potentially resulting in long distance dispersal (McMahon 1999, Sousa et al. 2009).

Physiology

Depending on location and growing season, adult clams grow to 50-70 mm in size and live for 3-4 years (McMahon 1999). The invasion success of the species can be attributed largely to its small size, rapid growth, early maturity and fecundity (Morton 1997). Adults produce ~100-570 juveniles per day, yielding an average annual fecundity of 68 000 pediveligers per adult per year (McMahon 1999). Moreover, its ability to self-fertilize can potentially cause a population to arise from a single individual (McMahon 1999). *C. fluminea* is extremely sensitive to hypoxia and low water temperatures; however, its high reproductive capacity allows it to recover from large mortality events (McMahon 1999, Werner and Rothhaupt 2008b). It is also adapted to living in a wide range of substrate types (Sinclair 1964) and thermal regimes (Mattice and Dye 1975). The upper lethal temperature of *C. fluminea* is approximately 38°C, whereas the optimal growth temperature is 22-24°C, and optimal reproductive temperature is above 15°C (McMahon 1999). Its lower lethal limit is reported to be 2°C (Mattice and Dye 1975), and this is often cited as a major factor limiting the latitudinal distribution of the species.

Reproduction, genetics and lineages

C. fluminea is a hermaphroditic species that reproduces through obligate androgenesis – a generally rare mode of reproduction in which all offspring inherit only the paternal nuclear genome (Pigneur et al. 2012b). Although sexual individuals exist, such lineages appear to be geographically restricted to their native range (Park 2003). *C. fluminea* is known to have very low genetic diversity within and between populations throughout its invaded range (Siripattrawan et al. 2000, Lee et al. 2005, Hedtke et al. 2008, Pigneur et al. 2012b). In North America, there are two distinct mitochondrial lineages: Form A, found primarily in eastern North America; and Form B, found in southwestern regions of the continent (Siripattrawan et al. 2000, Lee et al. 2005). In South America, both A and B exist with a third lineage (Form C); whereas, in Europe, there are three mitochondrial lineages (Forms R, S and Rlc) (Renard et al. 2000, Pigneur et al. 2012b). Populations of Form A from North America are genetically identical to Form R in Europe and *C. lena* in Japan (Hedtke et al. 2011, Pigneur et al. 2012b) indicating one widespread superclone (Pigneur et al. 2012b). Likewise Form S

from Europe and Form C from South America have the same haplotype, and Form Rlc from Europe has the same mitochondrial lineage as Form B in America and *C. fluminea* in Asia (Pigneur et al. 2012b).

It has been suggested that all lineages of *Corbicula* in both Europe and the Americas could be general-purpose genotypes derived from broad environmental tolerances and associated high levels of environmental plasticity (Vrijenhoek and Parker Jr 2009). A general-purpose genotype is described as a generalist lineage with flexible phenotypes that can operate across broad environments, and may evolve in asexual individuals, through androgenetic reproduction (Van Doninck et al. 2002). This could be a key to *C. fluminea's* invasion success, despite not having the high genetic diversity that is typically assumed necessary for broad invasions success.

Ecological and economic impacts

Bivalves are among the best examples of ecosystem engineers (reviewed by (Dame 1993)). In dense populations, *C. fluminea* can dramatically alter habitat conditions, nutrient cycling, sedimentation rates, and trophic interactions in lakes and rivers (Mattice and Dye 1975, Phelps 1994, Karatayev et al. 2002). The clam's distribution is restricted to the littoral zone, usually at depths above 4m (McMahon 1983), with highest densities occurring in sand and gravel sediments, and lowest densities in silts and rocky substrate (Karatayev et al. 2002). It can maintain thriving populations in a broad range of oligotrophic to eutrophic conditions (McMahon 1983).

C. fluminea is able to filter-feed from the water column using its siphons (Boltovskoy et al. 1995) and deposit-feed from the sediment using its foot to collect particles (Reid et al. 1992). It has been shown to compete for food and space with native bivalves (Karatayev et al. 2003, Werner and Rothhaupt 2007, Sousa et al. 2009). A dense population (10^3 clams/ m^2) can remove most suspended matter from the water column from lakes and river in a matter of days (McMahon 1999, Sousa et al. 2009). This can result in substantive reductions in phytoplankton (e.g. by 40-60% in some cases; Cohen et al. 1984) and enhanced light transparency (Karatayev et al. 2003, Sousa et al. 2013), with concomitant enhancement of macrophytes and shifts in primary

production from planktonic to benthic communities (Phelps 1994). Digested and undigested particles are deposited in the sediment as feces and pseudofeces, respectively, and provide a rich carbon source for benthic feeding organisms, but can also increase the release of NH_4 and ammonia (Atkinson et al. 2010).

C. fluminea can also negatively impact municipal, agricultural and industrial raw water systems (Sousa et al. 2009). Most significantly, *C. fluminea* can interfere with water supply systems through its biofouling activities; it often fouls the cooling water intake pipes and screens of electrical and nuclear power plants that draw water from rivers and reservoirs (Sousa et al. 2009). It has been estimated that nearly half of all nuclear power plants are infested with *C. fluminea*, and costs associated with its removal from such installations exceed $1 billion per year in the US (Pimentel et al. 2000).

Thesis Objectives

My work seeks to evaluate inter-population differences in the lower lethal temperature limit of *Corbicula fluminea* across a portion of its invaded range. To date, all of the literature on temperature tolerance of *C. fluminea* cites a lower lethal limit of 2°C, originally reported by (Mattice and Dye 1975), who tested individuals from only one population. In recent years invading populations of *C. fluminea* have been found to overwinter in north temperate lakes in the northeastern United States, challenging previous conclusions about its thermal tolerance. I examined variation in survivorship to cold tolerance across six geographically distinct populations of *C. fluminea* in eastern North America (three recently invaded northern populations, and three established southern populations) when exposed to winter conditions. Additionally, I sought to determine if acclimation to a low temperature prior to exposure would enhance survivorship. All populations of *C. fluminea* in North America are thought to be of the same lineage (Siripattrawan et al. 2000, Lee et al. 2005), and thus are believed to be genetically similar. This suggests that any variation seen among the populations would be a result of phenotypic plasticity or local adaptation. Adaptation to survival at low temperatures could permit *C. fluminea* to continue expanding its invasive range further north, posing a serious threat to Canadian aquatic ecosystems.

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CHAPTER 1

Variation in low-temperature tolerance among invasive populations of the Asian Clam *Corbicula fluminea*

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INTRODUCTION

The rate of spread of invasive species has accelerated over the last century, owing to human activities such as agriculture, aquaculture, and global transportation systems (Mills et al. 1993, Karatayev et al. 2007, Ricciardi 2007). Two long-standing research questions in invasion ecology are why some species are more successful than others and why the invasion success of given species can vary greatly across space and time. Two traits that can, in part, explain the success of invasive species when invading new habitats are broad environmental tolerances (Dukes and Mooney 1999, Kolar and Lodge 2001) and phenotypic plasticity (Pigliucci and Murren 2003, Price et al. 2003). Both traits may be altered by multiple introductions (Lockwood et al. 2005; Dlugosch and Parker 2008). By enhancing survivorship of individuals, phenotypic plasticity can increase the probability of establishment of a self-sustaining population (Chown and Terblanche 2006). Following establishment, plasticity can become adaptive and ultimately enhance fitness by shifting a species reaction norm to best suit its new environment (Price et al. 2003, Angilletta 2009). Eventually, individuals in the newly established population will exhibit a phenotype closer to the optimum for the novel habitat (Fig. 1) (Ghalambor et al. 2007).

Invasion success can often be attributed to a species predisposed broad environmental tolerance – which can be enhanced by an organism's capacity for acclimatization. Based on environmental conditions in a given range, species with a wide spread distribution can often exhibit variation in their tolerance limits among isolated populations (Elderkin and Klerks 2005, Fangue et al. 2006, Wallace et al. 2014). The ultimate tolerance limits of a species can change both temporally and spatially as organisms shift their range. This change can be accelerated by the lifting of thermal or geographical barriers, disturbances, or changes in climate.

Recent Spread of Corbicula fluminea

The spread of *C. fluminea* in temperate regions is believed to be limited by low winter water temperatures (Mattice and Dye 1975, French III and Schloesser 1991, French and Schloesser 1996). Overwintering has been reported in the St. Clair River

during a mild winter (French III and Schloesser 1991). and in the Connecticut River, where survivorship was positively correlated with the mean winter water temperature and negatively correlated with frequency of daily mean water temperatures ≤ 1°C (Morgan et al. 2003). However, until recently, most populations found in north-eastern USA and the Great Lakes were confined to the heated water discharge plumes of power plants (French and Schloesser 1996, Ward and Hodgson 1997).

A widely-cited laboratory study by Mattice and Dye (1975) concluded that the lower lethal temperature of *C. fluminea* was 2°C, based on the response of a single population. In other studies examining the overwintering capacity of *C. fluminea*, tested populations were observed downstream of power plants where clams lived in a thermal refuge throughout the year (French III and Schloesser 1991, French and Schloesser 1996). However, Müller and Baur (2011) reported that Asian clams from the River Altrhein, Germany, could tolerate colder temperatures than previously assumed; in lab experiments, some clams (17.5% of tested) could tolerate exposure to water temperatures of near 0°C.

Until recently, low water temperatures and winter ice formation have appeared to restrict the northern distribution of *C. fluminea* in North America. However, a recently established overwintering population in Lake George, New York, was discovered in 2010 and has persisted to date, spreading throughout the lake and surviving unsuccessful eradication attempts (Meg Modley, Lake Champlain Basin Program, pers. comm.). Established northern populations have also been found in the Seneca River, NY and Owasco Lake, NY (E. Cvetanovska, unpublished data). In Canada, *C. fluminea* established in the St. Lawrence River in a thermal plume generated by outflow water from Gentilly – 2 Nuclear Power Plant (G2NPP) in Bécancour, Quebec (Simard et al. 2012) by 2009. In December 2012, G2NPP was shut down permanently, thus halting the discharge of heated water and re-establishing winter conditions to that stretch of the river for the first time in decades. Following the plant closure and exposure to winter conditions, the population collapsed and appears to have been completely extirpated (Castañeda 2012; E. Cvetanovska, personal observation).

Although the direct lineages and invasion vectors of *C. fluminea* to northern locations are unknown, it is possible that the more recent northern introductions are a result of bait bucket dumping from anglers or by aquarium release. *C. fluminea* is sold (as "clams") through online aquarium stores and in some pet stores in the Great Lakes region (Becky Cudmore, Fisheries and Oceans Canada, personal communication).

Given the variation in environmental conditions (especially temperature) in habitats previously invaded by *C. fluminea*, it is of interest to compare the survivorship of different North American populations when exposed to winter temperatures in the lab. Through cold tolerance experiments, I tested the predictions that 1) overwintering Lake George, NY population would have a higher survivorship than the St. Lawrence River thermal plume population; 2) naturally overwintering northern populations in general will have a higher survivorship than southern populations; 3) acclimation to a lower temperature prior to exposure to winter conditions will enhance survivorship across all populations; and 4) larger clams will have a higher survivorship than smaller clams. Using survivorship as an experimental end point is arguably an extreme assessment of ecologically relevant thermal tolerance; exposure to new environmental conditions can produce non-lethal, but still compromising effects on feeding, growth and reproduction. Nonetheless survivorship is a useful method for comparing environmental tolerance across populations (Addo-Bediako et al. 2000) and is an indicator of the capacity for a species to establish in suboptimal habitats.

METHODS

Experimental animals and study site

My initial study site was in the St. Lawrence River at the outflow of the Gentilly 2 Nuclear Power Plant (G2NPP) in Béconcour Quebec (46°23'42.51"N, 72°21'23.53"W). Over the past two summers, four sampling stations (Fig. 2) along a 1-km section of the thermal plume were established and sampled repeatedly (by K. Hamelin and R. Castañeda). The four sites were chosen based on the temperature gradient created by

the warm water outflow of the power plant, which created an 11°C temperature difference between the two most distal sites (K. Hamelin, personal comm.).

In Fall 2012, samples of *C. fluminea* were collected at the four sites using a petite Ponar grab (152 x 152 mm) at 1.9 - 2.8 m depths. Three grabs were taken per site except where *C. fluminea* abundance was sparser thus more grabs were necessary. Living clams were temporarily stored in large coolers during transport to McGill University. These samples were divided by their site origin among large 50L aquariums (two per site). Each aquarium was supplied with ~3cm of pre-washed gravel and a charcoal filter. The aquaria were maintained at ~18°C with tri-weekly 20% water exchange and fed with ground spirulina flakes (5mg/100clams). In addition to this experimental population, sixty clams were collected from Lake George, NY (43°37′20″N 73°32′48″W) (Fig. 3) in October 2012, shipped to the lab and maintained in two additional tanks.

Experiment series I: St. Lawrence River and Lake George populations

In mid-December 2012, a short-term cold tolerance experiment was conducted for seven days, as a pilot study to test methodology as well as clam behavior and survivorship at a temperature lower than the putative lower tolerance limit for the species in North America (Mattice and Dye 1975). Ten clams from each of the four sites along the thermal plume in the St. Lawrence River and ten clams from Lake George were separated into five 16-L aquaria and acclimated at 1°C/hour to 1°C in the lab. The aquaria were kept in a temperature-controlled fridge at 1°C for seven days. Clams were not fed and were checked daily for mortality by gently forcing the tip of a dissection needle between posteriori valve margins in the region of the siphons; living clams resisted needle entry, whereas dead clams failed to close valve after needle insertion (McMahon et al. 1995); this type of testing causes no apparent harm the clams.

In January 2013, a long-term (eight-week) cold-tolerance experiment was initiated, following a similar protocol as outlined above (Table 2). To minimize disturbance to individuals, mortality was checked biweekly and water was changed

(20%) weekly. After eight weeks, temperatures were raised at the rate of 1°C/hour to 18°C, and recovery was tracked for an additional eight weeks. During recovery, water was changed and clams were fed with spirulina flakes tri-weekly.

Analysis of variance (ANOVA) was used to examine differences between the proportions of individuals surviving among all four populations and a *post-hoc* Tukey test was used to identify population differences. The statistical analysis was computed in SPSS Version 22 (IBM Corp. 2013).

Experiment series II: Long term cold tolerance experiments on northern and southern populations

In August 2013, samples of *C. fluminea* were collected from Park Lane site in Lake George, NY (43°37′20″N 73°32′48″W) (Fig. 3) using a sieve at depths ranging from 0.3–1.0 m. A limited size range was available at this location, ranging between 14.1–18.7mm, with an average length of 16mm. Living clams were placed in containers filled with lake water, and temporarily stored in large coolers during transport to the laboratory (McGill University).

In October 2013, live samples of *C. fluminea* were shipped to McGill overnight from Sinking Creek, VA (37°18' 11"N 80°29' 09"W), Upper Clinch River, VA (37° 5' 38.5146"N 81° 47' 7.7382"W) and Lake Cheston, TN (35°12' 36N 85°55' 49"W) (Fig. 3). Clams were transported in containers filled with water and sediment. No major die-offs (<2%) were observed during transport. Size of clams varied between populations, but not among populations (Table 2).

In November 2013, live clams were collected from 6 sites along the Seneca River (42° 56' 8.016"N 76° 46' 1.3794"W) (Fig. 3) using a petite Ponar grab (152 x 152 mm) at depths of 1.0–3.5m, and one site from Owasco Lake (42° 53' 35.052"N 76° 31' 50.736"W) (Fig. 3) using a sieve at depths of 0.3–1.0 m. Again, size of clams varied between the populations, but not among them (Table 2). Living clams were placed in containers filled with lake water, and temporarily stored in large coolers during transport to the laboratory. Upon arrival, samples from each location were placed in separate 50L

aquaria supplied with 3cm of pre-washed gravel and a charcoal filter. The aquaria were maintained at ~17°C with tri-weekly 20% water exchange and fed with ground spirulina flakes.

Experiment series II: 10°C Acclimation experiment

Two replicates of ten clams from each of the six sites were kept separately in twelve 2.5-L aquaria (Fig. 4). The ambient water temperature was reduced to 10°C at a rate of 2°C/day, a slower rate than was used previously. The aquaria were maintained in a temperature controlled fridge at 10°C for a 30-day low temperature acclimation period, during which time clams were fed and water was changed tri-weekly. Following acclimation, the ambient water temperature was reduced to 1°C at a rate of 2°C/day and the aquaria were maintained in a temperature-controlled fridge at 1°C for the 8-week experimental period; during this time clams were unfed and kept in complete darkness. During acclimation and exposure periods, water was changed tri-weekly. Clams were checked bi-weekly for mortality using the same procedure as in the first series of experiments. Live clams from all of the populations were maintained in a temperature controlled room at 18°C, following the same feeding and water changing regime as for the recovery period. These aquaria and were maintained for >6 months and served as a control for the experiment. No major die-offs (<2%) were observed during this time.

At the end of the 8-week exposure period, the temperature chamber used during the experiment experienced a calibration malfunction which exposed all of the experimental clams to water temperatures of ~0.1°C for 5 days. This resulted in the formation of a thin (5mm) ice cover in all of the tanks. After correcting the chamber's temperature calibration, the ambient water temperature was increased to 12°C at 2°C/day. All tanks were then moved to a temperature-controlled wet lab maintained at 16-18°C for an 8-week recovery period. Clams were fed and water was changed tri-weekly. Mortality was checked daily; dead clams were removed immediately and size parameters (length, height, width) were measured using Vernier digital calipers (resolution of 0.01mm). Water temperatures were monitored with a temperature logger

and all aquaria were moved around the temperature chamber in a random fashion once per week to avoid any temperature bias due to location inside the chamber.

Experiment series II: 18°C Acclimation experiment

In this experiment, four of the six previously mentioned populations were used because clams from Seneca River, NY and Lake Cheston, TN populations were depleted more rapidly than anticipated leaving an insufficient number to include them. An additional two replicates of ten clams from the remaining four sites were separated in twelve 2.5-L aquaria (Fig. 4) and the water temperature was increased to 18°C at 2°C/day. The aquaria were maintained in a temperature-controlled room at 18°C for a 30 day acclimation at the ambient (18°C) temperature. Immediately following acclimation, clams were moved to an experimental chamber and the water temperature was reduced to 1°C at a rate of 2°C/day. The aquaria were maintained at 1°C for 8 weeks following the same protocol as used in the previous experiment. However to allow comparisons between the two acclimation temperature experiments, the unplanned "freezing event" at the end of the previous experiment was replicated, so all experimental clams were exposed to water temperatures just above 0°C for five days. Following the end of the experimental period, water temperatures were raised to 18°C and all tanks were moved to the temperature-controlled lab for an 8-week recovery period, following the same protocol as in the previous experiment.

Statistical Analysis

Survivorship results were analyzed using a generalized linear mixed model (GLMM) with a binomial error distribution and logit link. This type of model was appropriate as it allows for the data to be nonnormal, contain random effects, and have a binary response variable (Bolker et al. 2009). In this case, the binary response variable was clam survivorship (alive or dead) after the duration of the exposure and recovery period, with a fixed factor of location and clam size (maximum length (mm)), and a random effect of tank. Interpolation differences were compared using a multiple comparisons Tukey's HSD *post hoc* test. A linear regression was used to determine if

there was a significant relationship between size (length (mm)) and survivorship for all populations and acclimation temperatures. All statistical analyses were computed in R using the glme4 and multcomp packages (R Development Core Team 2014).

