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Risk Assessment

A risk assessment of the golden mussel, *Limnoperna fortunei* (Dunker, 1857) for Ontario, Canada

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Editor's note:

This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (http://www.icais.org/html/previous19.html). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

The golden mussel, Limnoperna fortunei, is an epibenthic mytilid native to freshwaters of China and south-eastern Asia. It became established in Hong Kong in 1965, in Japan in the 1990's and South America in 1991 through ballast water discharge into the La Plata River basin in Argentina. It has since expanded to Bolivia, Paraguay, Uruguay, and Brazil in South America. Populations have steadily increased in these countries since their first reported incidence. The golden mussel is not yet present in North America. A Risk Assessment of golden mussel for the Province of Ontario. Canada was performed using a four step process, developed by the Ontario Ministry of Natural Resources and Forestry: While the potential impacts of an invasion of golden mussel in the Great Lakes has been well documented, the likelihood of a wide spread invasion (i.e., the likelihood of arrival, survival, establishment and spread) was further investigated. Uncertainty for each of the four stages of invasion was also estimated. The risk assessment ranked ballast water exchange of Atlantic transoceanic vessels from South America as the primary potential pathway for introduction of golden mussel directly into the Great Lakes. A "back door" entry into the Great Lakes was also considered via overland dispersal (e.g. trailered boats) following a ballast water exchange introduction by transoceanic vessels from Asia to the Pacific coast of North America. However, the overall probability of arrival through these two pathways was ranked low. The probability of survival, establishment, and spread of golden mussel in Ontario was deemed to be low, primarily because of its physiological intolerance of cold, winter waters. The level of uncertainty was considered moderate as there has been no golden mussel invasion reported from lakes that freeze over in Asia or South America for at least two months. Based on the current distribution of golden mussel being limited mostly to waters with minimum temperatures of 10 °C to 12 °C at 36° latitude, the Climatch analysis, and the number of degree days required for reproduction and establishment, the level of risk for invasion into Ontario waters is low and the level of uncertainty is moderate. This would likely apply to all of Canada and most of the USA north of the 36th parallel that is covered by snow or where lakes have ice cover for at least two months of the year. An analysis of the impact of climate change on golden mussel in Ontario suggests that summer temperatures in Lake Erie could rise to levels that would support reproduction and establishment by 2040. However, the lowest temperature reported to support establishment of mussels is 5 °C and it is estimated it would likely take more than 200 years to reach and maintain this temperature in Lake Erie.

Key words: aquatic invasive species, arrival, survival, establishment, spread

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Introduction

The golden mussel, Limnoperna fortunei (Dunker, 1857) (Figure 1) is a mytilid mussel related to the marine Blue Mussel (Mytilus edulis Linaeus, 1758) and has the same invasive characteristics as freshwater dreissenids. Like zebra (Dreissena polymorpha Pallas, 1771) and quagga mussels (Dreissena bugensis Andrusov, 1897) they secrete byssal threads and attach to solid substrates and foul any suitable natural or man-made surface causing macrofouling problems in industrial installations (Mackie and Claudi 2010). The golden mussel is purported to be native to China and may be native to neighbouring countries such as Vietnam, Thailand, Cambodia and Japan. In 1991 it was introduced to South America at Bagliardi Beach, Argentina (Pastorino et al. 1993). Oliveira et al. (2010a, b) predicted the potential distribution of golden mussel in Brazilian rivers and North American systems using exploratory analyses based on limnological data integrated into two niche models that have previously been used to predict alien species invasions: GARP (Genetic Algorithm for Ruleset Production), and Maxent (Maximum Entropy Method). Their analyses indicate that the probability of establishment and survival in North America is greatest in the Mississippi River below its confluence with Ohio River, as well as in the Colorado River and Rio Grande, where the minimum winter water temperature is above 5 °C. Kluza and McNyset (2005) and Campos et al. (2014) used other invasion models with similar results.

GLANSIS (Great Lakes Aquatic Nonindigenous Species Information System) (2015) reported that the golden mussel "has a high probability of establishment if introduced to the Great Lakes (Confidence level: High)". The present risk assessment was carried out to evaluate the potential risk in the event that golden mussel is introduced or released into Ontario waters, primarily through the ballast water pathway. Given the huge numbers of transoceanic vessels entering the Great Lakes, this risk assessment evaluates the suitability of conditions for survival and establishment of golden mussel within all of Ontario's portions of the Laurentian Great Lakes, including Lake St. Clair (Figure 2). Therefore, only the Great Lakes whose boundaries include Ontario are considered here; Lake Michigan is totally with the United States and not included but could collaterally be impacted. While the primary risk assessment is based on the short-term (< 10 years). longer term scenarios were also considered because of threats like climate change. There is no discussion of impacts of golden mussel to the natural environment because they are well described in the literature (e.g.