Daily variation in survivorship following the 10°C acclimation experiment was explored further using a Kaplan–Meier survivorship analysis, which allows for censored data –in which the time endpoint is reached without an event (Kaplan and Meier 1958). In this case an "event" is when an organism dies during the analysis. All censored data points are incorporated into the final analyses and not discarded, so sample sizes are equal among locations (n = 20). Along with the Kaplan-Meier analysis, the semi parametric Cox model is a widely used procedure for statistical analysis modeling the relationship of any independent variable (covariates) on the dependent variable (survivorship) (Cox and Oakes 1984). The logrank (Mantel-Cox) test – a non-parametric test appropriate for right-skewed and censored data (Mantel 1966) – was used to compare survivorship curves amongst populations. To determine exactly which populations differed in survival, a multiple comparisons Tukey's HSD *post hoc* test was used. These analyses are unusual for ecological applications (but see (Johnson and McMahon 1998, McNair et al. 2012)), although used extensively by medical researchers (Parmar and Machin 1995). All statistical tests used a probability level of <0.05 to determine significance among temperature treatments. All statistical analyses were computed in R using the survival package (R Development Core Team 2014).

RESULTS

Experiment series I: St. Lawrence River and Lake George populations

The pilot study was executed to test the experimental protocol and methods. All clams survived the 7 day exposure at 1°C.

After 8 weeks' exposure at 1°C, survivorship did not differ significantly between St. Lawrence River sites and the Lake George population (ANOVA, $F_{4,5}=1.028$, p=0.475). All clams from the Lake George population survived after 8 weeks, whereas St. Lawrence River clams suffered mortality across all four sites (Table 3, Fig. 5a). By

contrast, following the subsequent 8-week recovery period, there was a significant effect of site on survivorship (ANOVA, $F_{4, 5}$=18.773, p=0.003). *Post-hoc* tests revealed that the survival of clams from Lake George was higher than from the St. Lawrence River (Tukey's HSD, p<0.004), and the St. Lawrence River sites did not differ from each other (Table 3, Fig. 5b).

Experiment series II: Long-term cold tolerance experiments on northern and southern populations

A mixed effects model with fixed factors of acclimation temperature, location and clam size and random factor of tank nested in location was used to examine their effects on survivorship. Acclimation temperature had a significant effect on survivorship (GLMM, z=-3.35, p=0.0008) (Fig. 7). Survivorship was overall higher for clams acclimated at 10°C (average survivorship 38.75± 14.3%, than 18°C (11.25± 8.0%). Clams from Lake George and Upper Clinch River did not differ significantly from each other in survivorship (p= 0.116), but had an overall higher survivorship than the other populations (Fig. 6, Table 4). A linear regression model was used to show the effect of clam size, measured by the maximum length (mm) on survivorship. Clam size had a significant effect on survivorship during cold exposure, at both acclimation temperatures (GLMM, z= -2.283, p= 0.022); larger clams had an overall higher survivorship than smaller clams (Fig. 8).

Experiment series II: 10°C Acclimation

A mixed effects model with fixed factors of location and clam size and random factor of tank nested in location was used to examine their effects on survivorship. Six populations were tested in this experiment; three northern populations: Lake George, NY, Owasco Lake, NY and Seneca River, NY, and 3 southern populations: Sinking Creek, VA, Upper Clinch River, VA and Lake Cheston, TN. The 10°C-acclimated clams from all 6 populations survived the duration of the experiment (Table 5): overall 40.8± 12.9% of all clams (n=120) survived the duration of the experiment. Tolerance to cold temperatures varied substantially across populations. Clams from northern populations

had a significantly higher survivorship (55.0± 16.1%) than those from southern populations (26.7± 19.2%) (GLM, z=-3.597, p= 0.0003) (Fig. 9). Three of the 6 populations had significantly higher survivorship with over 50.0% (Table 6): Seneca River had the highest overall survivorship with 80.0±10.0%, followed by Upper Clinch River (65.0±15.0%) and Lake George (60.0± 10.0%). The remaining 3 populations had much lower survivorship; Owasco Lake had 25.0± 25.0%, followed by Lake Cheston (10.0±10.0%) and Sinking Creek (5.0±5.0%) (Fig. 10). Along with location, size of clams had a significant effect on overall survivorship (GLMM, z= -2.415, p= 0.016). A logistic regression of length (Fig. 11) revealed that the probability of survivorship increased with an increase in clam size (p= 0.014). The survival curves for the six populations in the 10°C acclimation experiment were found to be statistically different over the duration of the experiment and recovery period (log rank, χ^2= 47.93, p<0.005) (Fig. 12). A Tukey's HSD *post- hoc* test revealed that Lake George, Seneca River and Upper Clinch River did not differ from each other, but had a significantly higher probability of survivorship over time compared to Owasco Lake, Sinking Creek and Lake Cheston, which did not differ from each other (Table 7).

Experiment series II: 18°C Acclimation

Similar to the 10°C acclimation experiment, a mixed effects model was used to examine their effects on survivorship. Populations from Seneca River and Lake Cheston were not used in this experiment as explained previously. Only 11.3 ± 8.0% of all of the 18°C- acclimated clams (n=80) survived the duration of the experiment (Table 8). At this acclimation temperature, survivorship did not differ amongst populations – except for the Upper Clinch River population, whose clams had the highest overall survivorship (35.0 ± 5.0%), and were also the largest clams out of all the populations (range 19.9- 25.6mm, average 22.9mm). Clams from Lake George and Owasco Lake had 5.0 ±5.0% survivorship, and no clams from Sinking Creek survived the experiment. Neither location nor clam size were found to have a significant effect on survivorship (p> 0.05) (Fig. 13).

DISCUSSION

Variation in cold tolerance across populations

Temperature is a major constraint on the geographic distribution of ectotherms (Huey and Kingsolver 1989) and a key limiting factor for aquatic invaders in general (Rahel and Olden 2008). Conspecific populations that are geographically isolated can differ greatly in temperature tolerance, owing in part to the varying thermal conditions experienced in each location (Elderkin and Klerks 2005, Fangue et al. 2006, Wallace et al. 2014). Here I experimentally manipulated temperature to examine variation in cold tolerance across populations of *Corbicula fluminea*. The first set of experiments compared two populations which differed in water temperature: one population which was established in a year-round thermal refuge (St. Lawrence River, downstream of the G2NPP, and one overwintering population in a natural northern lake (Lake George, NY). As predicted, clams in the thermal refuge had a lower tolerance of prolonged exposure to near freezing conditions than the clams from the overwintering population. Importantly, differences in survivorship became most pronounced during the recovery period; some clams that endured the experimental treatment were too weak to persist. This finding underscores the importance of monitoring recovery from an applied stress, to avoid erroneous interpretation of experimental results; the recovery period is analogous to early spring transition from reduced activity to regular feeding and growth as water temperatures warm. Intriguingly, I was able to compare these results to field observations of the St. Lawrence River population's response to prolonged exposure to natural cold temperatures in the field. In December 2012, four months after clams were collected from the thermal refuge, the G2NPP was shut down permanently, thus re-establishing winter temperature in that section of the river for the first time since the 1970s. A major die-off of the *C. fluminea* population occurred over the following winter and spring, as evidenced by dense aggregations of recently spent shells observed during two intensive sampling periods that failed to locate any live clams in the summer. The St. Lawrence River population appears to have been completely extirpated, whereas the overwintering population in Lake George has persisted, corroborating the results of my experiments.

The second series of experiments examined cold tolerance across populations from unheated waterbodies in eastern North America (Fig. 3): three southern populations (Upper Clinch River, VA, Sinking Creek, VA and Lake Cheston, TN) which have been established for at least a few decades and that experience mild climatic conditions, and three northern populations (Lake George, NY, Seneca River, NY and Owasco Lake, NY) that have been established more recently and experience prolonged exposure to near freezing water temperatures every winter. This experiment further demonstrated that the species has a greater tolerance to lower temperatures than commonly reported. All populations, regardless of their location or acclimation history, had some individuals that survived exposure to 1°C. Most notably, there was strong variation in survivorship across populations. Acclimation to 10°C enhanced survivorship (Fig. 10) when exposed to winter thermal conditions, but acclimation to 18°C did not (Fig. 7). The northern populations exhibited greater cold tolerance than southern populations.

Behavioural adaptations for surviving stress

Although clams were not fed for the duration of the eight week exposure period, food limitation was dismissed as having a major influence on survivorship; *C. fluminea* is capable of surviving 154 days of starvation at room temperature (22-24°C) (Thorp and Covich 2009). Starvation is likely to be more stressful to clams during summer temperatures, because at lower temperatures clams close their valves to reduce metabolic demand (Guppy and Withers 1999, Ortmann 2003). During exposure to low temperature, I observed a unique behaviour of *C. fluminea*, where clams closed their valves tightly shut and sealed with a hardened mucus, thus preventing direct exposure of internal tissues to the external environment. This behaviour has previously been observed by Matthews and McMahon (1999), who noted that *C. fluminea* may remain anaerobic with the valves shut for a minimum of 3–4 days at high temperatures and for several weeks at low temperatures. They suggested that clams can remain like this until toxic anaerobic end products, such as acetate, propionate, and succinate accumulate, causing the clams to open their valves and move water over the gills to eliminate those

end products. Similarly Ortmann and Grieshaber (2003) studied the valve movement of *C. fluminea*, and reported that, during winter, the movement almost ceased and the clams remained closed for more than a week. During valve closure, *C. fluminea* can reduce its energy metabolism to less than 10% of the standard metabolic rate measured when the valves are open (Ortmann and Grieshaber 2003). Similar metabolic reductions have been described among other invertebrates facing severe stress (Guppy and Withers 1999). The ability to close the valves and reduce the metabolic rate can save a lot of energy and can be beneficial in protecting an organism from any major environmental stress, particularly during periods of starvation (Ortmann 2003). This behavior, which has been observed in some other bivalves ((Kramer et al. 1989, Borcherding 1992, Holopainen and Penttinen 1993) should enable *C. fluminea* to persist through limited periods of unfavourable conditions (McMahon 1999, Sousa et al. 2009).

Size dependent survivorship

The stress induced by exposure to cold temperatures is harsh on organisms when energy stores are generally more depleted, since energy requirements for physiological processes exceed the amount obtained through feeding. Without feeding externally, lipid reserves are used to fuel physiological demands, particularly during valve closure. When lipid reserves are depleted, mortality occurs from starvation (Cunjak 1988). Larger individuals probably have a better ability to store energy, and therefore have a better resistance against different stressors (such as low temperature in our experiment) than smaller clams. For example French & Schloesser (1991) observed higher overwinter mortality rates of first year clams, which are believed to be more susceptible to low temperatures than larger clams. In my experiments, I observed a size-dependent survivorship when clams were exposed to cold temperatures. The probability of survivorship increased with clam size (Fig.11). Size-dependent survivorship of cold temperatures by *C. fluminea* was also observed by Müller and Baur (2011), and has been reported for other bivalves (Wallis 1975, Karatayev et al. 2011).

Overwintering in the wild: the roles of acclimation, acclimatization and plasticity

Two major physiological mechanisms that may contributing to the invasive success of organisms when introduced to novel environments are broad environmental tolerance, and increased plasticity induced by acclimation (Terblanche and Chown 2006, Ghalambor et al. 2007). A combination of these mechanisms is likely responsible for overwintering survivorship of *C. fluminea*. In fluctuating or highly seasonal environments, organisms are often exposed to heat or cold stress (Bijlsma and Loeschcke 2005); logically, those with a broad tolerance or a higher adaptive capacity should be more likely to form persistent populations. However, a broad environmental tolerance alone does not explain the inter-population variation in survivorship that is observed in my experiments. Genetic differences cannot be ruled out, but are an unsatisfactory explanation for the observed variation, because only one genetic lineage of *C. fluminea* is known in eastern North America (Hedtke et al. 2011, Pigneur et al. 2012a).

Although the adaptability of a species is often linked to its genetic diversity (Frankham 2005), successful invasions do not always have to possess high genetic diversity (Roman and Darling 2007, Rollins et al. 2013). Although the genetic diversity of *C. fluminea* in its invaded range is low, its spread is not hindered by a lack of genetic variation because introduced individuals reproduce by androgenesis, a relatively rare mode of asexual reproduction (Hedtke et al. 2008, Pigneur et al. 2012a), that permits a species to quickly produce a clonal population (Pigneur et al. 2012a). In its native range, *C. fluminea* reproduces both sexually and asexually through parthenogenesis (Pigneur et al. 2012a). In its invasive range, which occupies a broader geographic area, populations of *C. fluminea* are strictly asexual (exhibiting geographic parthenogenesis *sensu* (Weider 1993)). Asexual populations of species in general tend to be distributed over broader latitudes than sexual populations, and can be found in more disturbed habitats (Peck et al. 1998), suggesting that they possess more broadly adapted "general purpose" genotypes (Van Doninck et al. 2002). The ability to reproduce

asexually could be one reason the general purpose genotype is apparently reserved in invasive populations of *C. fluminea* and similar species, aiding in their environmental tolerance and continuous spread to new environments (Hedtke et al. 2008, Pigneur et al. 2012a).

Variation in cold tolerance across populations could conceivably be the result of plasticity in response to different acclimation histories. A few studies have shown that isolated populations of a single species that have developed under different thermal conditions actually differ in thermal tolerance (Huey and Kingsolver 1989, Elderkin and Klerks 2005, Sorte et al. 2011, Wallace et al. 2014). This could in part be due to differences in the acclimation ability between populations (Sorte et al. 2011). Intraspecific variation can play a key role in the persistence of invasive populations in new environments (Kelley et al. 2011) by enhancing a species' survival. For example poorly adapted populations can be replaced by propagules from populations with similar thermal conditions, which are already adapted to the new conditions.

Acclimation – the rapid alteration in physiology of an organism that occurs as a response to environmental change (Huey et al. 1999) – is assumed to benefit the performance of an organism in a new thermal environment, if it produces an overall advantage in fitness over another sympatric organism that has not had the opportunity to acclimate (*beneficial acclimation hypothesis* (Leroi et al. 1994)). Although not explicitly tested in the present study, one can infer that acclimation potential, if it continually enhances survivorship, and thus increases overall fitness of the organisms, can become adaptive over a period of time. Many studies on Drosophila species have tested the hypothesis that adult acclimation can be beneficial in the survivorship of desiccation, heat and cold stress; adult acclimation for cold resistance in *D. melanogaster* increases lifespan, reduces mortality and recovery time after exposure (Hoffmann 1990, Kristensen et al. 2008, Colinet and Hoffmann 2012). In my experiments acclimation to a colder temperature increased cold tolerance across populations, but variation in survivorship was pronounced. Some of the northern populations, or larger sized individuals had a better acclimation potential than some of

the southern populations – with the exception of the populations from Upper Clinch River population, which suggests that even some southern populations, if properly acclimated, can persist through near freezing winter conditions.

Acclimation can also happen naturally due to seasonality in the wild. This process is known as acclimatization (Kelly et al. 2012), which can trigger physiological mechanisms that prepare organisms to withstand temperature stress. Acclimatization through the exposure of non-lethal conditions can initiate plastic changes in an organism, which can over time increase resistance when exposed to that particular stressor (Hoffmann and Watson 1993). I hypothesized that acclimatization is an important factor that creates inter-population variation in cold tolerance. The northern populations likely experience a seasonal acclimatization period each year, whereas the southern populations do not have the same triggers. It has been suggested that prior exposure to a mild stressor can then improve an organism's tolerance to higher levels of that stressor (Costantini 2014). One can argue that if a population is introduced at the right time, with adequate time for acclimatization to occur, seasonal temperature drops will first expose an invader to mild levels of temperature stress, which will then enhance survivorship when exposed to higher levels of the temperature stress. Similarly acclimatization can happen temporally: a more mild winter can result in survivors, which in turn are better acclimated to winter conditions, thus enhancing overall survivorship over time.

To explore this further I compared historical daily air temperature data (USGS 2014) as a proxy for water temperature, because seasonal water temperature data are lacking for most of the waterbodies involved in my experiment. I summed the total number of days which had air temperatures below 0°C at each location (Table 9). Lake George had the highest number of days below 0°C, followed by Seneca River. Surprisingly, one of the least cold-tolerant populations from our study (Sinking Creek, VA) had the same number of <0°C days as Seneca River. Perhaps more important than the total number of days below 0°C could be the wide range of low temperatures, and also the amount of days at the extreme low temperatures. For example the coldest

temperature at Lake George was -27°C, whereas Lake Cheston was -10°C. Furthermore, the northern locations had temperatures <10°C steadily for the months of January and February, whereas in the southern locations although negative, temperatures fluctuated around 0°C. To further examine temperatures experienced in the wild, temperatures loggers should be placed overwinter in each location.

Table 9. Total number of days in 2013 as recorded by the USGS with air temperature below 0°C at the six experimental locations

Location	Number of days below 0°C
Lake George, NY	152
Seneca River, NY	121
Owasco Lake, NY	103
Sinking Creek, VA	121
Upper Clinch River	89
Lake Cheston, TN	75

Overwinter mortality of *C. fluminea* has been reported at the edge of its northern range, likely due to deteriorating physiological condition (McMahon and Williams 1986), resulting from insufficient food reserves (French III and Schloesser 1991). They may have relatively good survival and recruitment in a more mild winter, as was observed in a study of *C. fluminea* in the discharge waters downstream of an electrical power plant in the St. Clair River in the winter of 1989-1990 (French and Schloesser 1996) but can also experience high mortality in a cold winter, as was observed in the same study (French and Schloesser 1996) the following year. I also observed high mortality at the G2NPP in the winter of 2012-2013 in the present study, to the point where the population was extirpated.

However, overwinter survivorship has also been observed in unheated waterbodies, including the Clinton River in Michigan (Janech and Hunter 1995), Lake Tahoe (Wittmann et al. 2008) and more recently in Lake George, Owasco Lake and

Seneca River in New York State (pers. obs.). Therefore it seems that the populations of *C. fluminea* at these locations operate at the edge its lower lethal limit, since a significant proportion of individuals are capable of tolerating the low winter water temperatures. Even when such northern populations of *C. fluminea* suffer substantial winter mortality, a small number of surviving individuals could possibly have increased fitness and produce offspring that possess an even higher tolerance to harsh winter conditions. However, northward expansion might not be limited solely by adult tolerance to low winter temperatures. *C. fluminea* requires temperatures of 14-15°C for reproduction, thus low spring temperatures could be limiting reproduction (McMahon 1999, Werner and Rothhaupt 2008b), although such barriers may be lifted in some regions as a result of ongoing climate change.

Implications for further invasion

Most species introductions fail to establish sustainable populations (Williamson and Fitter 1996), because in spite of high propagule pressure, individuals might be incapable of surviving in the new environment, or conditions might not be optimal for reproduction, or a population just might not be able to maintain stable numbers (Zenni and Nuñez 2013). Even well-established populations can suffer major crashes (Simberloff and Gibbons 2004); any population, even if locally adapted, is at risk of extinction if it is unable to adapt to changing conditions (Atkins and Travis 2010, Sanford and Kelly 2011), as exemplified by the *C. fluminea* population in the St. Lawrence River. For years we observed a successfully reproducing population; however it was sustained to a small area, in a thermal plume. Following the closure of the nuclear power plant which was providing the thermal plume, the environmental conditions necessary for survival of this population changed, and the population suffered regional extinction. However one might expect that poorly adapted populations that become extirpated can be replaced by propagules from populations that have been acclimated to the extreme thermal conditions in the target habitat. This is one explanation of why successful establishment may follow multiple failed introductions by the same species (Zenni and Nuñez 2013). It also serves as a caution for the St.