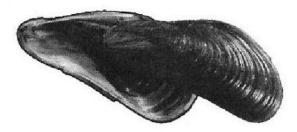


Figure 1. Golden mussel has an inner purple nacreous layer and a light to very dark brown outer shell. Photo courtesy Dr. Gustavo Darrigran.

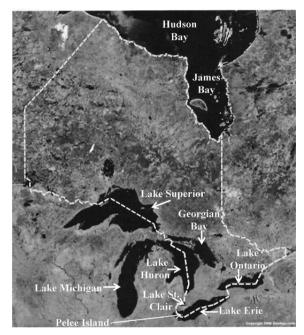


Figure 2. The province of Ontario showing its boundaries through the Great Lakes. Lake Michigan is totally with the USA. Hudson Bay and James Bay are marine systems. The southern limit of Pelee Island is at 41°43.602′N, 82°40.331′W.

Boltovskoy et al. 2006; Uliano-Silva et al. 2013; Boltovskoy and Correa 2015 in South America; Nakano et al. 2011, 2014 in Japan; Karatayev et al. 2015 globally).

The risk assessment used is adapted from a process developed by the Ontario Ministry of Natural Resources and Forestry (OMNR 2013), which in turn has been adapted from the Fisheries and Oceans Canada detailed level risk assessment methodology of Mandrak et al. (2012). The impacts of a widespread invasion of golden mussel in locations where it becomes established are well documented (Boltovskoy et al. 2009; Mackie 2015). Most accounts seem to agree that the potential for negative impacts is high; and thus, the potential impacts of an invasion

will not be further assessed for Ontario. Instead, this study will focus on the probability of an invasion occurring in the Great Lakes or other waters of Ontario, specifically considering the four stages of invasion through the conditional probabilities of arrival, survival, establishment and spread. For each estimated probability, an estimate of uncertainty will be considered. Much of the information here is extracted from Mackie (2015), with the exception of the assessment of the probability of establishment, which also examines the effect of degree days at reproductive temperatures (16–17 °C) and degree days for establishment at 21 °C. A long term assessment of the effect of climate change on the probability of invasion is also included.

Methods

Risk Assessment Process

Ricciardi and Rasmussen (1998) provide guidelines for identifying potential aquatic invaders by first identifying potential donor regions and dispersal pathways. The most likely donor region of golden mussel for Ontario is South America. The likelihood is based, in part, on the proximity of each country to the Great Lakes and the numbers of transoceanic voyages linking ports in each country (Mackie 2015).

Estimating the probability of invasion by golden mussel:

The overall probability of a wide spread invasion is estimated through the combination of four conditional probabilities:

Probability of arrival – The first step is assessing the likelihood of arrival through primary (e.g. transoceanic transport) and secondary pathways (e.g. trailered boats). "Secondary" implies the transport of propagules from an intermediate location rather than from the native range (Rup et al. 2010; DFO 2014).

Probability of survival – Assuming a species has arrived, the next step is determining the likelihood of its survival. The probability of survival includes survival of propagules in ballast water and survival upon release to the wild in a recipient port. Survival in recipient ports considers the distances travelled via primary and secondary pathways and ecological and physiological requirements of the mussel. The likelihood of survival in recipient waters of Ontario can be approximated by determining the most northern occurrence in Asia and southern occurrence in South America. The search focused on more northern locations reported in the literature in Asia e.g. Lake Paldang, South Korea (37°31.342′N; 127°17.978′E), Lake Ohshio (36°13.233′N; 138°52.783′N) and

Lake Takenuma (36°13.833′N: 139°01.417′E). Japan. and southern occurrences in South America e.g. Punta Piedras, Argentina (35°23.728'S; 57°08.714'W), to determine the coldest zones occupied by golden mussel. The occurrences do not include "linear occurrences", e.g. all sites sampled in a river system. The latitudes and longitudes can be found in Mackie 2015). If linear occurrences are reported, then in Argentina alone there are approximately 100 different locations where the mussel has been reported at different times, for Brazil (Oliveira et al. 2015) there probably are more than 150 locations, with a similar number for Japan (Ito 2015), and in China there are at least 30 locations where this species has been introduced (Xu 2015). To date no occurrences have been reported in Asia between 0 and 10°N and in South America between 0 and 8.5°S of the equator (Barbosa et al. 2016).

Probability of establishment – This step considers the likelihood of not only surviving but also reproducing and maintaining a population. The probability of establishment of golden mussel assumes that arrival and survival has occurred (i.e. a conditional probability). In order to estimate the probability of establishment, the likelihood that golden mussel can successfully reproduce and establish a self-sustaining population in Ontario waters was determined not only by its ability to survive low temperatures (see "probability of survival" above), but also considers:

- 1. The most northern latitude at which *L. fortunei* is known to be established.
- The similarity in climates between donor and recipient regions using Climatch, a user-friendly climate matching model that allows the prediction of the potential range of a species by matching climate data from weather stations in donor and recipient regions (Australian Bureau of Rural Sciences 2008).
- 3. The degree days required for reproduction to occur between 16 and 17 °C, the generally accepted threshold temperature for golden mussel reproduction, with a mean of 16.5 °C used here (see *Determining Degree Days for Reproduction and Establishment*). Growing degree days (GDD) could not be calculated because data to determine the average of the daily maximum and minimum temperatures compared to a base temperature (usually 10 °C) were not available for most lakes.
- 4. The degree days required for golden mussel to develop, settle and grow into juveniles and adults (see *Determining Degree Days for Reproduction and Establishment*).

Probability of spread – For the final conditional probability, the likelihood of golden mussel to spread within Ontario or across all of Ontario was estimated.

The overall probability of an invasion was then determined by using the lowest estimate of the four conditional probabilities. The definitions for the estimated probabilities of all four stages of invasion and the final overall probability are: Very high (96–100% likelihood of occurrence); high (61–95%); moderate (41–60%); low (6–40%); and very low (0–5%).

Uncertainty analyses:

An estimation of uncertainty was assigned to each probability. The criteria used to determine the level of uncertainty are: Very high (little or no scientific information or no supporting data); high (limited scientific information or circumstantial evidence); moderate (moderate level of scientific information or first hand, unsystematic observations); low (substantial scientific information or expert opinion); very low (extensive scientific/systematic information; peerreviewed data sources/information).

Potential impact of climate change

Impacts of climate change are being observed in the Great Lakes basin with a 1.3 °C increase in air temperatures between 1968 and 2002. A 1 to 3 °C increase is projected by 2050 (Kahl and Stirratt 2012). Some of the most evident impacts include warming temperatures, changing precipitation patterns, decreased ice coverage and lower than average water levels (Mortsch et al. 2003). Trumpickas et al. (2008) used two scenarios to predict temperature increases in the Great Lakes over three time periods, 2011–2040, 2041-2070, and 2071-2100, using 1971-2000 as a baseline. One scenario is characterized by high population growth and energy use, whereas the other reflects moderate population growth and energy use. The average of the two scenarios is used here to estimate a future state for the Great Lakes.

Determining Degree Days for reproduction and establishment

The threshold temperature for golden mussel reproduction is considered here to be between 16 °C and 17 °C (e.g. Cataldo and Boltovskoy 2000 in South America; Xu et al. 2013 in China). An average of 16.5 °C was selected for determining degree days for reproduction. However, the number of degree days at the threshold temperature may be just as important as the temperature itself. The method for determining degree days is shown in Figure 3 for

tropical, subtropical and temperate lakes. The number of degree days for reproduction was the sum of Julian days from onset at 16.5 °C in the fall to the end of 16.5 °C in the spring for both tropical and subtropical lakes of the southern hemisphere (Figure 3A). For temperate lakes in the northern hemisphere the number of degree days for reproduction was the sum of Julian days from onset at 16.5 °C in the spring to the end of 16.5 °C in the fall (Figure 3B). To determine which of the Great Lakes fell within the 16.5 °C threshold value for reproduction, the means and ranges of degree days at or above 16.5 °C from 2010 to 2015 were plotted against latitudes of the lakes (see Figure 8). The means and ranges of degree days present in the Great Lakes at 16.5 °C were determined from the Great Lakes Surface Environmental Analysis databases (http://coastalwatch.glerl.noaa.gov) from 2010 to 2015, inclusive, and the averages and ranges were plotted against latitudes of the Great Lakes to determine which lakes fell within the threshold value of 16.5 °C.