Lawrence River, which could be re-invaded by *C. fluminea* if it receives individuals from an overwintering source population, especially if they arrive at a time when environmental conditions allow for natural acclimatization before the onset of critically low temperatures. Moreover invasion success of 'subtropical' species such as *C. fluminea* will doubtlessly be enhanced by the inevitable increases in global temperatures due to climate change by producing conditions with shorter and milder winters.

TABLES

Table 1. Size range and average length of clams used in cold tolerance experiments from populations from six locations in eastern North America

Location	Size range (length mm)	Average length (mm)
St. Lawrence River, QC	9.7-37.4	18.8
Lake George, NY	14.1–18.7	16.6
Seneca River, NY	22.4–28.1	25.5
Owasco Lake, NY	10.8–14.1	12.2
Sinking Creek, VA	10.7–16.7	12.7
Upper Clinch River	19.9–25.6	22.9
Lake Cheston, TN	18.4–28.9	23.8

Table 2. Experiment parameters during exposure and recovery for all cold tolerance experiments

Experiment parameter	Experiment	Recovery
Duration	8 weeks	8 weeks
Temperature	1°C	18°C
Feeding	no	Tri-weekly
Water change	weekly	Tri-weekly
Mortality check	Bi-weekly	Tri-weekly

Table 3. Average survivorship values at 4 sites in St. Lawrence River at the Gentilly-2 NPP, and 1 site at Lake George, NY. Survivorship values are shown immediately after 8 week exposure at 1°C, and after 16 weeks, which includes exposure at 1°C for 8 weeks and recovery at 18°C for 8 weeks. Values shown with 1 standard error.

Location	Survivorship after 8 weeks (%)	Survivorship after 16 weeks (%)
St. Lawrence R. 1	75 ± 5.0	25± 5.0
St. Lawrence R. 2	85 ± 5.0	35± 5.0
St. Lawrence R. 3	80± 20.0	25± 15.0
St. Lawrence R. 4	90± 0.0	30 ± 0.0
Lake George	100± 0.0	100 ± 0.0

Table 4. Tukey's *post-hoc* for GLMM showing significant differences in survivorship of full model with 10°C – acclimated and 18°C – acclimated clams from 4 locations: 2 northern populations Lake George, NY, Owasco Lake, NY, and 2 southern populations: Upper Clinch River, VA and Sinking Creek, VA. Bolded p-values indicate significant differences (p<0.05).

Location	z-value	p- value
OW-LG	-2.994	**0.01166**
SC-LG	-3.569	**0.00145**
UCR-LG	2.125	0.11593
SC-OL	-1.365	0.45187
UCR-OL	2.835	**0.01850**
UCR-SC	3.356	**0.00346**

Table 5. Average survivorship values of 10°C – acclimated clams from 6 locations: 3 northern populations Lake George, NY, Owasco Lake, NY and Seneca River, NY, and 3 southern populations: Upper Clinch River, VA, Sinking Creek, VA and Lake Cheston, TN. Survivorship values are averages between 2 repetition tanks and showing final survivorship after 8 week exposure at 1°C, and 8 week recovery at 18°C. Values shown with 1 standard error.

Location	Average survivorship (%)
Lake George, NY	60 ± 10.0
Owasco Lake, NY	25 ± 25.0
Seneca River, NY	80± 10.0
Average Northern	**55± 8.3**
Upper Clinch River, VA	65± 15.0
Sinking Creek, VA	5 ± 5.0
Lake Cheston, TN	10± 10.0
Average Southern	**26.67± 10.0**

Table 6. Tukey's post-hoc for GLMM showing significant differences in survivorship of 10°C – acclimated clams from 6 locations: 3 northern populations Lake George, NY, Owasco Lake, NY and Seneca River, NY, and 3 southern populations: Upper Clinch River, VA, Sinking Creek, VA and Lake Cheston, TN. Bolded p-values indicate significant differences ($p < 0.05$).

Location	z-value	p- value
LG-SR	-1.216	0.82351
OL-SR	-2.892	**0.04224**
UCR-SR	-0.941	0.93328
LC-SR	-3.543	**0.00499**
SC-SR	-3.529	**0.00531**
OL-LG	-1.92	0.3806
UCR-LG	0.289	0.99972
LC-LG	-2.739	**0.06497**
SC-LG	-2.844	**0.04844**
UCR-OL	2.161	0.24835
LC-OL	-1.112	0.87248
SC-OL	-1.524	0.64006
LC-UCR	-2.938	**0.03716**
SC-UCR	-3.009	**0.02999**
SC-LC	-0.565	0.99295

Table 7. Tukey's *post-hoc* for Cox-analysis showing significant differences in survivorship of 10°C – acclimated clams from 6 locations: 3 northern populations Lake George, NY, Owasco Lake, NY and Seneca River, NY, and 3 southern populations: Upper Clinch River, VA, Sinking Creek, VA and Lake Cheston, TN. Bolded p-values indicate significant differences (p<0.05).

Location	z-value	p- value
OL-LG	2.755	0.06137
SR-LG	-1.194	0.83338
SC-LG	4.014	**<0.001**
UCR-LG	-0.375	0.99897
LC-LG	2.818	**0.05184**
SR-OL	-3.436	**0.00737**
SC-OL	1.487	0.66167
UCR-OL	-3.053	**0.02588**
LC-OL	-0.031	1.00000
SC-SR	4.397	**<0.001**
UCR-SR	0.856	0.95435
LC-SR	3.482	**0.00620**
UCR-SC	-4.246	**<0.001**
LC-SC	-1.566	0.60950
LC-UCR	3.115	**0.02137**

Table 8. Average survivorship values of 18°C – acclimated clams from 4 locations: 2 northern populations Lake George, NY and Owasco Lake, NY, and 2 southern populations: Upper Clinch River, VA and Sinking Creek, VA. Survivorship values are averages between 2 repetition tanks and showing final survivorship after 8 week exposure at 1°C, and 8 week recovery at 18°C. Values shown with 1 standard error.

Location	Average survivorship (%)
Lake George, NY	5 ± 5.0
Owasco Lake, NY	5 ± 5.0
Average Northern	**5± 5.0**
Upper Clinch River, VA	35± 5.0
Sinking Creek, VA	0± 0.0
Average Southern	**17.5± 2.5**

FIGURES
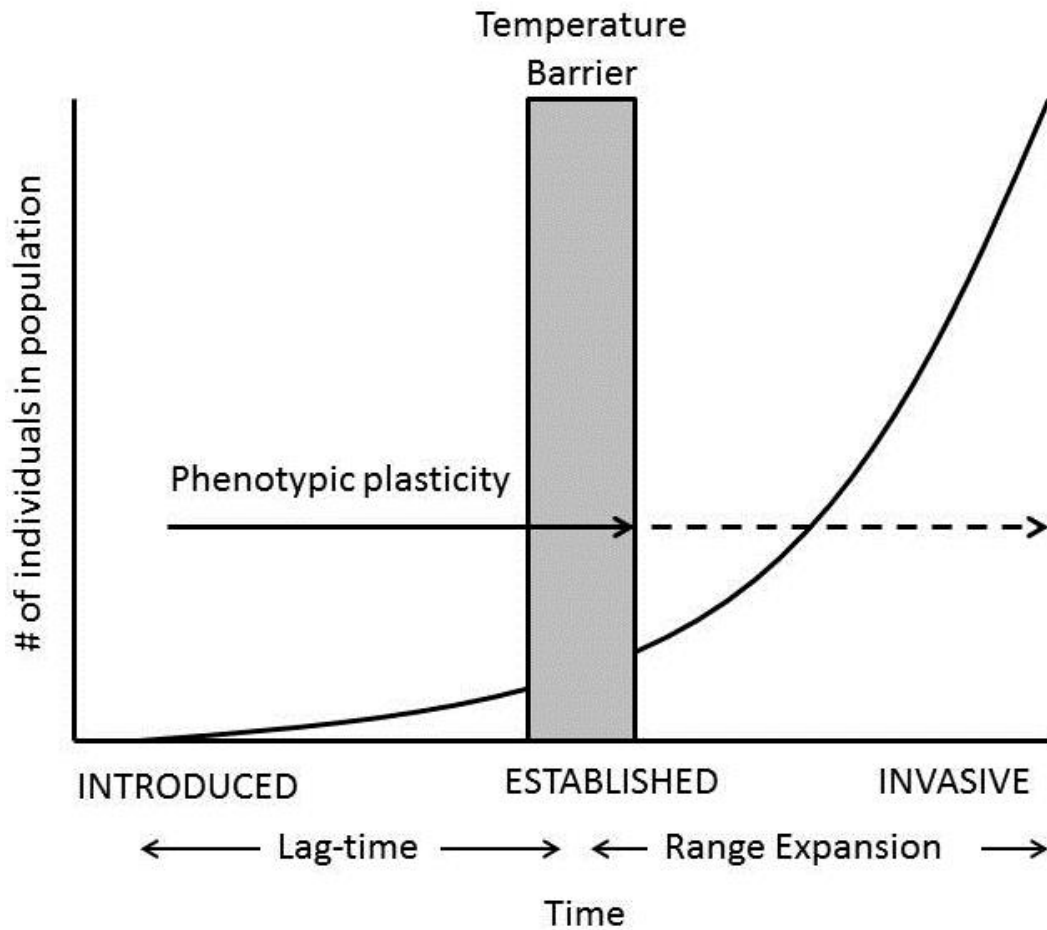
Figure 1. Invasion process depicting phenotypic plasticity in response to a temperature barrier. Initial introduction is followed by a lag phase in early stages of establishment until plasticity elicits changes in the population to overcome the temperature barrier. If plasticity allows the population to persist, the number of individuals can increase exponentially, increasing its range expansion and become invasive. Figure adapted from (Sexton et al. 2002).

Figure 2. Location of sample sites for first pilot experiment at the discharge plume of Gentilly-2 Nuclear Power Plant (G2NPP), Béconcour, QC. Sites established by K. Hamelin (2012).

Figure 3. Locations of sample sites for experiments. Series I experiment G2NPP location in black; northern sites indicated in blue: Lake George, NY, Owasco Lake, NY, Seneca River, NY; southern sites indicated in red: Sinking Creek, VA, Upper Clinch River, VA, Lake Cheston, TN.
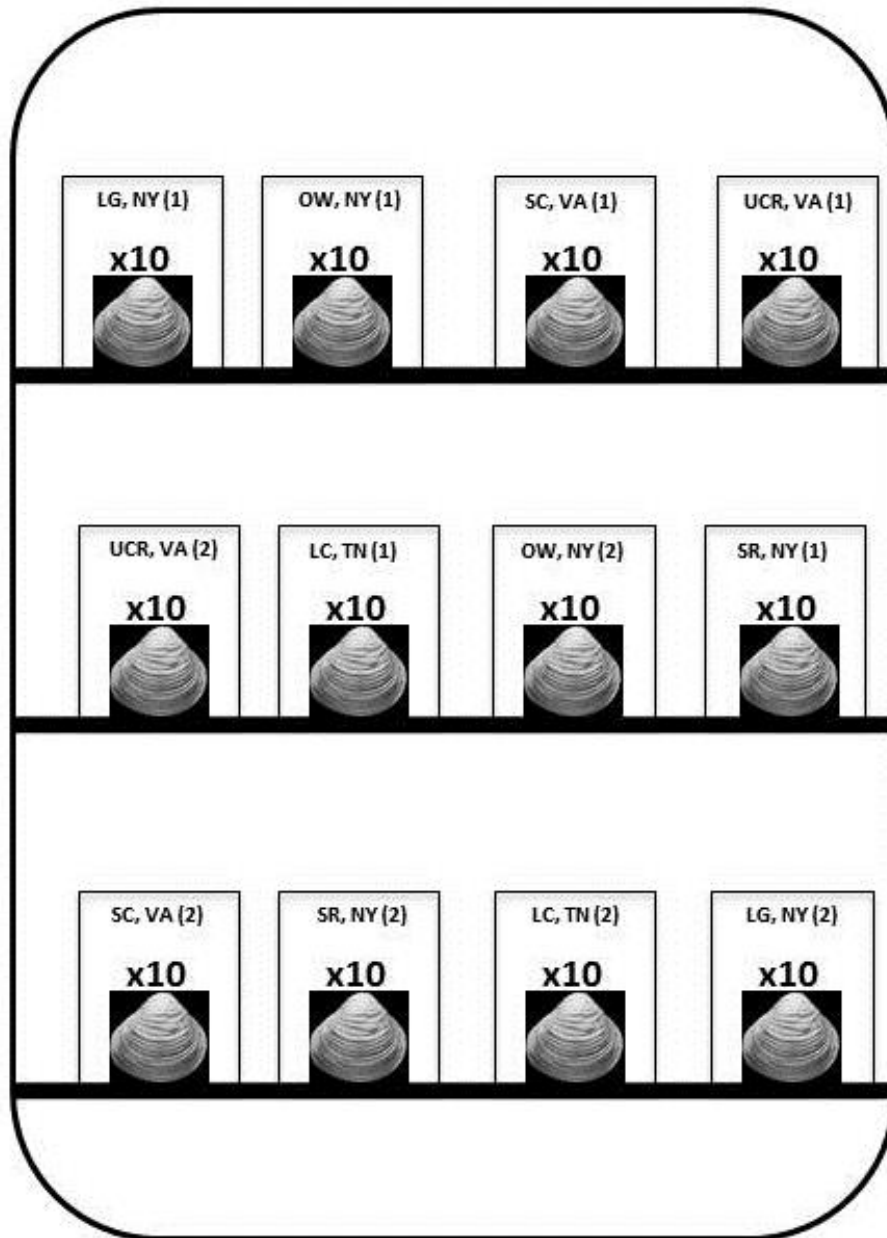
Figure 4. Experimental set up in temperature chamber of two replicate tanks for each of the six experimental locations (LG=Lake George; OW=Owasco Lake; SC=Sinking Creek; UCR=Upper Clinch River; LC=Lake Cheston; SR=Seneca River; see locations in Fig. 3). Numbers in parentheses are the replicate number. Ten clams were used in each tank, and the tank position was randomized within the temperature chamber. Temperature inside the chamber was varied depending on stage of experiment/ recovery.
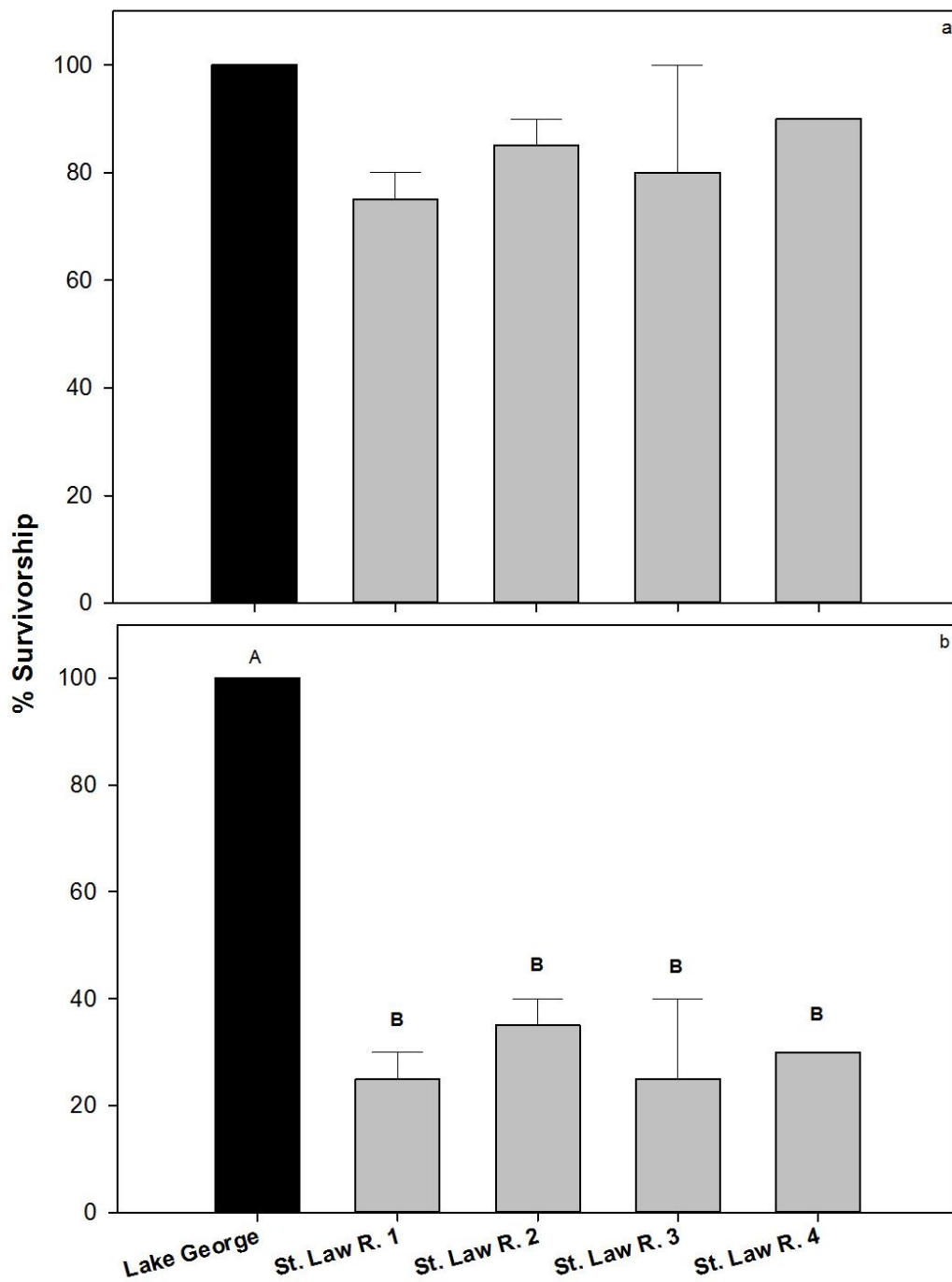
Figure 5. Variation in survivorship (%) of clams after a) 8 weeks' exposure at 1°C and b) an additional 8 weeks' recovery at 18°C at four sites along the St. Lawrence River (1-4) and one site from Lake George. Error bars are 1 standard error. Different letters indicate p<0.05.
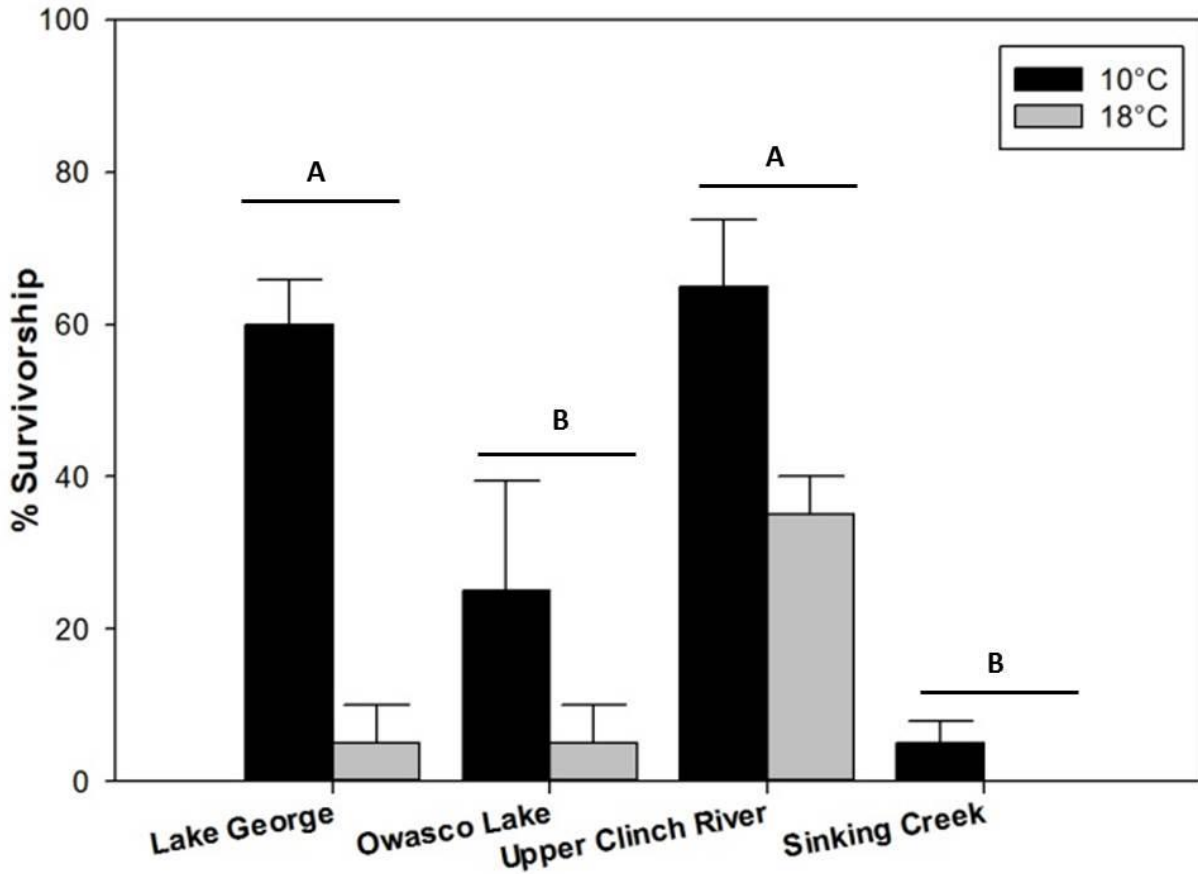
Figure 6. Full model comparison of variation in survivorship (%) of clams post exposure and recovery showing average survivorship at both experimental acclimation temperatures. Sample sites are organized from left to right to reflect the distribution of populations from north to south. Black bar is 10°C acclimation, grey bar is 18°C acclimation experiment (n=40 for each acclimation treatment).
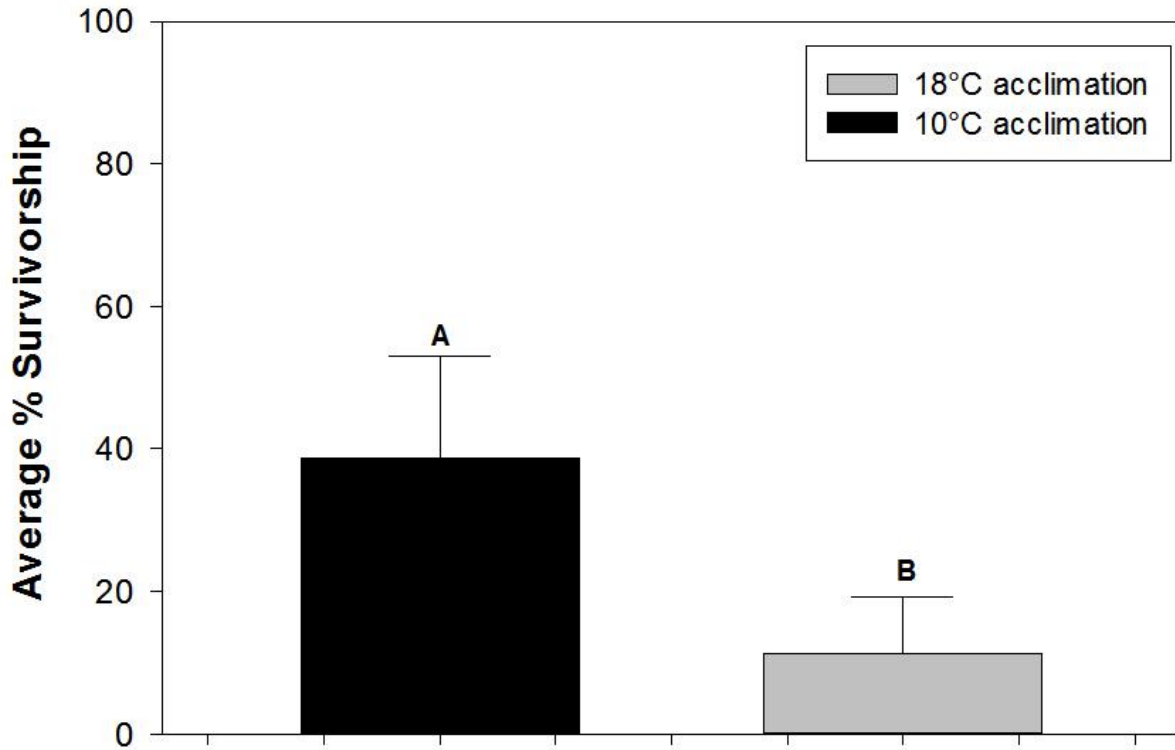
Figure 7. Variation in survivorship (%) of clams post exposure and recovery showing average survivorship at both experimental acclimation temperatures: black bar is 10°C acclimation, grey bar is 18°C acclimation experiment (n=40) (p<0.005). Error bars are 1 standard error. Different letters indicate p<0.05.
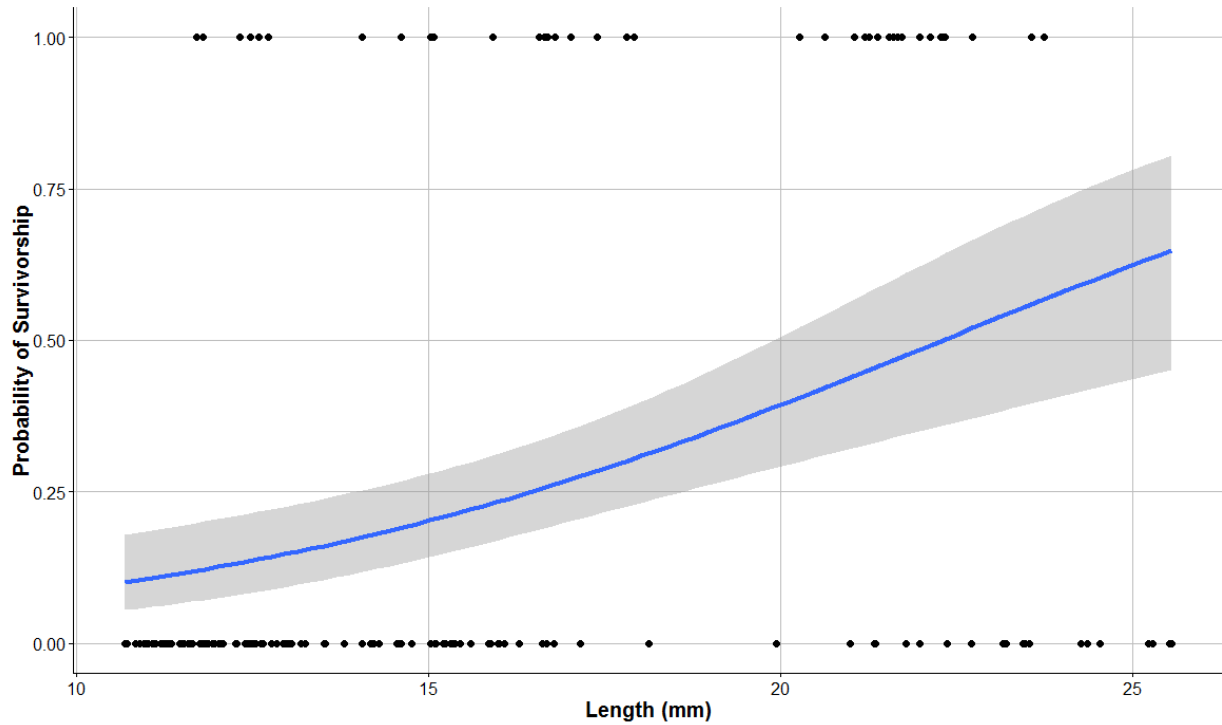
Figure 8. Probability of survivorship from full model including both 10°C acclimation and 18°C acclimation experiment based on size of clam measured as the maximum length (mm). Line fitted by a binary logistic regression (p=0.02). Shaded grey area represents the 95% confidence intervals.
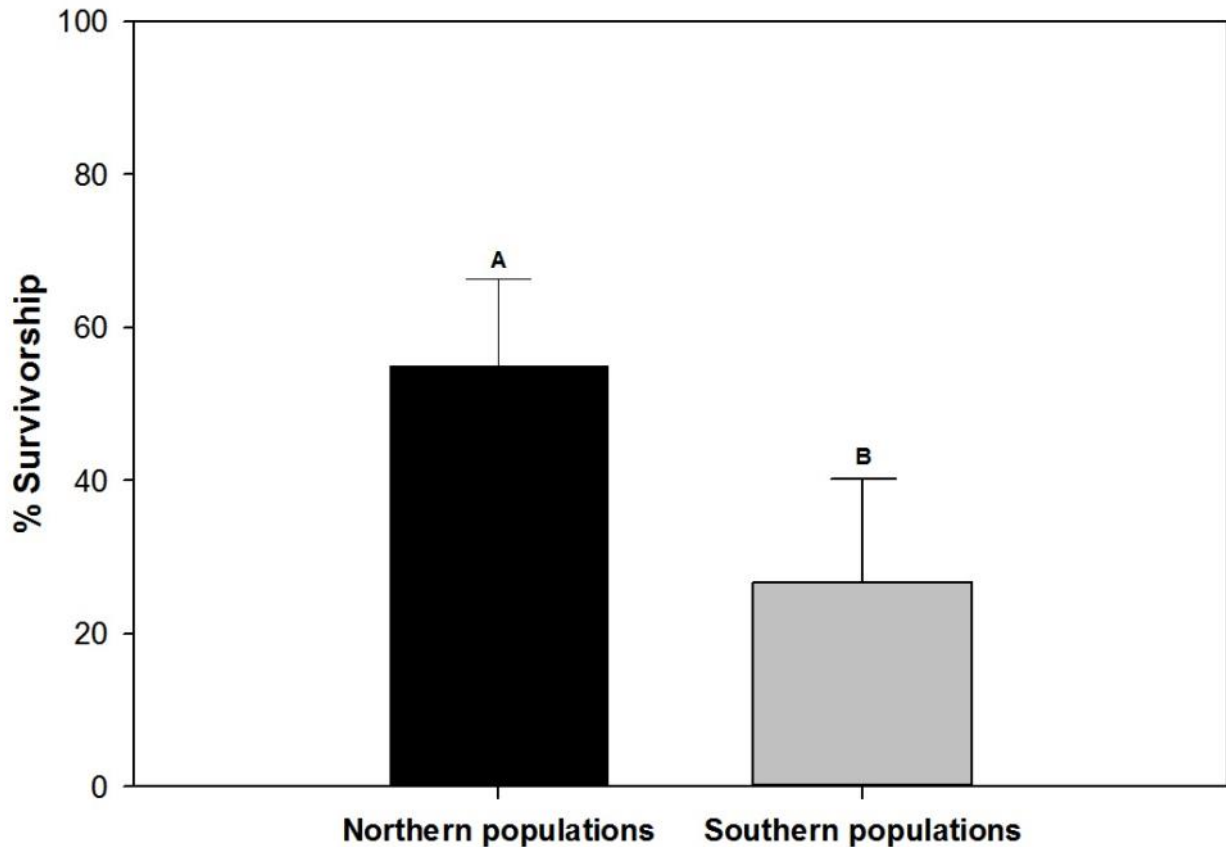
Figure 9. Variation in survivorship (%) of clams post exposure and recovery from 10°C acclimation experiment. Northern populations (indicated in black: Lake George, NY, Owasco Lake, NY) are pooled together and southern populations (indicated in grey: Sinking Creek, VA, Upper Clinch River, VA) are pooled together. Each bar represents the mean survivorship from each geographic bracket (n=40). Error bars are 1 standard error. Different letters indicate p<0.05.
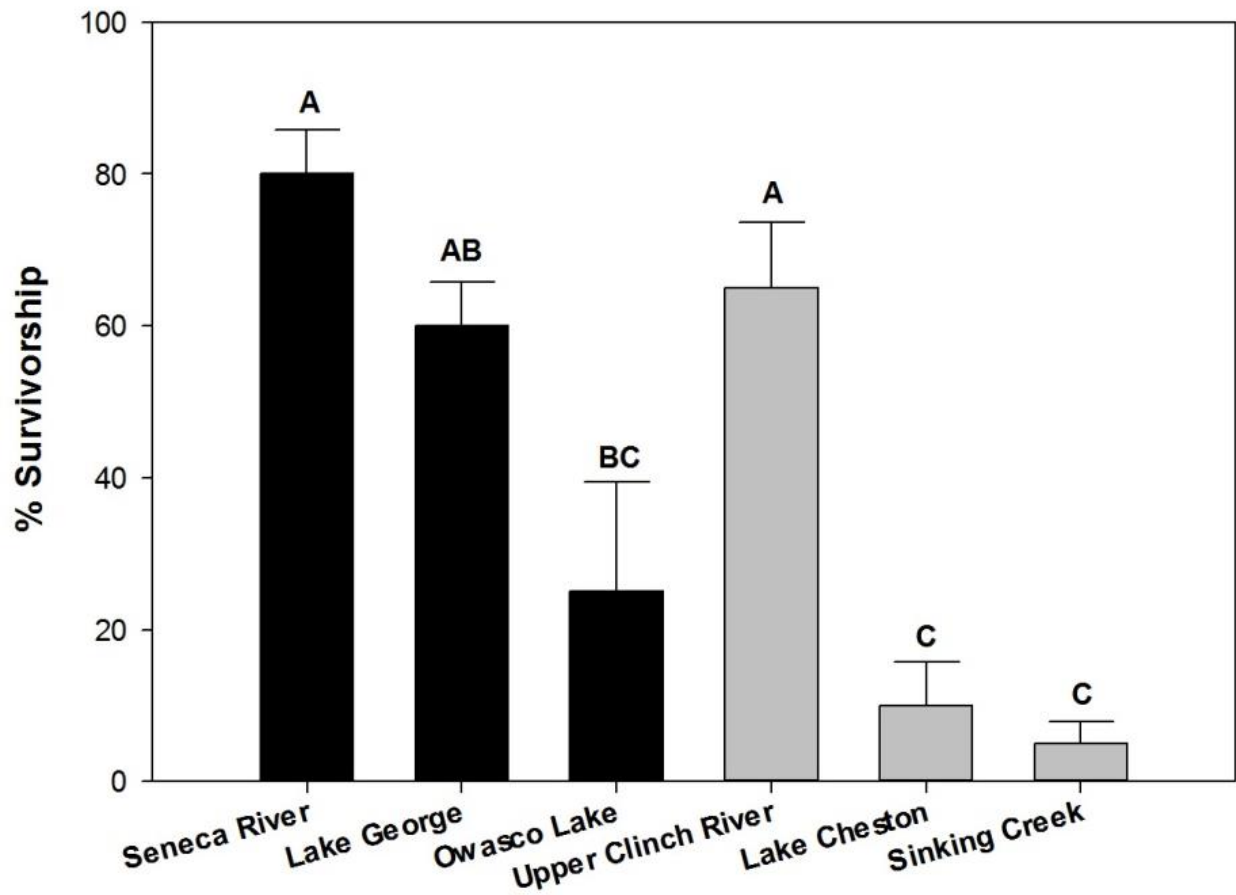
Figure 10. Variation in survivorship (%) of clams post exposure and recovery from 10°C acclimation experiment. Each bar represents the mean survivorship from each site (n=20). Error bars are 1 standard error. Different letters indicate p<0.05.
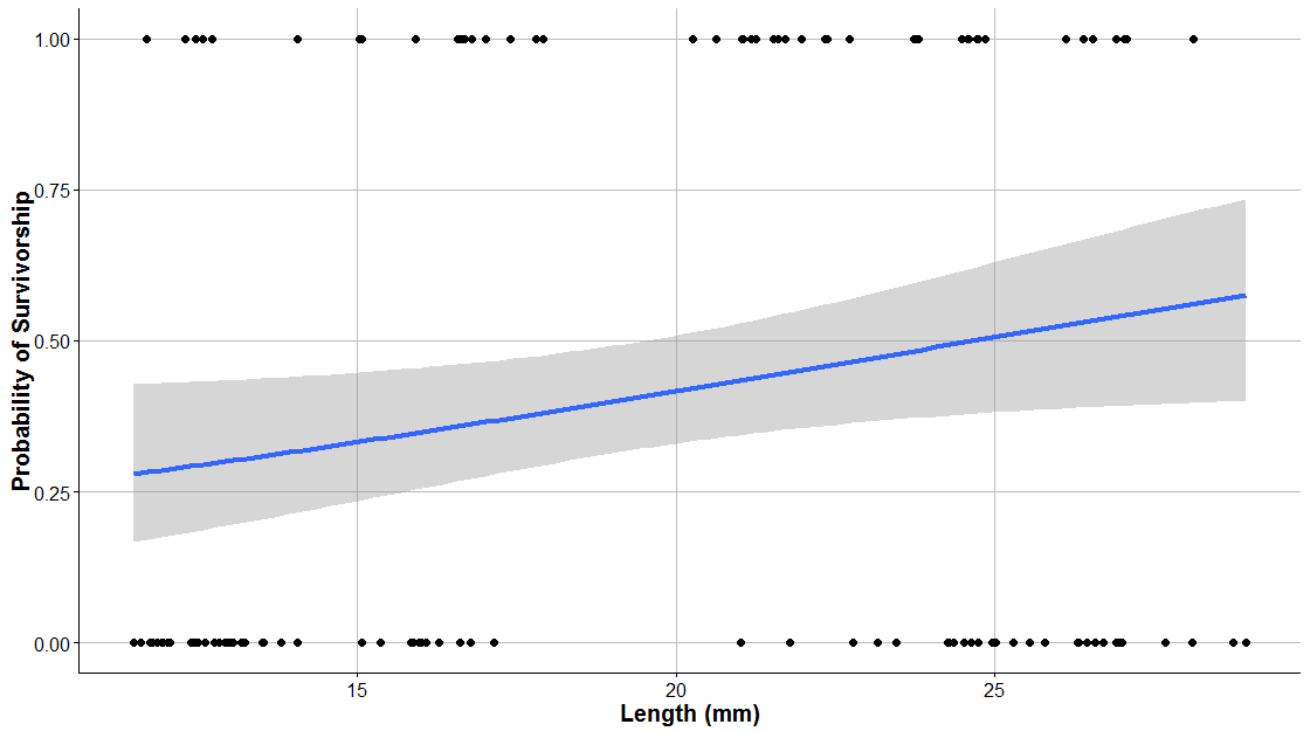
Figure 11. Probability of survivorship from 10°C acclimation experiment based on size of clam measured by as the maximum length (mm). Line fitted by a binary logistic regression (p= 0.01). Shaded grey area represents the 95% confidence intervals.
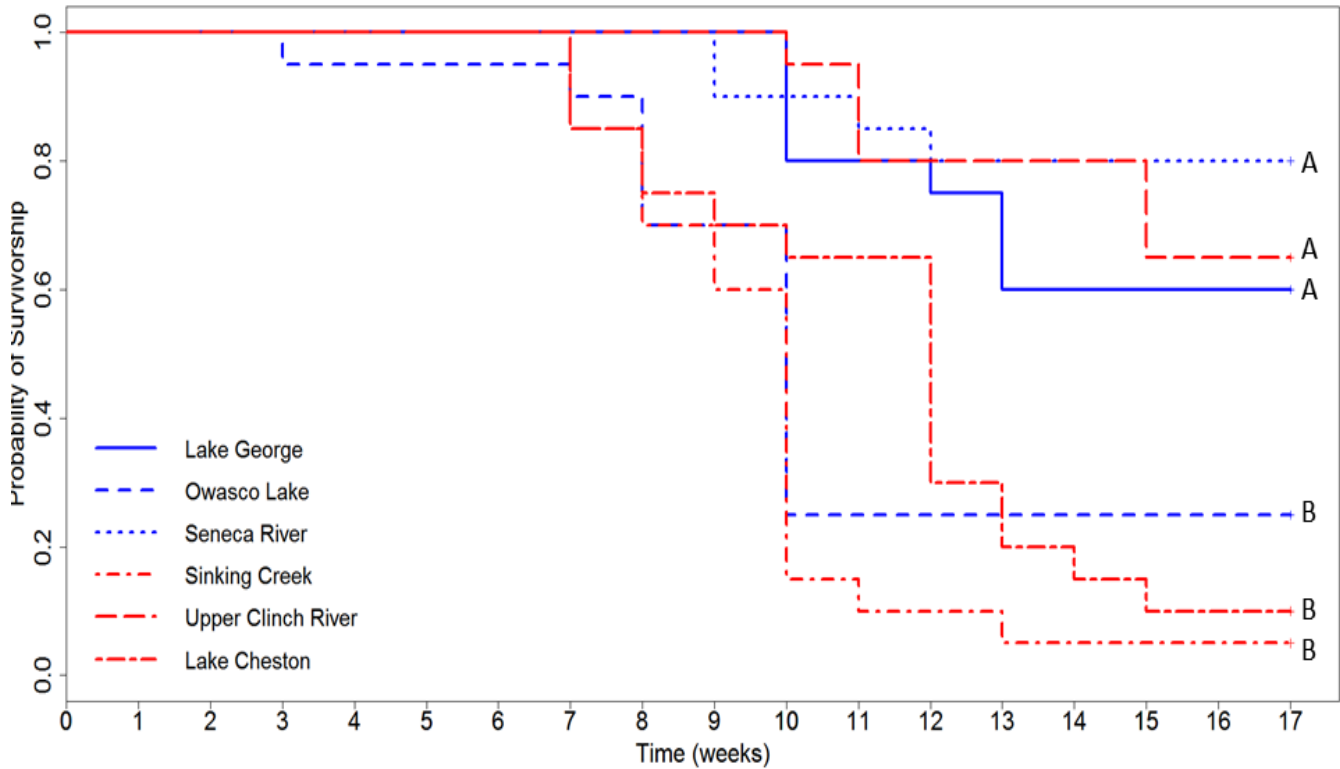
Figure 12. Kaplan–Meier survivorship curve over duration of entire 16 week 10°C acclimation experiment. In the first 8 weeks clams were exposed to experimental temperature of 1°C, in the next 8 weeks clams were held at 18°C for the recovery period. Survivorship curves represent the mean survivorship from each site (n=20). Plus signs indicate censored observations (i.e. clams that did not die during the course of the experiment).
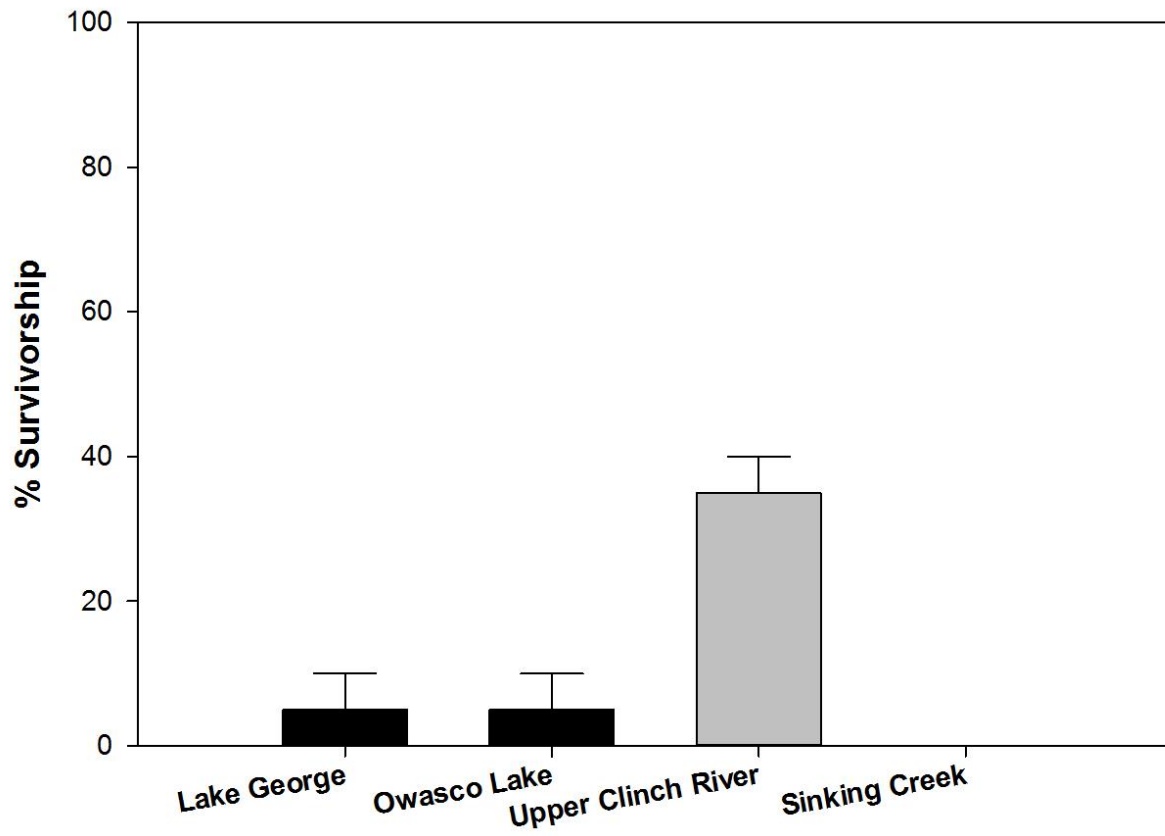
Figure 13. Variation in survivorship (%) of clams post exposure and recovery from 18°C acclimation experiment. Each bar represents the mean survivorship from each site (n=20). Error bars are 1 standard error. The survivorship of the four populations was not significant (p>0.05).