The literature indicates that the average temperature for establishment of golden mussel is 21 °C. The same methods used for degree days to reproduction were used for degree days to establishment (Figures 3A, B). To determine which of the Great Lakes fell within the 21 °C threshold value for establishment, the means and ranges of degree days at or above 21 °C from 2010 to 2015 were plotted against latitudes of the lakes (see Figure 8).

Results and discussion

Probability of invasion

Probability of invasion was assessed by estimating the probability of arrival, survival, establishment, and spread, as described sequentially below.

Probability of arrival

Golden mussel is not yet in Canada, or the USA, but the most likely receptor region from transoceanic vessels in Canada is the Great Lakes, which are partially in Ontario. The potential donor regions are South America (with a previously invaded distribution of Argentina, Uruguay, Paraguay, Bolivia, and Brazil), and Southeast Asia, with a purported native distribution in China (Xu 2015), although IUCN (2014) also lists Thailand, Vietnam and Cambodia as native. Xu (2015) lists the latter three countries as introductions as well as Japan and Laos. The literature review indicates the primary pathways from which golden mussel could enter Ontario waters are ballast water in transoceanic vessels entering the Great Lakes. An alternative pathway is via

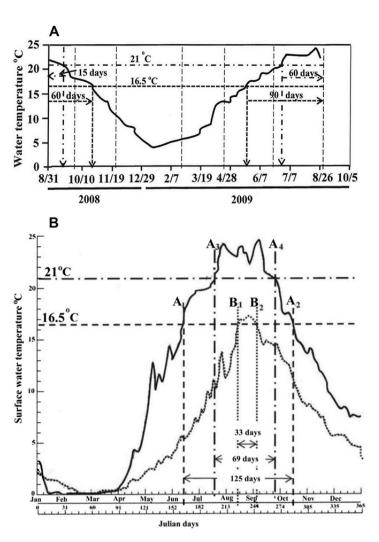


Figure 3. Methods used to determine number of degree days (based on the Julian calendar) for reproduction at 16.5 °C and establishment at 21 °C in (A) tropical and subtropical lakes (e.g. Lake Ohshio, Japan (Nakano et al. 2011)) and (B) temperate lakes (e.g. Great Lakes). The dashed line is for estimating degree days at 16.5 °C and the dot-dashed line is for estimating degree days at 21 °C in Figures 3A and 3B. The dotted line shows the threshold for reproduction in hypothetical Lake B in Figure 3B; however, there are zero degree days at 21 °C the threshold for establishment in Lake B in Figure 3B.

trailered boats if the species is first introduced into the USA, or into other Canadian provinces, such as British Columbia (BC) with its seaports, and then transported overland into the Great Lakes. Secondary invasions will most likely occur in ballast water or hulls of coastal and inland ships, as well as by trailered boats from lakes and rivers already infested with golden mussel. There is a remote possibility of golden mussel arrival via the unauthorized release of freshwater plants and animals with mussels attached, as in the aquarium trade. Very few freshwater plants and animals arrive to Canada from South America, with virtually all of the trade from South America involving wild terrestrial specimens, as cultivation of freshwater plants in this region is considered uneconomical (UNEP-WCMC 2007). The ballast water and trailered boat pathways are further evaluated below.

Ballast water: The probability of arrival of golden mussel into Ontario waters through this pathway depends on the frequency of transoceanic vessels from donor regions, the proportion of NOBOBS (No Ballast on Board) to BOBs (Ballast on Board) vessels or tanks, and efficacy of BWE (Ballast Water Exchange) or treatment. Transoceanic vessels operate outside of the Canadian and U.S. exclusive economic zones (EEZs). Currently, all transoceanic vessels entering the Great Lakes are operating under foreign flags (National Research Council (U.S.) 2008).

There are several factors that affect the probability of arrival and survival of propagules. The likelihood of ships taking on ballast water containing golden mussel larvae increases with the number of ships leaving ports of rivers in which the species is known to occur. Mackie (2015) provided a list of occurrences of golden mussel in ports of donor

countries of Asia and South America. He listed only freshwater ports because propagules of golden mussel are more likely to be entrained in freshwater ballast water than sea water from marine ports. Assuming the risk potential for introductions increases with an increasing number of riverine ports, China, Japan, and Vietnam are the most likely donor countries in Asia, and Argentina and Brazil are the most likely donor countries in South America. However, Lo et al. (2012) estimated >1800 vessel arrivals to the Great Lakes between Nov. 1, 2006 and Oct. 31, 2007; and none were from these potential donor regions.