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LINKING STATEMENT

The Asian Clam *Corbicula fluminea* has invaded many freshwater systems in North America and in Europe. It is expanding its range further north than was believed possible, based on its distribution in its native range and a presumed lower lethal limit of 2°C (reported from an experiment on a single southern population (Mattice and Dye 1975)). However in recent years, *C. fluminea* has invaded north temperate regions in North America, challenging the prevailing notion regarding its temperature tolerance.

In Chapter 1, I tested the cold tolerance of *C. fluminea* and found that, contrary to prevailing opinion, the species tolerates near-freezing temperatures. Moreover, survival during long-term exposure to such low temperatures varies among different populations. I concluded that multiple populations should be tested when examining thermal tolerance – and perhaps tolerance to other stressors, particularly when the end goal is to use such data in risk assessment of future spread. My finding begs the question: how common are inter-population comparisons, and to what extent are such data available for risk assessment for invasive species of concern? I examine this question in the next chapter.

CHAPTER 2

Inter-population differences in environmental tolerance within aquatic invasive species: What data are available for risk assessment?

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INTRODUCTION

A major limiting factor for aquatic invasive species is temperature, which governs reproductive success, recruitment, growth, performance and survivorship, and, ultimately their biogeographical distribution (Angilletta Jr et al. 2002). As such, all organisms survive within a range of temperatures defined by the upper and lower lethal temperatures limits. These critical temperatures can vary between conspecific populations subjected to different seasonal temperature acclimation or latitude (Hoffmann and Watson 1993). Although physiological barriers may limit the distribution of a species across different climates (Parmesan and Yohe 2003, Somero 2005), most organism have the ability to alter their tolerances by acclimating or adapting to temperatures outside of their thermal reaction norms (Chown and Terblanche 2006, Angilletta 2009)}. In general, organisms with a broader tolerance, or those capable of adapting quickly, will be able to exploit new opportunities for invasion (Lee 2002). Species that are widely distributed are likely to include isolated populations that are exposed to different temperature regimes(Fangue et al. 2006, Tepolt and Somero 2014), and thus may have developed different tolerances that have implications for their respective abilities to colonize habitats in other climates (Chown et al. 2007, Ghalambor et al. 2007).

Owing to their potentially widespread distribution and ability to thrive in different environments, invasive species are useful model organisms to study variation in thermal limits among conspecific populations. Their success across a range of temperatures is often attributed to their ability to acclimate to new conditions (Yeh and Price 2004, Richards et al. 2006). Variation across different populations can be generated from a population's plasticity based on environmental conditions experienced throughout its history. This capacity for plasticity can result in non-heritable changes in its physiology (West-Eberhard 2003). Alternatively intraspecific variation can have a genetic basis and reflect adaptation (Lee 2002, Somero 2010). In any case, intraspecific variation in thermal tolerance could be a major reason why some species successfully invade and survive in different environments (Tepolt and Somero 2014).

In the previous chapter, I showed that there can be substantial variation in environmental tolerance across conspecific populations within the non-native range of a widespread invader, suggesting that invasion risk assessments based on observations from a single population are potentially misleading. It is not clear whether suitable tolerance data exist to support risk assessments even for invasive species of greatest concern. Here, I review published environmental tolerance limits, particularly thermal tolerances, to determine the extent to which comparisons across conspecific populations have been made for aquatic invasive animals.

METHODS

I conducted a literature search of tolerance studies on aquatic animals in the last 40 years, to determine how many studies examined multiple populations experimentally. The following terms were searched from Web of Science: (temperature OR thermal OR dissolved oxygen OR desiccation OR aerial exposure OR salinity OR conductivity OR acidity OR pH OR alkalinity OR calcium) AND (lower limit OR upper limit OR thermal limit OR tolerance OR survivorship OR critical limit OR LD50 OR LT50 OR LOE) AND (freshwater OR marine OR intertidal OR subtidal OR estuarine OR aquatic OR fish OR mollus* OR crustacean OR invertebrate) AND (invas* OR nonnative OR alien OR nonindigenous OR exotic). The resultant 498 studies were narrowed down to 78 that experimentally derived a tolerance, and stated how many populations were used and where they were located. Studies that compared closely related species were not included in my analysis.

A second dataset was created to examine the thermal tolerance limits of 20 invasive aquatic animals. The Invasive Species Specialist Group (ISSG) – a global network of scientific and policy experts on invasive species – produced a list of "100 of the World's Worst Invasive Alien Species", those considered to be among the most problematic in terms of containment and impact. Among the 100, are 17 aquatic animals to which I added 3 more that are also considered to be highly invasive on a global scale. Searches through the Web of Science (1900-2014, accessed on August 11 – August 22 2014) were conducted by combining each of the 20 species with the following search

terms: (temperature OR thermal OR temperature range OR thermal range OR range) AND (lower limit OR upper limit OR thermal limit OR tolerance OR survivorship OR critical limit OR LD50 OR LT50 OR LOE) AND (Organism's scientific name). I considered only original sources for derived limits and I excluded any papers that reported a thermal tolerance without location or indication of whether the value was obtained experimentally. Only adult tolerances were recorded, for consistency. All reported thermal limits were recorded, regardless if two studies concluded the same value. From each of these studies the following information was obtained: native or invasive range, experimental laboratory study or observational study (often derived by observation of winter or summer die offs or maximum and minimum temperatures in a particular waterbody that a species occupies). It was also noted whether organisms were acclimated prior to exposure to thermal stress. I collected only data on lethal limits i.e. death; sublethal indicators such as cessation of growth or feeding were not appropriate limits for this analysis. Duration for exposure to lethal temperature varied with each study.

RESULTS

Remarkably, only 5.5% of published experimental studies on environmental tolerance compared multiple conspecific populations (3% of studies tested three or more conspecific populations; Table 1).

Of all the thermal tolerance studies available on the 20 high profile invaders, 3.4% compared multiple populations (Table 2). Thermal tolerances of the 20 invaders are presented in detail in Appendix Table 1. Even when pooling data from published studies concerning the 20 high-profile invaders, for most of these species thermal tolerance data (experimental or observational) could be found for <8 distinct populations (Fig. 1). Less than half (47%) of these studies reported thermal limits that were experimentally derived (Table 3); of those, 64% included some type of acclimation prior to exposure to experimental temperature. Upper and lower thermal limits are unavailable for seven (of the 17) species listed among the "world's worst" invaders in the ISSG database.

The number of studies per species varied greatly (Fig. 3) with zebra mussel *Dreissena polymorpha* having the highest number, compromising 13% (23/178), followed by rainbow trout *Oncorhynchus mykiss* with 11% (19/178), and brown trout *Salmo trutta* with 8.5% (15/178). Most experimental studies focused on fish, whereas must observational studies focused on molluscs (Table 2; Fig 2). Finally, 72% of studies derived tolerance limits from species in their invasive range.

DISCUSSION

Experimental data on environmental tolerance of aquatic invasive animals is limited and highly skewed. Most studies derived tolerance limits from species in their invasive range, suggesting that the identification of species limits becomes increasingly more important when a species shifts its range. Not surprisingly, more thermal tolerance studies were found for economically important species, and those with a wider distribution or longer invasion history. For example, all of the fishes with the exception of the round goby, are of commercial or recreational value; as such they are often intentionally stocked in lakes and rivers (Heidinger 1999), and their tolerance limits have been studied more thoroughly to determine which habitats are suitable for stocking (Cowx et al. 1998). The most number of studies for a single species was found for the zebra mussel *Dreissena polymorpha*, which has created devastating effects and has spread widely in the Great Lakes following its introduction in the late 1980s (Strayer 2008). This was followed by rainbow trout, *Oncorhynchus mykiss*. In contrast rainbow trout is often purposely stocked for commercial or recreational fishing, thus its thermal limits are of value when performing stock assessments (Molony 2001). Additionally, the list of twenty species chosen in this review is actually not a random sample of invasive species, but rather a list of some of the most high profile invaders around the world. Yet, although such species might be expected to be well studied, their thermal tolerance data available are insufficient for risk assessment.

Different studies on the same species can provide a range or average of tolerance limits for a given species; however, even though the same species is used, the treatment and derivation of the thermal limit value is not standard and these studies

are often not comparative and can therefore be misleading. For example, estimates of thermal tolerance derived from laboratory experiments are often based on relatively short time periods of less than seven days, assuming that effects from short term exposure would be similar in long term exposure. However, some studies have suggested that exposure to temperatures that are sublethal during acute tests might actually become lethal during longer exposure times (Selong et al. 2001), and thus long-term duration of exposure might result in a more realistic measure of thermal tolerance in natural situations. Finally, it can be argued that studies with an acclimation prior to exposure to a certain temperature reveal more accurate results because it prepares the animal to engage physiology mechanisms in response to, or in anticipation of an environmental change (Huey et al. 1999). It has been suggested that acclimation in the direction of exposure can have beneficial effects when an organism is subsequently exposed to an extreme temperature (Leroi et al. 1994, Bennett and Lenski 1997, Colinet et al. 2012).

Implications for prediction and risk assessment

A species biogeographic distribution is set by its specific temperature tolerance limits (Rahel and Olden 2008), through restrictions on physiological performance and fitness (Kingsolver and Huey 1998). These barriers can be diffused through organisms' capacity for plasticity (Chown et al. 2007) and their acclimatization potential (Somero 2010). Through variation in climates, and the plasticity and acclimation potential of organisms, widespread species often display different tolerance limits among isolated populations (Huey and Kingsolver 1989, Elderkin and Klerks 2005, Sorte et al. 2011, Wallace et al. 2014). This provides a challenging problem in applying tolerance limits to risk assessment and for predicting species distributions.

To best predict the risks posed by invasive species it is important to be able to accurately estimate tolerances of species (Tepolt and Somero 2014). Although intraspecific variation is recognized, tolerance is often studied with the implicit assumption that any variation between populations is negligible (Strayer et al. 2006). Ecologically significant variation is muted when tolerances are generalized across

different populations of each species. To simplify risk assessment and management, it might be alluring to produce a single absolute value for each environmental tolerance; however, it is more important to recognize the variation in tolerances that is created when different populations inhabit different environments. These differences offer a better understanding of how intraspecific variation can affect invasion outcomes.

Just over half (53%) of the thermal tolerance studies simply determined thermal limits based on the lowest or highest observed temperature experienced either in their native or invasive range. Observed thermal limits can provide a preliminary understanding of the tolerance capacity of a species. However this approach is limited in that it does not consider the ultimate limits of a species' tolerance, just those observed in a particular location at a particular time. As such, this over simplified approach can lead to an underestimation of their potential distribution.

Traditional ecological approaches estimate potential distributions of invasive populations based on the characteristics of a species' native range by using climate-matching models such as CLIMEX (Williamson 1996, Stohlgren et al. 2005, Hayes and Barry 2008). This type of model works by predicting the range over which a species could be invasive, based on physiological tolerances in its native range. However, it does not take into account changes or variation in tolerances owing to acclimation or adaptation. Moreover, native range limits may be set by various local factors, thus the observed niche of a species could be smaller than the species actual tolerance capabilities (Sexton et al. 2009). Even species with large and discrete native ranges have been found to invade well outside of their predicted range (reviewed by (Sax et al. 2007)).

Determining thermal limits by simple observation is not sufficient to predict the extent of invasive species distributions and the range of habitat conditions that they can invade, or to assess risks associated with anthropogenic changes in water temperature (Eaton and Scheller 1996). Thermal limits should be assessed experimentally and compared across conspecific populations. That said, there is no standardized method to determine the absolute tolerance limits of a species. Variation can exist based on characteristics of the species or population in question, particularly its plasticity,

capacity for acclimation and adaptation. Observed variation may also be a result of the experimental methods: acclimation and exposure time can vary from minutes to months and the endpoint of the experiment can vary from sub lethal stress to death. Moreover, lethal tolerance data alone may not be sufficient for understanding physical environmental constraints on the establishment and persistence of a species. A more accurate analysis may require information on environmental limits and optimal ranges for reproduction and growth as well as inter-population variation in these parameters.

TABLES

Table 1. Summary of literature review of studies (published since 1900) that test upper or lower tolerance of environmental stressors on invasive aquatic animals. The number of studies that compare multiple conspecific populations is reported

Num. of different populations	Num. of studies	Percent of total
1	67	91.7
2	4	5.5
4	1	1.4
7	1	1.4

Table 2. Summary of literature review of studies that test thermal tolerance of 20 selected aquatic invasive species. The number of studies that compare multiple conspecific populations is reported.

Num. of different populations	Num. of studies	Percent of total
1	172	96.6
2	3	1.7
3	3	1.7

Table 3. Summary of the number of studies reporting thermal tolerances for the 20 selected aquatic invasive species of concern. Numbers in brackets are percentages of experimental or observational studies.

Type of organism	Total num. experimental studies	Total num. observation studies
Fish	49 (27.5%)	23 (12.9%)
Mollusc	20 (11.2%)	35 (19.7%)
Crustacean	14 (7.9%)	22 (12.4%)
Echinoderm	0	6 (3.4%)
Ctenophore	1 (0.6%)	8 (4.5%)
Total	84 (47.2%)	94 (52.8%)

FIGURES
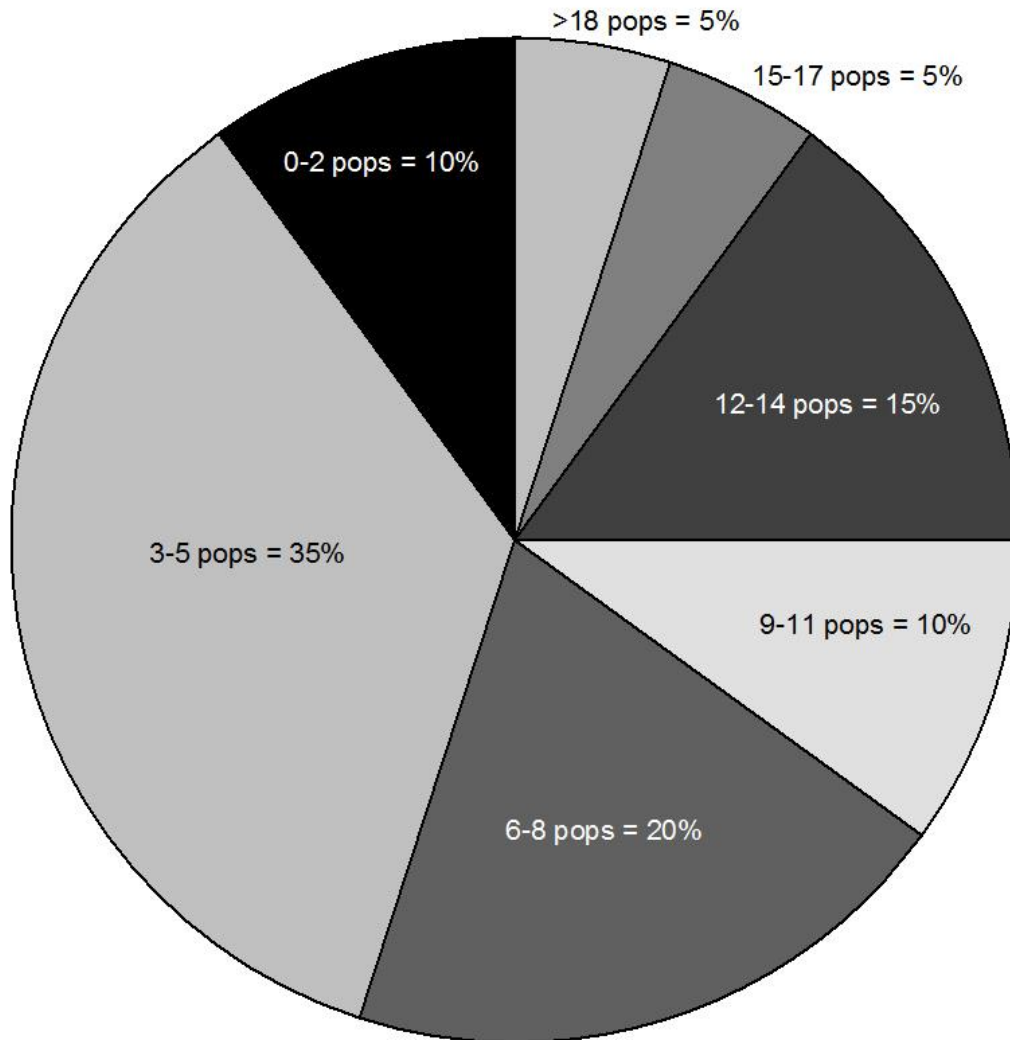
Figure 1. Total amount of populations per species summed across all studies collected (n=178) from species on list of 20 selected aquatic invasive animals for upper or lower thermal tolerance data. Counterclockwise from the top, first segment is representative of 2/20 species that had data available from 0-2 different populations; 2nd =7/20 species had data available from 3-5 different populations, 3rd= 4/20 species had data available from 6-8 different populations, 4th=2/20 species had data available from 9-11 different populations, 5th= 3/20 species had data available from 12-14 different populations, 6th= 1/20 species had data available from 15-17 different populations, and finally 7th=1/20 species had data available from <18 different populations.
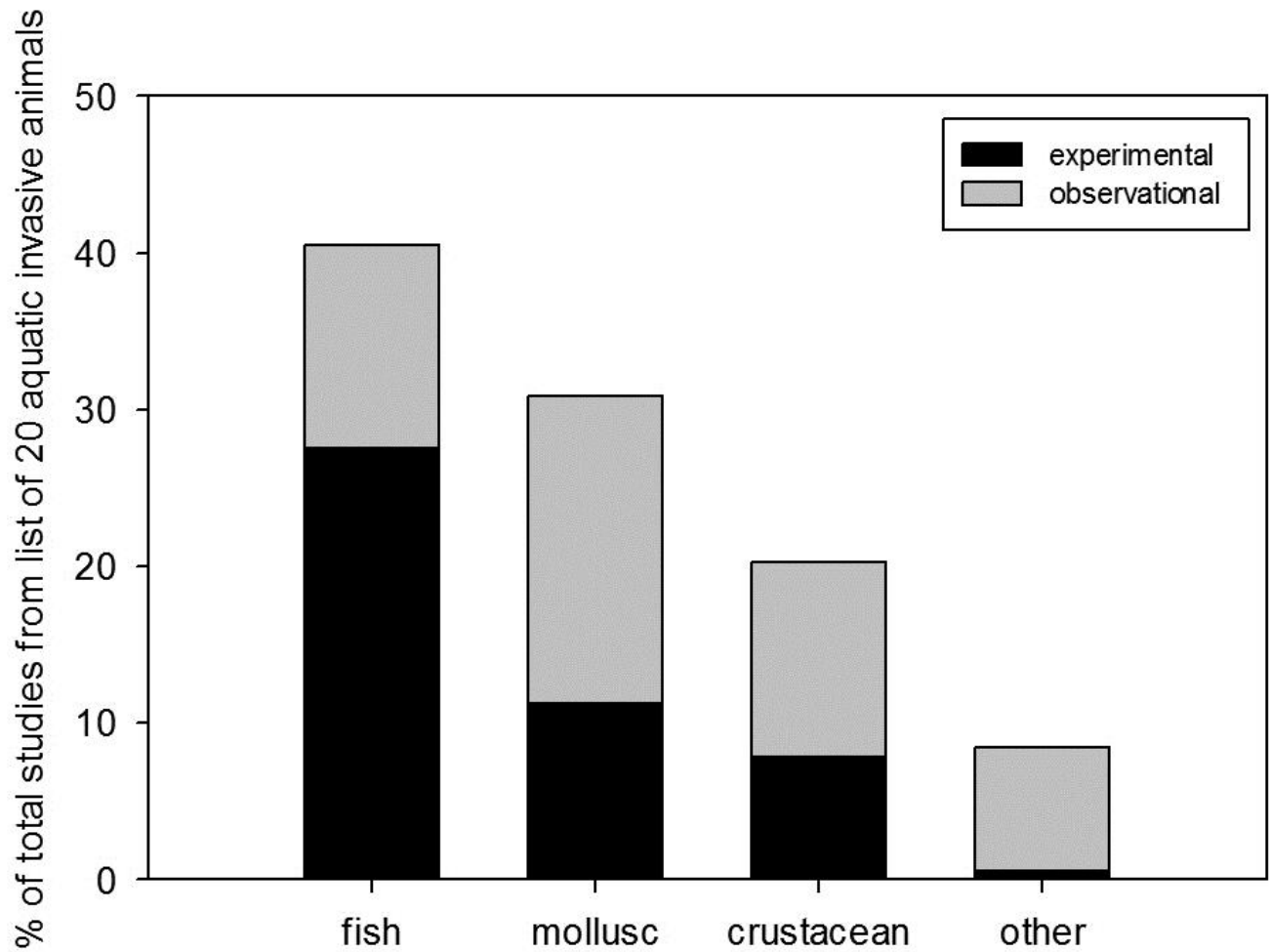
Figure 2. Percent of total studies (n=178) compiled from species on list of 20 selected aquatic invasive animals, separated according to organismal group: fishes (n=9), molluscs (n=5), crustaceas (n=4), and other (1 ctenophore and 1 echinoderm).
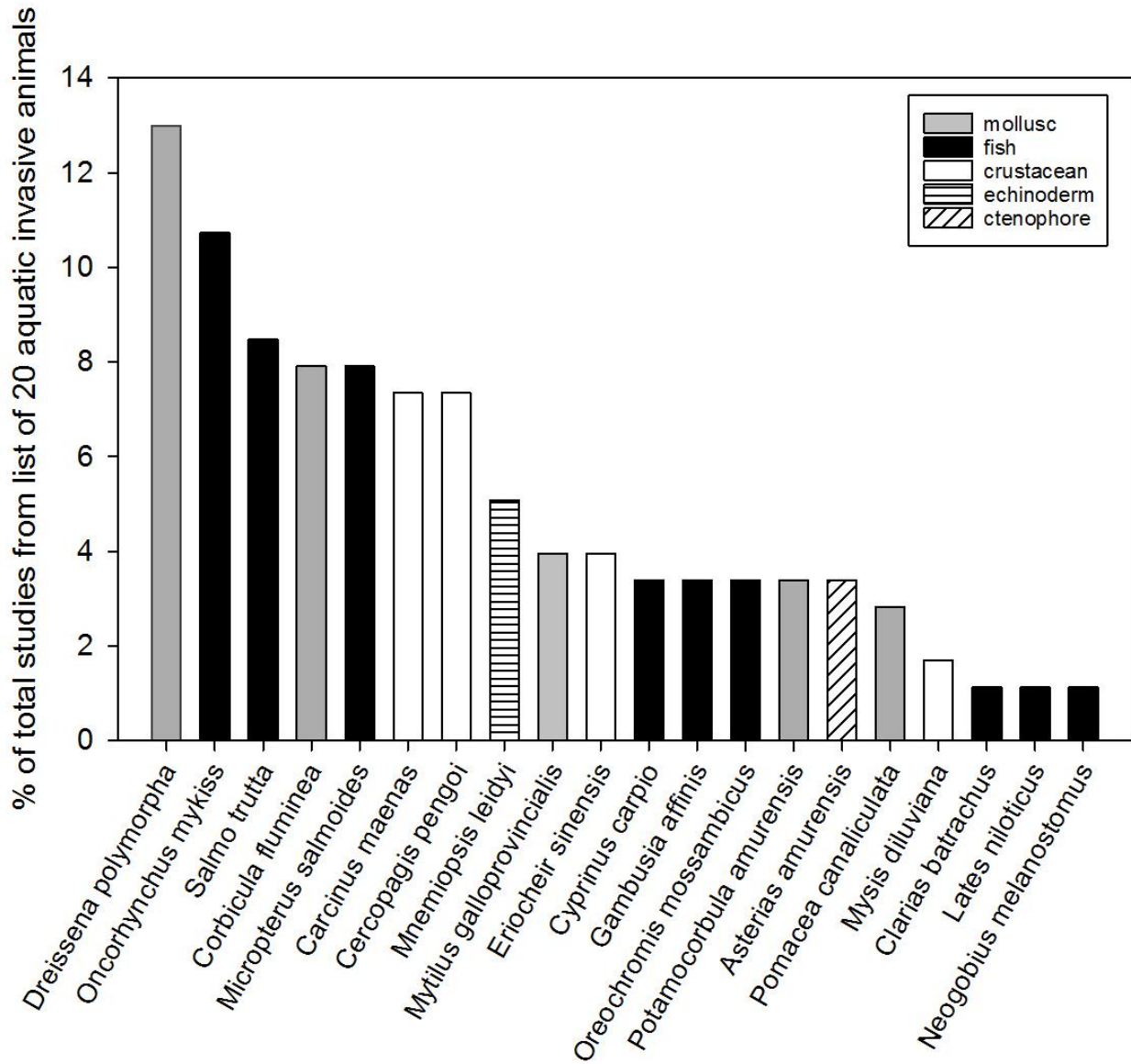
Figure 3. Percent of total studies (n=178) per species from list of 20 selected aquatic invasive animals

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GENERAL CONCLUSIONS

Tolerance to cold temperature is likely to be a major factor limiting the current and future distribution of *Corbicula fluminea*. To date, this species has not been considered a major invasive threat to Canadian waterbodies, owing to its assumed intolerance to temperatures below 2°C. However my experiments demonstrate that, if properly acclimated, *C. fluminea* can survive exposure to near freezing temperatures (~1°C) for at least two months. Individuals from each of the tested populations from six eastern North American locations, can not only survive overwintering, but can later return to normal functioning (exemplified by their conspicuous feeding activity) during the recovery period. These results are consistent with the recent range expansion of *C. fluminea* in eastern North America and the persistence of overwintering populations in northern waterbodies such as Lake George.

To further demonstrate full recovery, subsequent growth and reproduction of clams would need to be confirmed; however, *C. fluminea* has never been carried through a reproductive cycle in an artificial culture (Thorp and Covich 2009). Nevertheless, evaluating survival potential is a critical first step in determining the capacity for the species to spread into atypical or suboptimal habitats. The observed interspecific variation of cold tolerance begs the question of whether such variation exists among other common stressors, such as hypoxia, salt tolerance, heat tolerance, etc. It would be interesting to test the hypothesis that tolerances to different stressors are correlated across populations. Furthermore, it would be interesting to test if thermal tolerance of the same population change over short time scales, such that there could be a temporal variation in thermal tolerance as a result of acclimatization. Another intriguing, but more difficult investigation would be to test the effects of multiple simultaneous stressors.

This study indicates that populations of *C. fluminea* – despite apparently belonging to the same genetic lineage – are not physiologically equal, and that population variation might be crucial in determining patterns of spread. This has two

major implications for invasive species management. First, risk assessments based on data derived from a single population can be erroneous. My literature review suggests that the potential for such errors is high, even for invasive species of global concern. Second, where suitable vectors exist, there is a non-negligible risk of invasion by introductions from cold-adapted populations in habitats previously thought to be inhospitable to *C. fluminea* and where previous introductions have failed. Repeated introductions during peak windows of opportunity, when environmental conditions are tolerable in these habitats could result in invasion. As shown in my experiments, even individuals from some southern populations have the potential to invade northern waterbodies.

My results should motivate researchers to survey other northeastern North American lakes for *C. fluminea* populations, as I expect that several may remain undetected – in part because their presence in such habitats is unexpected. As it has been observed over the last few years in Lake George, NY, and consistent with my experiments, even harsh winters with substantial die-offs could be survived by sufficient numbers of individuals that is large enough to sustain an initial population which then has the potential to continue to grow each year. The processes by which such populations could adapt to enhance their cold tolerance certainly merit more attention. Finally, climate change can provide conditions with more mild winters, aiding in the acclimatization of invading populations, and ultimately enhancing survivorship.

APPENDIX

Table 1. Summary of all studies reporting a minimal lethal temperature and maximum lethal temperature for aquatic invasive animals on the list of 20 worst invaders (as specified by the Invasive Species Specialist Group (ISSG) chosen by criteria outlined in methods

	Organism	Common Name	Native Range	Invasive Range	Minimum lethal temperature (°C)	Maximum lethal temperature (°C)
1	*Asterias amurensis*	Japan-eese Sea star	Japan, North China, Korea, Russia, and far North Pacific waters	South-eastern Australia, Tasmania and Victoria	ISSG: 0°C (NIMPIS, 2002) Observed native 1) 0°C ambient Japan (Ino et al. 1955) Observed invasive 1) In Tasmania no death after 10 days at 1 °C (Marsh 1993). 2) In the Mediterranean, found in 11 °C (Giangrande and Petraroli 1994)	ISSG: 25°C (NIMPIS, 2002) Observed native 1) Sampled A. amurensis from waters of 25 °C in Tokyo Bay (Ino et al. 1955) 2) 25 °C (Park and Kim 1985) Observed invasive 1) in Tazmania 23 °C (Davenport and McLoughlin 1993)
2	*Carcinus maenas*	Green crab	Belgium, Denmark, France, Germany, Iceland, Ireland, Mauritania, Morocco, Netherlands, Norway, Portugal, Spain, Sweden, United Kingdom, Western Sahara	Argentina, Australia, Canada, Japan, South Africa, United States	ISSG: 0°C (Bravo, Cameron & Metaxas, 2007) Observed native 1) -1.0 °C Based on temperature tolerance in native range (Cohen et al. 1995) 2) Balgzand westernmost part of the Wadden Sea (53 ° N, 5 °E) 1985= -2.1°C; 1986=-2.0°C; 1987=-1.9°C (Beukema 1991) 3) Kvarnbukten Bay, Gullmar Fjord, Sweden 1975= -0.3°C (Eriksson and Edlund 1977) 4) LynaesIsefjord Denmark 1979= 0°C indirect observation(Lützen 1984)	ISSG: 33°C (Bravo, Cameron & Metaxas, 2007) Observed invasive 1) Can tolerate max temperatures of 33°C, Washington (Washington Department of Fish and Wildlife 2001) 2) 26°C, Southern Gulf of St. Lawrence, Canada (Audet et al. 2008) 3) San Francisco Bay, CA 26°C (Cohen et al. 1995) Laboratory native with acclimation 1) Isle of Cumbrae, Scotland = up to 24°C with acclimation (Wallace 1973) 2) North Sea, near Hartlepool, Co. Durham 1996; Acclimated at 8= summer

#	Species	Common name	Native range	Invasive range	Cold tolerance	Heat tolerance
					Observed invasive 1) -2°C in the southern Gulf of St. Lawrence, Canada for a very short time towards the end of the winter months (Audet et al. 2008) 2) Boothbay Harbor Maine Coast 1956= 3.4°C mass mortality in winter (Welch 1969) Laboratory invasive with acclimation 1) 5°C when acclimated to 6°C for duration of winter in Sitka, BC to predict northern range expansion (Kelley et al. 2013)	31.8±0.27°C autumn 33.5±0.5°C winter31.3±0.24°C; Acclimated at 15= autumn 34.2±0.12 °C winter32.5±0.2 °C; Acclimated at 22= summer 35.4±0.26 °C autumn 35.8±0.11 °C (Cuculescu et al. 1998) Laboratory invasive with acclimation 1) **2 populations** Seadrift Lagoon, California, USA (37°54′27.82″N, 122°40′19.56″W) acclimated to 6°C = mean CTmax of 34.7 °C; acclimated to 23°C =mean CTmax of 36.2 °C and Pipestem Inlet, Vancouver Island, British Columbia, Canada (49°02.3′N, 125°12.2′W) acclimated to 6°C = mean CTmax of 31.7 °C; acclimated to 23°C =mean CTmax of 35.5 °C (Kelley et al. 2011)
3	*Cercopagis pengoi*	Fishhook waterflea	Southern Europe - Ponto-Aralo-Caspian basin, i.e. Caspian, Black and Azov seas	Eastern Europe, Baltic Sea, Laurentian Great Lakes, Finger Lakes (North America)	ISSG: 3°C (c.f. Gorokhova et al. 2000) Observed native 1) 13°C in Caspian Sea Observed invasive 1) 8°C in Baltic Sea (Gorokhova et al. 2000) 2) 8°C (MacIsaac et al. 1999) 3) 12°C in Baltic Sea (Gorokhova et al. 2000) Laboratory native without acclimation 1) 3°C in Caspian Sea (Gorokhova et al. 2000) 2) 3°C in Black and Azov Sea	ISSG: 38°C (c.f. Gorokhova et al. 2000) Observed invasive 1) 20°C in Baltic Sea (Gorokhova et al. 2000) 2) 26°C in Baltic Sea (Gorokhova et al. 2000) 3) 30°C (MacIsaac et al. 1999) Laboratory native without acclimation 1) 32°C in Caspian Sea (Gorokhova et al. 2000) 2) 36°C in Aral Sea (Gorokhova et al. 2000) 3) 38°C in Black and Azov Sea (Gorokhova et al. 2000)