Of the two continents, propagules are more likely to arrive to Ontario in ballast water of vessels from South America than from Asia, mostly due to the length of time spent at sea. The longer the voyage, the lower the survival rate of propagules. Thus, survival potential of propagules is greater in ballast drawn at latitudes and longitudes closer to the Great Lakes than for those further away. Additionally, the greater the quantity of ballast water drawn and released the greater the probability of introduction into the Great Lakes. Further, the quality of ballast water and the condition of propagules also affects probability of survival. Generally, younger life cycle stages are more sensitive to stress than older stages (Mackie and Claudi 2010). If the quality of ballast water is high, it may be possible for golden mussel to complete its entire life cycle in the ballast tank. Usually 2 to 3 weeks is required for golden mussel to complete its planktonic stage and begin to settle. Many transoceanic vessels require longer times to complete a voyage.

As the release of ballast water from vessels departing South America has been historically low, the probability of arrival in the near future via this pathway is estimated to be low in the current study. However, the literature has not targeted frequency or volumes of ballast water releases from transoceanic vessels, so more research is needed to reduce uncertainty.

Calas-Monroy et al. (2014) conducted a national risk assessment to better understand the relative invasion risk posed by ballast water discharges across Canada by examining 11 different merchant shipping pathways that involve transoceanic, coastal and domestic voyages. Two of the 11 are Arctic pathways and are not considered further here. The remaining nine pathways and their definitions are given in Mackie (2015). Within the pathways, two primary taxa were identified, phytoplankton and zooplankton, but only zooplankton are considered further because it encompasses all the larval swimming stages of *L. fortunei*. Two timescales, per-event and annual, were also examined by Mackie (2015).

Current risk was assessed under present ballast water exchange (BWE) requirements (Government of Canada 2004, 2006) and future risk under international ballast water discharge standards. The potential for introduction of NIS and the magnitude of consequences of introduction were estimated considering shipping activity (ballast volume discharged). propagule pressure (based on biological sampling surveys), environmental similarity between donor and recipient ports (based on salinity and climate), the number of high impact NIS in donor ecoregions, and the effects of mitigation strategies (Mackie 2015). On an annual basis the invasion risk currently posed by International Transoceanic vessels in the Great Lakes-St. Lawrence River (GLSLR) region was used as the "lowest risk" benchmark, because BWE is thought to be particularly effective for mitigating this pathway and no new ballast-mediated NIS have been reported from the Great Lakes since 2006 (Calas-Monroy et al. 2014). They caution, however, even the lowest risk pathways still pose a small, but not negligible risk of invasion.

Upper salinity tolerance for golden mussel varies from <3 to 14‰ (Boltovskoy et al. 2006). Sylvester et al. (2013) found no significant mortality (31% after 30 days) at a constant salinity of 2‰, increasing to 45 and 57% at 5 and 10%, respectively. No significant mortality was observed in mussels exposed to a salinity cycle with abrupt salinity changes ranging from 1-23‰ (mean 2.68‰) over a month. Angonesi et al. (2008) tested specimens under salinities of 2, 4, 6, 8 and 12‰, and exposed them for periods of 24, 48, 72, 96 and 240 hours. They found "the mussel can survive (90%) up to a salinity shock of 2‰ for periods of at least 10 days. Considering the influence of climatic and stochastic events and the chemical instability of Patos Lagoon estuarine regions, they concluded it unlikely that populations could survive for longer periods (more than a year) in this area."

Of the nine donor pathways examined by Calas-Monroy et al. (2014), on a per event basis the three most likely to be primary pathways for golden mussel are (1) GLSLR International Transoceanic (GLSLRIT), (2) Eastern Coastal Domestic (ECD), and (3) Lakers, the latter two of which could also act as secondary invasion pathways. Three others are potential primary invasion pathways, Atlantic International Coastal U.S., Atlantic International Exempt, and Atlantic International Transoceanic (the definitions for all of these pathways are given in Mackie 2015). While each of these pathways could carry golden mussel larvae from Asia or South America to the GLSLR, it is possible for golden mussel to enter Ontario from any Atlantic International pathway if first introduced to any Atlantic coast port, and then into GLSLR.

The three Pacific International pathways are unlikely to introduce *L. fortunei* to GLSLR.