				(Gorokhova et al. 2000) 3) 4°C in Aral Sea (Gorokhova et al. 2000)	
4	*Clarias batrachus*	Thailand catfish	Southeastern Asia	Indonesia (Sulawesi), USA, Hong Kong, Taiwan, China, UK, Papua New Guinea, Guam, Taiwan, Thailand	ISSG: 9.8°C Observed native 1) 9.4-12.8°C depending on thermal history (Pardue 1070) Laboratory invasive without acclimation 1) Florida (invasive) 9.8+/- 0.6°C (Shafland and Pestrak 1982)	No tolerance reported in ISSG
5	*Corbicula fluminea*	Asian clam	southeastern China, Korea, southeastern Russia, and the Ussuri Basin	1930s USA, Europe, South America	USSG: 2°C Observed invasive 1) low winter water temperature in around St. Clair PP in St. Clair River created major mortality event (cited (Cherry et al. 1980) (French III and Schloesser 1991) 2) lowest limit is <2°C (Britton and Morton 1982) 3) New River, VA contrasting thermal plume with normal water when temp dropped <2°C, clams mass mortality (Graney et al. 1980) 4) Mesocosm study with clams from Lake Constance (central Europe) clams died during winter	USSG: 30°C Observed invasive 1) highest temperature observed in the field (Texas) is 36°C (McMahon and Williams 1986) Laboratory invasive with acclimation 1) Tennessee River, Alabama upper lethal temperature 38°C when acclimated to 22.5°C (Habel 1970). Laboratory invasive without acclimation 1) population from Clinch River, Tennessee 50% mortality at 34°C (30°C Acclimation for 1 wk) (Mattice and Dye 1975) 2) reviewed in (Karatayev et al. 2005) 36-

#	Species	Common name	Native range	Habitat	Lower temperature	Upper temperature
					exposure to temps <2°C (Werner and Rothhaupt 2008a) 5) Tennessee River, Alabama observed to survive temperatures as low as 2°C (Habel 1970). 6) lower lethal is 2°C based on observation of extensive winter die offs on northern edge of NA range (McMahon 1983) 7) Clinton River in Michigan survived overwinter (Janech and Hunter 1995) 8) no successful dispersal below 2°C (Scholl 2000 in (Galil et al. 2007)) Laboratory invasive with acclimation 1) population from Clinch River, Tennessee mortality at 2°C (20°C Acclimation for 1 week) (Mattice and Dye 1975) Laboratory invasive without acclimation 1) population from River Rhine, Germany in 0°C 17.5% survivorship after exposure for 9 weeks (Müller and Baur 2011)	37°C from (Dreier and Tranquilli 1981, Britton and Morton 1982)
6	*Cyprinus carpio*	Common carp	Europe in rivers around the Black Sea and the Aegean basin, especially	tropical, subtropical and temperate freshwaters throughout the world	ISSG: 3°C Observed invasive 1) 12.3°C in Belgium (Meuwis and Heuts 1957) Laboratory invasive with acclimation	ISSG: 32°C Observed invasive 1) 38°C in Belgium (Meuwis and Heuts 1957) 2) 40.6°C Druzba Lake Poland (Horoszewicz 1973)

	the Danube			1) Khopoli fish seed farm Mumbai acclimated for 30days LTmin: 25°C accli=8.2°C+/- 0.05; 30°C accli= 8.4°C +/- 0.04; 35°C accli= 10.1°C +/- 0.05 (Chatterjee et al. 2004)	<u>Laboratory invasive with acclimation</u> 1) Khopoli fish seed farm Mumbai acclimated for 30days LTmax: 25°C accli= 39.8°C +/- 0.06; 30°Caccli= 40.9°C +/- 0.07; 35°C accli= 42.9°C +/- 0.04 (Chatterjee et al. 2004) 2) BC Twin Lakes acclimated at 26°C 24h survival: all at 34.1°C; 50% at 35.7°C; 0% 36.9°C (Black 1953)
7	*Dreissena polymorpha*	Zebra mussel	Black, Caspian, Aral and Azov seas	1920s north-west Russia, central and western Europe, Scandina via, Britain, Ireland and North America; South America, South Africa, Australia and New Zealand is possible	<u>ISSG:</u>-20°C (DAISIE 2006) <u>Observed invasive</u> 1) Lake Erie growth ceases below 9°C (Aldridge et al. 1995) 2) Lake St. Clair min temperature for growth 8-10°C (Claudi and Mackie) 3) Lower Mississippi, Louisiana 6-9°C (4°C) (USGS 1986-1993) 4) Britain – no growth below 10°C (Morton 1969) 5) Rhine River – min temp of 3°C for shell growth (Smit et al. 1993) 6) Lake Ijsselmeer area The Netherlands - can survive in wild with average winter temperatures as low as 6°C (de Vaate 1991) <u>Laboratory invasive with acclimation</u> 1) adult NA(invasive) zebra mussels min freezing temp for mortality -3°C suggesting a lack of tissue or homolymph antifreeze agents (McMahon 1996)	<u>ISSG:</u> 40°C (DAISIE 2006) <u>Observed invasive</u> 1) Lukomskoe Lake= 32°C (Karatayev 1998) 2) Zaporozhskoe Reservoir = 31.5°C (Karatayev 1998) 3) Canal of the Pridnieprovskaya PP =32°C (Karatayev 1998) 4) KuchurgansklyLiman 32°C (Karatayev 1998) 5) Cooling reservoir in South-Ukranian Nuclear station = 33°C (Karatayev 1998) 6) Cooling reservoir of the Cherenobyl Nuclear Station = 34°C (Karatayev 1998) 7) Lonin Lakes = 29°C (Karatayev 1998) 8) Canal Dnieper – Krivoi-Rog= 30°C (Karatayev 1998) 9) Europe 29°C = 100% mortality (Griebeler and Seitz 2007) 10) Netherlands 34°C = 100% mortality (Jenner and Janssen-Mommen 1993) 11) temperatures >26-32°C can kill larvae or adults (Great Lakes) (Strayer 1991) <u>Laboratory invasive with acclimation</u> 1) Lake Ontario near Olcott NY - 35°C

					when acclimated to 20°C, all died at 39°C (Spidle et al. 1995) 2) Niagara River acclimated at 32°C survived for 32 days (Aldridge et al. 1995) 3) acclimated at 30°C max tolerance 32°C for 150h (McMahon et al. 1995) 4) Lake Erie and St. Clair acclimated at 25°C max tolerance 30°C for 95 hr (Iwanyzki and McCauley 1993) Laboratory invasive without acclimation 1) **3 locations** along the Mississippi River: Lake Pepin, Minnesota (latitude 44.47 N., longitude 92.29 W.); Alton, Illinois (latitude 38.90 N., longitude 90.15 W.); and Baton Rouge, Louisiana (latitude 30.45 N., longitude 91.13 W) at 32°C southernmost location (Louisiana) had a significantly higher TTD than mussels from the Minnesota and Illinois locations (Elderkin and Klerks 2005)	
8	*Eriocheir sinensis*	Chinese mitten crab	Yellow Sea China, Hong Kong to North Korea border	Europe and California <u>First invasion</u>: 1912 - Germany	No tolerance reported in ISSG <u>Observed native</u> 1) Adults can live through winter under ice at 4°C (Hymanson et al. 1999)in (Hanson and Sytsma 2005) <u>Laboratory invasive without acclimation</u> 1) requires temperatures above 9°C to survive northern Germany population (Anger 1990) (Anger 1991)**main study 2) Can survive 0°C for 7 days and	No tolerance reported in ISSG <u>Observed native</u> 1) can survive in temperatures of 27-29°C in Singapore (Veldhuizen and Stansih 1999) 2) water systems with adult mitten crabs range up to 25°C in the summer (Hanson and Sytsma 2005) <u>Laboratory invasive without acclimation</u> 1) No growth greater than 30°C (Rudnick et al. 2000)

				then recover when placed in warmer waters (Veldhuizen and Stansih 1999) 3) No growth at less than 7°C (Rudnick et al. 2000)	
9	*Gambus iaaffinis*	Western mosquito fish	Southern USA and northern Mexico	pan-global distribution	ISSG: 12°C (FishBase 2003) Observed invasive 1) Southern lake Michigan 1942– fish overwintered with snow and ice cover for 4 months (Krumholz 1944) Laboratory invasive with acclimation 1) **2 populations** Indian Hot Springs, Arizona fish acclimated to 5°C and 10°C for 30 days had an estimated lower incipient lethal temperature of 2.7°C. Utah(cold acclimated cold adapted) fish acclimated to 5°C and 10°C had an estimated lower incipient lethal temperature of 0.5°C.(Otto 1973)	ISSG: 29°C (FishBase 2003); 42°C (McCullough 1998) Laboratory invasive with acclimation 1) **2 populations** Indiana Hot Springs Arizona and Utah - when acclimated to 20°C upper lethal temperature was 38°C (Otto 1973) 2) Aravaipa Creek, Bonita Creek, San Pedro River, Verde River, and Buenos Aires National Wildlife Refuge stock tanks, Arizona acclimated to 25°C: LOE=39.5°C ±0.3; Death=40.7°C ±0.2; acclimated to 30°C: LOE=41.7°C ±0.3; Death=42.1°C ±0.4 (Carveth et al. 2006) Laboratory invasive without acclimation 1) 37.3°C (Hart 1952) in (Jobling 1981)
10	*Lates niloticus*	Nile perch	Nile system, Lake Mariout, and most major West African river systems	Lakes Kyoga, Nabugabo and Victoria in East Africa	No tolerance reported in ISSG Observed native 1) in own range Nile Delta <10°C(8°C) 1973(Hashem and Hussein 1973) Laboratory native without acclimation	No tolerance reported in ISSG

				2) 12-15°C (Midgley 1968) in (Barlow and Lisle 1987)	
11	*Micropterus salmoides*	Large-mouth bass	St. Lawrence and Great Lakes, Hudson Bay (Red River), and Mississippi River basins from southern Quebec to Minnesota and south to the Gulf; Atlantic Slope drainages from North Carolina to Florida; Gulf Slope drainages from southern Florida into northern Mexico	UK, Europe, Russia, Middle East, North Africa, Continental US, Caribbean territories, South America, Asia, Southeast Asia, Hawai'i, Mauritius, Madagascar, Fiji, Guam, New Caledonia and the US Virgin Islands	ISSG: <10°C Observed invasive 1) 4.5°C (Bell 1990) Laboratory native without acclimation 1) **3 populations**: Alabama, Ohio, Wisconsin all exposed to "winter conditions" at ~6°C slow feeding (Fullerton et al. 2000) 2) Temp <5°C in winter makes fish more susceptible to predation (Miranda and Hubbard 1994) 3) 3-6°C in winter in Mississippi (Miranda and Hubbard 1994) 4) Ohio population growth and food consumption ceased <6°C (Garvey et al. 1998) Laboratory invasive with acclimation 1) Oklahoma population acclimated to 20°C =3.2±0.27°C; 25°C =7.3±0.52°C; 30°C =10.7±0.61°C (Currie et al. 2004)	ISSG: 37°C(FishBase) Laboratory native with acclimation 1) BC Twin Lakes when acclimated to 20-21°C 24h survival: all at 35.2°C; 50% at 28.9°C; 0% 30.4°C (Black 1953) 2) Florida acclimated to 20°C =32.5°C (Hart 1952) 3) Ohio acclimated to 25-26°C = 32.5°C (Hart 1952) 4) Lake Mendota, Wisconsin acclimated at 30°C =36°C (Hathaway 1928) Laboratory invasive with acclimation 1) Aravaipa Creek, Bonita Creek, San Pedro River, Verde River, and Buenos Aires National Wildlife Refuge stock tanks, Arizona; acclimated to 25°C: LOE=37.8°C ±0.3; Death=39.1°C ±0.4 (Carveth et al. 2006) 2) Arizona population acclimated to 25°C for 14d CTM=35°C (Recsetar et al. 2012) 3) Oklahoma population acclimated to 20°C =35.4±0.47°C; 25°C =36.7±0.59°C; 30°C =38.5±0.34°C (Currie et al. 2004)
12	*Mnemiopsis leidyi*	American comb jelly	temperate to subtropical estuaries along the	1980s Black Sea, Azov, Marmara,	ISSG: 4°C Observed native 1) 0°C in Narragansett, Rhode Island (Purcell et al. 2001)	ISSG: 31°C Observed native 1) 30°C in Chesapeake Bay, Maryland (Purcell et al. 2001)

		Atlantic coast of North and South America	the Aegean and the eastern Mediterranean	2) 2°C in Chesapeake Bay, Maryland (Purcell et al. 2001) Observed invasive 1) 1.3°C (Burrell Jr and Van Engel 1976) 2) Can't survive below 4°C in Black Sea overwinter (Shiganova et al. 2001) 3) 4°C in Mediterranean winter (Purcell et al. 2001)	2) 32°C in Biscayne Bay, Florida (Purcell et al. 2001) Observed invasive 1) 31°C in Mediterranean summer (Purcell et al. 2001) Laboratory native without acclimation 1) 32°C (Baker 1973)
13	*Mysis diluviana*	Opossum Shrimp			No tolerance reported in ISSG	No tolerance reported in ISSG Laboratory invasive with acclimation 1) Trout Lake, Cook County, Minnesota population 50 % mortality for M. relicta in 16°C waters when acclimated to 7.5°C for 5 hrs (Smith 1970) 2) Lake Michigan population the upper lethal limit for mysids acclimated to 5°C was about 22°C (DeGraeve and Reynolds 1975) 3) Cayuga Lake, New York 40% mortality after 8hr at 18°C (Rudstam et al. 1999)
14	*Mytilus galloprovincialis*	Bay mussel	Mediterranean Sea, Black Sea, and Adriatic Sea.	Southern Africa, east and west North America, Hawaii, and north-eastern Asia	No tolerance reported in ISSG Laboratory native without acclimation 1) Oosterschelde (Holland) population survived over 70 days at -1.4°C (Korringa 1968) Laboratory invasive without acclimation 2) Penn Cove Shellfish (Coupeville, WA, USA) average low temperature in water is 2.5°C (Fly | No tolerance reported in ISSG Laboratory native with acclimation 1) area of Halastra in the Thermaikos Gulf, Greece, 2 wk of acclimation to 18°C, 20% died at 28°C; 80% died at 30°C (Anestis et al. 2007) Laboratory invasive with acclimation 1) Bodega Bay, CA, USA population when acclimated to 14°C =27°C; when acclimated to 21°C = 31°C (Lockwood and Somero 2011)

				Elizabeth and Hilbish 2002)	2) San Francisco Bay population 4 week acclimation at 13°C = 32°C upper thermal limit due to temperature induced oxidative stress (Tomanek 2012) 3) Penn Cove Shellfish (Coupeville, WA, USA) with 1 day acclimation at 10°C, 54% mortality at 30°C (Fly Elizabeth and Hilbish 2002) Laboratory invasive without acclimation 1) 30°C (Korringa 1968)
15	*Neogobius melanostomus*	Round goby	Asia and parts of Europe (ANSRP, 2003)	North America (ANSRP, 2003) and parts of Europe (Baltic Sea Alien Species Database, 2004)	No tolerance reported in ISSG 1) 0-30°C in FishBase from (Baensch and RRiehl, 1991) Observed invasive 1) Trent-Severn Waterway, a tributary that empties into the Bay of Quinte, Lake Ontario population survived overwinter where temperatures drop to 1-5°C (Houston et al. 2014)	No tolerance reported in ISSG Observed invasive 1) 30 (Moskal'kova 1996) in (Charlebois PM., Wolfe, DL. Jude, and S. Rudnika. 1997. The round goby, Neogobius melanostomus (Pallas), a review of European and North American literature. Illinois – Indiana Sea Grant Program and Illinois Natural History Survey. INHS Special Publication No. 20. 76pp
16	*Oncorhynchus mykiss*	Rainbow trout	Eastern Pacific, from Alaska to Baja, California and Mexico.	Introduced worldwide	ISSG: 10°C (FishBase, 2003) Observed invasive 1) 0°C (Bell 1990) 2) 0-4°C no growth observed reviewed in (Jonsson and Jonsson 2009) Laboratory invasive with acclimation 1) Oklahoma population acclimated to 20°C =<0.0°C; 25°C =0.2±0.16°C; 30°C =2±0.22°C (Currie et al. 2004)	ISSG: 24°C (FishBase, 2003) Observed native 1) 29.4°C (Bell 1990) Observed invasive 1) Santa Clara River basin population highest stream temp observed 31.5°C (Sloat et al. 2012) 2) short-term exposure to stream temperatures as high as 29°C in southwestern Idaho (Zoellick 1999) Laboratory native with acclimation

1) southern California 25°C max observed temperature (Matthews and Berg 1997)
2) Eagle Lake, California wild population acclimated to 10°C CTM =27.6°C; acclimated to 14°C CTM =28.6°C; acclimated to 19°C CTM =30.1°C; acclimated to 22°C CTM =31°C; acclimated to 25°C CTM =32°C (Myrick and Cech Jr 2000)
3) Mt Shasta, California wild population acclimated to 10°C CTM =27.7°C; acclimated to 14°C CTM =28.4°C; acclimated to 19°C CTM =29.7°C; acclimated to 22°C CTM =30.2°C; acclimated to 25°C CTM =31.5°C (Myrick and Cech Jr 2000)

Laboratory invasive with acclimation
1) Western Australia population compared 2 strains of trout "H-line" can survive at 27°C for 14-19hr and "S-line" can survive at 27°C for 7 hrs (Molony et al. 2004)
2) Upper incipient lethal temperature of the sample of rainbow trout acclimated to 15°C was 25.6°C (Hokanson et al. 1977)
3) Oklahoma population acclimated to 20°C =<28.0±0.36°C; 25°C =29.1±0.27°C; 29.8°C =2±0.32°C (Currie et al. 2004)
4) BC population 23-25°C (Black 1953)
5) Arizona population acclimated to 18°C for 14d CTM=31.5°C (Recsetar et al. 2012)
6) Great Lakes population acclimated to 15°C for 3 days, upper lethal temp was