The risk analyses performed by Calas-Monroy et al. (2014) ranked Lakers as highest risk for zooplankton arrivals at any individual port on both a per-event and annual basis (although with low certainty); GLSLRIT was ranked as higher in both time frames: ECD was ranked lowest annually and intermediate for per-event probability of arrival. Atlantic International arrivals of zooplankton were highest for transoceanic vessels at both time scales and low to intermediate for coastal USA vessels on per-event and annual basis. Similar rankings likely apply for L. fortunei propagules arriving from South America as well. The Pacific international vessels ranked highest to intermediate for arrival of zooplankton on a per-event and annul time scale from Asian countries, but the likelihood of arrival of L. fortunei to Ontario via this pathway is very low.

Overland Transport: The trailered boat pathway is a potential primary vector for golden mussel to enter the Great Lakes if a transoceanic introduction occurs elsewhere in North America. There are several ports in the southern USA that could serve as entry points, but mussels would have to be carried by overland transport to Ontario, as discussed earlier under Probability of Arrival. If Pacific transoceanic vessels empty ballast water into USA ports or BC ports first, then once in these jurisdictions, trailered boats can disperse the mussel to other states and provinces. Of the total volume of ballast discharged in BC waters (16,323 tonnes), 47.2% were from Japan, 11.7% from China, 0.8% from Taiwan, 0.1% from Thailand (Lo et al. 2012). If golden mussel is to arrive to the Great Lakes from the west, it would have to be through the "back door" and travel a considerable distance (about 12500 km), first by the transoceanic pathway from Asia (e.g. Japan, to BC, North America (~7600 km) and then via overland transport (e.g. trailered boats) east from Vancouver, BC to the west end of Lake Superior (~3600 km), where survival is unlikely (see next section) or down Lake Huron to Lake Erie (an additional ~1300 km), where survival is possible (see discussions below). While more than 80° in longitudes must be trespassed, 90° (9990 km) in latitudes need be traveled from South America to 41°N, the most southerly limit of populated Ontario (Pelee Island, Figure 2), which is well within the temperate zone where summer water temperatures in Lake Erie are sufficient to support reproduction (discussed further below). If adults are attached to trailers, the likelihood of survival across the Prairies is low to nil. Darrigran et al. (2004) exposed specimens to air without humidity control (49 to 63% relative humidity) and the mussels did not survive more than 120 hours, while those held in an elevated humidity environment survived up to 168 hours. Smaller mussels reached 100% mortality before larger ones. However, Montalto and Ezcurra de Drago (2003) reported survival between 3 and 11.5 days when exposed to air.

Overland transport from an Atlantic port involves potentially greater latitudes to travel, depending on the location of an Atlantic port, but fewer longitudes. Still, Lo et al. (2012) report little or no ballast water discharges from transoceanic vessels from South America to Atlantic ports. The arrival of golden mussel by overland transport is considered low because either the distance traveled overland (i.e. from west coast to Great Lakes) is too great for survival of propagules, or because there are few ballast water discharge events from ships arriving at Atlantic Ocean ports. However, some caution is warranted because the probability of dispersal of zebra mussels into western United States was assessed as a low probability event, but we now know this prediction was incorrect.

Probability of arrival summary

The estimate from the pathway with the highest risk was taken to estimate the overall probability of arrival. Ballast water from BOBs and NOBOBs from international source ports must now be exchanged or flushed on the open ocean for GLSL vessels, which dramatically reduces potential propagule supply to Canadian ports (Bailey et al. 2011, 2012; Chan et al. 2012) by these vessels. Therefore, ballast water discharged by transoceanic vessels may no longer play a prominent role in introducing AIS from foreign sources, which gives a low probability of arrival via GLSLIT. However, risk analyses for survival in ballast water of eight other oceanic pathways indicate high risk. Nevertheless, ballast water from international source ports must also be exchanged or flushed on the open ocean for Atlantic coastal-bound vessels (i.e. not including GLSRIT). It remains to be confirmed if these other vessels comply as strictly as those in GLSLIT vessels. However, inspections continue to ensure that 100% of vessels entering the Seaway at Montreal contain no freshwater ballast (i.e. all tanks have a salinity of at least 30%). The probability of arrival in ballast water of transoceanic vessels is therefore considered low, with considerable (moderate to high) uncertainty because of dependence upon humans for ensuring control of ballast water; the same probability and level of uncertainty apply for overland transport (Table 1). The lack of European occurrences especially in countries adjacent to Asia for this

species is further evidence to support the low risk to Ontario.