					25-26°C (Bidgood and Berst 1969)
					7) Benner Springs Pennsylvania domestic population acclimated to 9.8°C CTM =27.6°C (Carline and Machung 2001)
					8) USA Fish and Wildlife Service's Williams Creek National Fish Hatchery, Arizona population acclimated at 10°C CTM= 28.45°C; acclimated at 20°C CTM= 29.35°C (Lee and Rinne 1980)
					9) Fish and Temperature Database Matching System (FTDMS) used to estimate thermal requirements for fish estimated 24.0°C (Eaton et al. 1995)
					10) upper incipient lethal temperature is 26.2°C reviewed in (Jonsson and Jonsson 2009)
					11) Norway population 50% mortality at 26.3°C after 14d exposure (Grande and Andersen 1991)
17	*Oreochromis mossambicus*	Common tilapia	Lower river parts and coastal regions of southern Africa	Various tropical and subtropical waters all over the world	<u>No tolerance reported in ISSG</u> <u>Laboratory invasive with acclimation</u> 1) Florida population when acclimated to 15 mean upper lethal temp was 15°C (Stauffer 1986) <u>Laboratory invasive without acclimation</u> 1) 7°C (Chervinski and Yashouv 1971) 2) mean lower temperature was 10.10°C and complete mortality at 8.6°C with Egyptian strains of fish (Charo-Karisa et al. 2005) 3) can tolerate temperatures of 6-	<u>No tolerance reported in ISSG</u> <u>Laboratory invasive with acclimation</u> 1) Florida population when acclimated to 35 mean upper lethal temp was 37°C (Stauffer 1986) <u>Laboratory invasive without acclimation</u> 1) 42°C (Chervinski and Yashouv 1971)

				8°C for a few days (Wohlfarth et al. 1983)	

| 18 | *Pomacea canaliculata* | Golden apple snail | temperate Argentina northwards to the Amazon basin. | southern, eastern and south-east Asia, including the Philippines, Japan, Taiwan, Vietnam, Cambodia, Thailand, Laos, Korea, Sri Lanka, parts of Indonesia and Malaysia, southern China, Singapore; also Hawai'i, Guam, and Papua New Guinea; the | No tolerance reported in ISSG

Observed native
1) Southern Buenos Aires province population can tolerate temperature as low as 4-5°C (Martin et al. 2005)

Observed invasive
1) North Kyushu, Japan snails collected in winter survived more than ten days at 0°C (Oya et al. 1987)
2) snails overwintering outdoors under aquatic conditions showed survivorships at 0°C of 67.5% (± 7.5; S.E.) and 22.5% (± 7.5) for five and 10 days, respectively more tolerant than snails collected in summer Japan (Wada and Matsukura 2007)

Laboratory invasive with acclimation
1) comparison of **3 populations** when cold acclimated to 10, and exposed to 0 for 5 days Kyushu=65% survived , Luzon 68.3% survived, Mindanao 73.3% survived; without acclimation survival was 36.7%, 50%, 48.8% from Japan and Philippines | No tolerance reported in ISSG

Laboratory native without acclimation
1) Curamalal Grande, Argentina stream population At 35 there was 52.6% activity (Seuffert et al. 2010) |

			Dominican Republic; the USA	(Wada and Matsukura 2011)	
19	*Potamocorbula amurensis*	Amur River Clam	Japan, China and Korea	San Francisco Bay in the USA	No tolerance reported in ISSG Observed native 1) 0 °C Ambient in Asia (Sverdrup et al 1974). Observed invasive 1) 8°C San Francisco Bay (Carlton et al. 1990) 2) 3.5 °C sediment temp of intertidal (Koh and Shin 1988)	No tolerance reported in ISSG Observed native 1) 28°C Ambient in Asia (Sverdrup et al 1974). Observed invasive 1) 23 °C San Francisco Bay (Carlton et al. 1990) 2) 37.8 °C sediment temperature of intertidal (Koh and Shin 1988)
20	*Salmo trutta*	Brown trout	Europe, northern Africa, and western Asia	North America and South America, United Kingdom, Europe, Russia, Middle East, Australia, New Zealand, North Africa, South Africa,	No tolerance reported in ISSG Observed native 1) Logan River, Utah survived winter lows of 3.56°C but did not grow (Budy et al. 2008) 2) River Leven, NW England population minimum temperature of approximately 3.6°C required for growth (Elliott et al. 1995) Observed invasive 1) Estenstaddammen Lake, Norway ice-covered during winter (about 200 days), and hypolimnic water temperature during winter was about 4°C - fish survived but	No tolerance reported in ISSG Observed native 1) Humber River tributary ON population max summer temperature =25°C (Bowlby and Roff 1986) 2) Wyoming stream population can tolerate max summer temperature of 24.2-26.3°C (Binns and Eiserman 1979) 3) Fish and Temperature Database Matching System (FTDMS) used to estimate thermal requirements for fish estimated 24.1°C (Eaton et al. 1995) Observed invasive 1) Norway population 50% mortality at 27.8°C after 7 days exposure (Grande

throughout Asia and Fiji.	mean mass specific protein decreased (Berg et al. 2000) 2) Lake Krokavatn, Iceland survive winter temperatures in the range 3–5°C (Borgstrom and Museth 2005) 3) Finland Brown trout of the Rautalampi strain are those that are most commonly used for stocking natural water bodies in Finland: lower temperature limits for feeding and growth in brown trout are slightly above 0°C (Koskela et al. 1997) Laboratory invasive without acclimation 1) North-east Coast of NA population -0.8°C (Elliott and Elliott 2010) 2) 0-4°C no growth observed reviewed in (Jonsson and Jonsson 2009)	and Andersen 1991) Laboratory invasive with acclimation 1) Acclimated 20°C CTM=26–30°C (Elliott and Elliott 2010) 2) Michigan and Wisconsin streams population the 7-d upper tolerance limit was 25.4°C (Wehrly et al. 2007) 3) Old Creek Fort Apache Indian Reserve, Arizona population acclimated at 10°C CTM= 28.96°C; acclimated at 20°C CTM= 29.85°C (Lee and Rinne 1980) 4) upper incipient lethal temperature acclimated at 28°C is 24.7°C reviewed in (Jonsson and Jonsson 2009)

Table 2. Summary of studies reporting a tolerance limit for aquatic invasive animals in the last 40 years

	Title of Study	Multiple pop (Y/N)	Species	Reference
1	Thermal tolerance of *Crepidula fornicata* (Gastropoda) life history stages from intertidal and subtidal subpopulations	N	*Crepidula fornicata*	(Diederich and Pechenik 2013)
2	Temperature acclimation of upper and lower thermal limits and freeze resistance in the nonindigenous brown mussel, *Perna perna* (L.), from the Gulf of Mexico	N	*Perna perna*	(Hicks and McMahon 2002a)
3	Respiratory responses to temperature and hypoxia in the nonindigenous Brown Mussel, *Perna perna* (Bivalvia: Mytilidae), from the Gulf of Mexico	N	*Perna perna*	(Hicks and McMahon 2002b)
4	Exploring the Survival Threshold: A Study of Salinity Tolerance of the Nonnative Mussel *Mytella charruana*	N	*Mytella charruana*	(Yuan et al. 2010)
5	Environmental tolerance of *Caprella mutica*: Implications for its distribution as a marine non-native species	N	*Caprella mutica*	(Ashton et al. 2007)
6	Fluctuating salinity improves survival of the invasive freshwater golden mussel at high salinity: implications for the introduction of aquatic species through estuarine ports	N	*Limnoperna fortunei*	(Sylvester et al. 2013)
7	Comparative environmental tolerances of threatened delta smelt (*Hypomesus transpacificus*) and introduced wakasagi (*H. nipponensis*) in an altered California estuary	N	*Hypomesus transpacificus, H. nipponensis*	(Swanson et al. 2000)
8	Comparison of Upper Thermal Tolerances of Native and Nonnative Fish Species in Arizona	N		(Carveth et al. 2006)
9	Behavioral aspects of cold tolerance in blackchin tilapia, *Sarotherodon melanotheron*, at different salinities	N	*Sarotherodon melanotheron*	(Jennings 1991)
10	Upper Thermal Tolerances of Rio Grande Cutthroat Trout under Constant and Fluctuating Temperatures	N	*Oncorhynchus clarki*	(Zeigler et al. 2013)
11	Tolerance of nonindigenous cichlid fishes (*Cichlasoma urophthalmus, Hemichromis letourneuxi*) to low temperature: laboratory and field experiments in south Florida	N	*Cichlasoma urophthalmus, Hemichromis letourneuxi*	(Schofield et al. 2010)

12	Potential for Naturalization of Nonindigenous Tilapia *Orechromis sp.* in Coastal Louisiana Marshes Based on Integrating Thermal Tolerance and Field Data	N	*Oreochromis sp*	(Green et al. 2012)
13	Respiratory responses to salinity, temperature and hypoxia of six caridean shrimps from different aquatic habitats	N	*Probreviceps macrodactylus*	(González-Ortegón et al. 2013)
14	Salinity tolerance of non-native suckermouth armoured catfish (Loricariidae: *Pterygoplichthys*) in south-eastern Mexico: implications for invasion and dispersal	N	*Silurus callichthys*	(Capps et al. 2011)
15	Thermal Tolerance of the Invasive *Belonesox belizanus*, Pike Killifish, Throughout Ontogeny	N	*Belonesox belizanus*	(Kerfoot and ROY 2012)
16	Acute upper thermal limits of three aquatic invasive invertebrates: hot water treatment to prevent upstream transport of invasive species	N	*Dreissena polymorpha; Dreissena rostriformis bugensis; Bythotrephes longimanus*	(Beyer et al. 2011)
17	Temperature and relative humidity effects on water loss and emersion tolerance of *Perna perna* (L.)(Bivalvia: Mytilidae) from the Gulf of Mexico	N	*Perna perna*	(Hicks and McMahon 2003)
18	Effects of temperature on chronic hypoxia tolerance in the non-indigenous brown mussel, *Perna perna* (Bivalvia: Mytilidae) from the Texas Gulf of Mexico	N	*Perna perna*	(Hicks and McMahon 2005)
19	Salinity tolerance of brown mussel *Perna perna* (L.) from the Gulf of Mexico: An extension of life table analysis to estimate median survival time in the presence of regressor variables	N	*Perna perna*	(Hicks et al. 2000)
20	Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans*/miles complex) on the east coast of the United States	N	*Pterois volitans*	(Kimball et al. 2004)
21	Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*	N	*Tarebia granifera*	(Miranda et al. 2010)

22	Salinity tolerance of non-native Asian swamp eels (Teleostei: Synbranchidae) in Florida, USA: comparison of three populations and implications for dispersal	Y	*Monopterus albus*	(Schofield and Nico 2009)
23	Tolerance of the invasive tunicate *Styela clava* to air exposure	N	*Styela clava*	(Hillock and Costello 2013)
24	Temperature and salinity tolerances of Stage 1 zoeae predict possible range expansion of an introduced portunid crab, *Charybdis japonica*, in New Zealand	N	*Charybdis japonica*	(Fowler et al. 2011)
25	Salinity tolerance of the invasive round goby: experimental implications for seawater ballast exchange and spread to North American estuaries	N	*Neogobius melanostomus*	(Karsiotis et al. 2012)
26	Low temperature as a limiting factor for introduction and distribution of Indo-Pacific damselfishes in the eastern United States	N	*Pomacentridae sp.*	(Eme and Bennett 2008)
27	Temperature tolerance of red-rim melania *Melanoides tuberculatus*, an exotic aquatic snail established in the United States	N	*Melanoides tuberculatus*	(Mitchell and Brandt 2005)
28	Low-Temperature Tolerance and Critical Thermal Minimum of the Invasive Oriental Weatherfish *Misgurnus anguillicaudatus* in Idaho, USA	N	*Misgurnus anguillicaudatus*	(Urquhart and Koetsier 2014)
29	Variation in salinity tolerance among larval anrans: Implications for community compositionand the spread of an invasive, non-native species	N	*Hyla spp.; Lithobates spp.; Anaxyrus terrestris*	(Brown and Walls 2013)
30	Hypoxia tolerance of three native and three alien species of bitterling inhabiting Lake Kasumigaura, Japan	N	*Rhodeus ocellatus ocellatus*	(Morosawa 2011)
31	Using temperature and salinity tolerances to predict the success of the Shimofuri Goby, a recent invader into California	N	*Tridentiger bifasciatus*	(Matern 2001)
32	Effects of temperature on the survival and growth of age-0 least chub (*lotichthys phlegethontis*)	N	*Lotichthys phlegethontis*	(Billman et al. 2006)
33	Desiccation tolerance in a wild population of the invasive oriental weatherfish *Misgurnus anguillicaudatus* in Idaho, USA	N	*Misgurnus anguillicaudatus*	(Koetsier and Urquhart 2012)

34	Linkage of cold hardiness with desiccation tolerance in the invasive freshwater apple snail, *Pomacea canaliculata* (Caenogastropoda: Ampullariidae)	N	*Pomacea canaliculata*	(Wada and Matsukura 2011)
35	Effects of Developmental Acclimation on Adult Salinity Tolerance in the Freshwater-Invading Copepod *Eurytemora affinis*	N	*Eurytemora affinis*	(Lee and Petersen 2003)
36	Do invaders always perform better? Comparing the response of native and invasive shrimps to temperature and salinity gradients in south-west Spain	N	*Palaemon macrodactylus*	(Lejeusne et al. 2014)
37	Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats	N	*Blennioidei spp.*	(Plaut 1998)
38	Intraspecific variation in thermotolerance and morphology of the invasive European green crab, *Carcinus maenas*, on the west coast of North America	Y	*Carcinus maenas*	(Kelley et al. 2011)
39	Salinity tolerance in the early larval stages of *Carcinus maenas* (Decapoda, Brachyura), a recent invader of the Bras d'Or Lakes, Nova Scotia, Canada	N	*Carcinus maenas*	(Bravo et al. 2007)
40	Effects of temperature, body size, and chela loss on competition for a limited food resource between indigenous rock crab (Cancer irroratus Say) and recently introduced green crab (*Carcinus maenas* L.)	N	*Carcinus maenas*	(Matheson and Gagnon 2012)
41	Comparisons of upper thermal tolerances between the invasive Argentine ant (Hymenoptera: Formicidae) and two native Australian ant species	N	*Linepithema humile*	(Walters and Mackay 2004)
42	Physiological tools to predict invasiveness and spread via estuarine bridges: tolerance of Brazilian native and worldwide introduced freshwater fishes to increased salinity	N	*Clarias gariepinus, Ictalurus punctatus, Oreochromis niloticus and Cyprinus carpio*	(Gutierre et al. 2014)
43	The effects of temperature and pressure acclimation on the temperature and pressure tolerance of the shallow-water shrimp *Palaemonetes varians*	N	*Palaemonetes varians*	(New et al. 2014)

44	Low specific conductivity limits growth and survival of the New Zealand mud snail from the Upper Owens River, California	N	*Potamopyrgus antipodarum*	(Herbst et al. 2008)
45	Temperature Tolerance and Stress Proteins as Mechanisms of Invasive Species Success	N	*Diplosoma listerianum*	(Zerebecki and Sorte 2011)
46	Cold tolerance of the invasive *Carcinus maenas* in the east Pacific: molecular mechanisms and implications for range expansion in a changing climate	Y	*Carcinus maenas*	(Kelley et al. 2013)
47	Tolerance of *Venerupis philippinarum* to salinity: Osmotic and metabolic aspects	N	*Venerupis philippinarum*	(Carregosa et al. 2014)
48	Assessing Lethal Dissolved Oxygen Tolerance for Invasive Tunicate *Ciona savignyi* in Puget Sound	N	*Ciona savignyi*	(Pool et al. 2013)
49	Survival of the exotic Chinese mystery snail (*Cipangopaludina chinensis malleata*) during air exposure and implications for overland dispersal by boats	N	*Cipangopaludina chinensis malleata*	(Havel 2011)
50	Variation in Desiccation Tolerance in Freshwater Snails and Its Consequences for Invasion Ability	N	*Thiaridae spp.*	(Facon et al. 2004)
51	Tolerance to salinities shocks of the invasive mussel *Limnoperma fortunei* under experimental conditions	N	*Limnoperma fortunei*	(Angonesi et al. 2008)
52	Effects of desiccation on two life stages of an invasive snail and its native cohabitant	N	*Bithynia tentaculata*	(Wood et al. 2011)
53	Effects of Salinity on the Reproductive Cycle of Female Freshwater Shrimp, *Palaemonetes argentinus*	Y	*Palaemonetes argentinus*	(Ituarte et al. 2010)
54	Thermal constraints for range expansion of the invasive green mussel, *Perna viridis*, in the southeastern United States	N	*Perna viridis*	(Urian et al. 2011)
55	Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*?	N	*Trichocorixa verticalis*	(Van de Meutter et al. 2010)
56	Temperature and pressure tolerances in dispersal stages of the genus Echinus (Echinodermata: Echinoidea): prerequisites for deep-sea invasion and speciation	N	*Echinus spp.*	(Tyler and Young 1998)
57	Thermal tolerance experiments help establish survival probabilities for tilapia, a group of potentially invasive aquatic species	N	*Tilapiini*	(Wilson et al. 2009a)

58	Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina)	N	*Pomacea canaliculata*	(Seuffert et al. 2010)
59	Genotype-by-Environment Interaction for Salinity Tolerance in the Freshwater-Invading Copepod *Eurytemora affinis*	N	*Eurytemora affinis*	(Lee and Petersen 2002)
60	Salinity adaptation of the invasive New Zealand mud snail (*Potamopyrgus antipodarum*) in the Columbia River estuary (Pacific Northwest, USA): physiological and molecular studies	Y	*Potamopyrgus antipodarum*	(Hoy et al. 2012)
61	High tolerance to abiotic stressors and invasion success of the slow growing freshwater snail, *Melanoides tuberculatus*	N	*Melanoides tuberculatus*	(Weir and Salice 2012)
62	Distribution, population structure and salinity tolerance of the invasive amphipod *Gmelinoides fasciatus* (Stebbing) in the Neva Estuary (Gulf of Finland, Baltic Sea)	N	*Gmelinoides fasciatus*	(Berezina and Panov 2004)
63	Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (*Brachidontes pharaonis* - Mollusca: Bivalvia) within the Mediterranean sea	N	*Brachidontes pharaonis*	(Sarà et al. 2008)
64	Stress resistance in two colonial ascidians from the Irish Sea: The recent invader *Didemnum vexillum* is more tolerant to low salinity than the cosmopolitan *Diplosoma listerianum*	N	*Didemnum vexillum; Diplosoma listerianum*	(Gröner et al. 2011)
65	Salinity tolerance of Great Lakes invaders	N		(Ellis and MacIsaac 2009)
66	Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*	Y	*Carcinus maenas*	(Tepolt and Somero 2014)
67	Salinity Tolerance of Larval *Rapana venosa:* Implications for Dispersal and Establishment of an Invading Predatory Gastropod on the North American Atlantic Coast	N	*Rapana venosa*	(Mann and Harding 2003)

68	Thermal tolerance varies in tropical and sub-tropical populations of barramundi (*Lates calcarifer*) consistent with local adaptation	Y	*Lates calcarifer*	(Newton et al. 2010)
69	Seasonal Changes In Cold Hardiness Of The Invasive Freshwater Apple Snail, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae)	N	*Pomacea canaliculata*	(Wada and Matsukura 2007)
70	Thermal tolerance of the invasive oyster *Crassostrea gigas*: Feasibility of heat treatment as an antifouling option	N	*Crassostrea gigas*	(Rajagopal et al. 2005)
71	Survival of the Invasive Clam *Corbicula fluminea* (Müller) in Response to Winter Water Temperature	N	*Corbicula fluminea*	(Müller and Baur 2011)
72	Effects of upper-limit water temperatures on the dispersal of the Asian clam *Corbicula fluminea*	N	*Corbicula fluminea*	(Rosa et al. 2012)
73	Hot Water Treatment (Chronic Upper Lethal Temperature) Mitigates Biofouling by the Invasive Asian Mussel *Limnoperna fortunei* in Industrial Installations	N	*Limnoperna fortunei*	(Perepelizin and Boltovskoy 2011)

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