Probability of survival

Probability of survival of propagules may differ between those in ballast water and those released to the wild in a recipient port. Survival in ballast water depends on its water quality and efficacy of ballast exchange or flushing. Calas-Monroy et al. (2014) rank survival of zooplankton in recipient ports, as highest for all nine oceanic pathways, except GLSLIT (lowest) and Atlantic International Exempt vessels (intermediate) on both per-event and annual bases. While this likely applies to *L. fortunei* arriving from South American ports, survival ultimately depends on the ecological and physiological requirements of the mussel.

Ecological and physiological requirements and tolerances

Table 2 summarizes limits in chemical and physical variables of golden mussel in Asia and South America and gives ranges in values for Ontario lakes based on https://www.ontario.ca/data/provincial-stream-waterquality-monitoring-network (Accessed January 13. 2017) and authors' unpublished data. In terms of pH and calcium tolerances, the golden mussel survives and reproduces in pH of 5-10 and [Ca] of 1-50 mg·L⁻¹. which spans the ranges reported in most lakes and rivers throughout Ontario. Indeed, it can survive and reproduce in waters where zebra and quagga mussel cannot, at least with respect to lower pH and calcium content. Likewise, golden mussel has been reported from wide ranges in conductivity (14 to 1470 µS·cm⁻¹) and salinity (0.104 to 14 ppt). Although it can tolerate short term exposures up to 23% (Sylvester et al. 2013), golden mussel typically grows and reproduces in salinities of 0 to 3 ppt. The golden mussel seems tolerant of some organic enrichment and can survive short periods at 0.5 mg dissolved oxygen L-1 but annual averages are usually within the range of 3.7 to 11.2 mg·L⁻¹. Total suspended solids (TSS), including organic and inorganic material, range from 0.10 to 3000 mg L⁻¹. Based on all these criteria, golden mussel could establish itself throughout most of Ontario. It could not only spread widely in Ontario but more quickly than zebra mussel and quagga mussel because of larger numbers of suitable locations in close proximity to one another. Its rate of spread in South America was recorded as up to 240 km·yr⁻¹ (Boltovskov et al. 2006). However, the main limiting variable is cold water temperature, discussed next, which will likely limit or prevent its spread, and will slow the rate of spread.

Table 1. Summary of the estimated probability of arrival for golden mussel into Ontario.

Pathway of	Probability of	Level of
introduction	arrival	uncertainty
Ballast Water	low	high
Overland Transport	low	high

Thermal/climatic requirements of golden mussel

There has been considerable research on the optimal temperatures for growth and reproduction in golden mussel and its upper thermal tolerance limits. The literature reports 15-17 °C (Morton 1977; Brugnoli et al. 2011), 16-17 °C (Cataldo and Boltovskoy 2000; Giglio et al. 2016; Karatayev et al. 2015); 17 °C (Nakano et al. 2010a); 15-18 °C (Boltovskov et al. 2015) being required for reproduction and > 5 °C for long-term survival. The warmer the water, the more cohorts are produced. In China, the golden mussel completes three generations per year, with the first at 20–22°C, a second at 24–26°C, and a third at 30–33°C (Morton 1977; Boltovskov and Cataldo 1999; Cataldo and Boltovskoy 2000; Spaccesi 2013; Xu et al. 2013). In tropical and subtropical South America, larval output is more or less continuous up to 10 months of the year, often with a major peak in spring-early summer, and a smaller one in the late summerautumn. Larvae are very scarce during the winter (at temperatures ~10-13 °C), but rarely totally absent (Boltovskov et al. 2015). In Japan, at considerably lower water temperatures, larval production is limited to 1–2 months centered around summer (Boltovskov et al. 2015).

The southern limit of Ontario's boundary in Lake Erie is 41°40.528′N with the southernmost shoreline being Pelee Island at 41°48.5'N, which is about 4° further north of the current northernmost occurrence of golden mussel in Lake Paldang, South Korea (Choi and Kim 1985; Choi and Shin 1985; Park et al. 2013). The 37°31.342′N of Lake Paldang parallel runs through the northern portion of North Carolina. Tennessee, Arkansas, Oklahoma, Texas, New Mexico, Arizona, and California, which likely includes the most northern states in the subtropical zone of the eastern USA that will support golden mussel infestations. The 41°N latitude corresponds to a mean annual isotherm of 10 °C (50 °F) and 37°30'N to a mean annual isotherm of 16 °C (60 °F) based on a map on Florida Center for Instructional Technology (FCIT) (2015) web site, USF. http://etc.usf.edu/maps [Accessed March 7, 2015]. While the most southern occurrence in South America is Punta Piedras at 35°23.728'S, it is acknowledged that suitable invasion sites may not be available further south due to limiting factors other than temperature.

Table 2. Ranges in values for some chemical and physical parameters of surface waters in which golden mussel have been reported in South America and Eastern Asia. Some of the temperature, salinity, and TSS data are based on laboratory experiments TSS = total suspended solids (organic + inorganic). Sorted by ascending author name. The bottom row gives a range in values that are typical for Ontario waters, taken from the Ontario Provincial Stream Water Quality Monitoring Network and the authors' unpublished data.

Source	pН	[Ca] mg·L ⁻¹	Dissolved Oxygen mg·L ⁻¹	Temp °C	Conductance µS/cm	Salinity ‰	Turbidity NTU Secchi depth m	TSS mg·L ⁻¹	Depth m
Boltovskoy et al. (2006)	5	2-10	0.5		60-80	14			
Cataldo and Boltovskoy (2000)	6.4-7.4		5.1-9.8	12.5-29	118-160	<5	1.4-5.5 m	17-104	
Campos et al. (2014)	5.8-7.9	1.7-23.5	5.1-10.4	18.0-30.4	14.0-58.0		0.3-7.0m		
Campos et al. (2014)	6.8-6.9	3.4-4.7	7.6-7.8	24.5-28.0	38.5-39.6		2.0-3.0m		
Darrigran (2002)	6.2-7.4	3.96		15.3-32.6		<3			
Darrigran et al. (2007, 2011)	6.2-7.0	2-46.0	6.8-7.5	26.2-29.7		0.10-0.31		66-88.0	40
Darrigran et al. (2012)	6-7.9	3-50	2.8-14	25.5-35.9	44-1470	0.05-0.86	Bed-1.5m	5-327	1-1.5
Dos Santos et al. (2008)	6.2-7.5		3.91-11.33	12-27.5	30-90		0.08-10 m	0.10-1.4	12.2
Nakano et al. (2010a,b)			< 0.5-12	<5-24.5		0	0-150		~25
Nakano et al. (2011)	7.5-9.0	17.8-23.6	7.3-12.7	4.2-23.6	160-250	0	9-28		>9
Oliveira et al. (2006, 2010a)	5.0-7.8	1.0-11.6	0.2-1.3	5-31.8		<12			
Oliveira et al. (2011)	5.4-7.8	1.0-23.0	0-8.0	8.0-35.0			0.10-1.90m	20-120	
Pareschi et al. (2008)	7.3-10.0		6.8-10	19.9-33.1	44-232		3-62.4		
Sylvester et al (2013)						<2*, 23**			
Tokumon et al. (2015)								3000	
Ontario lakes	5.5-9.3	2.0-104	2.5-14	0-25.5	24-550	0	0.4-15.5m	2-85	1-406

One may argue that perhaps the most northern occurrences of the golden mussel have yet to be discovered. However, a good surrogate of survey effort is demonstrated in determining the distribution of the Asian clam, *Corbicula fluminea* (O. F. Müller, 1774), which is often associated with the golden mussel (Morton 1996; Darrigran and Pastorino 1995, 2004; Magara et al. 2001; Darrigran and Damborenea 2006; Darrigran et al. 2012). Figure 4 shows the global distribution of the Asian clam up to 2015 (Gama et al. 2016). Its isolated occurrence in Ontario is probably due to its broader tolerance of low temperatures than golden mussel, and despite this, Asian clam has not become widespread in Ontario.

Nakano et al. (2011) examined growth rate and recruitment of *L. fortunei* in Lake Ohshio, Japan and discussed differences in the biological characteristics of the species inhabiting reservoirs of temperate, subtropical, and tropical zones. In Lake Ohshio there was very little mussel growth in the first year (median shell length = 1.2 mm), but the growth rate increased in the second year and the median shell length reached 16 mm by September 2009. Larval densities rose from 26,000 ind.m⁻³ in first year to 80,000 ind.m⁻³ in second year, with water temperature varying between 4.2 °C in winter to 23.6 °C in the summer. From these observations Nakano et al. (2011) estimated the lower temperature limit for growth to be between 5 and 10 °C, based on the rela-

tionship between water temperature and growth rate. The mean shell length after one year in Lake Ohshio was 9.8 mm, which is half that in most subtropical lakes (e.g. 20 to 22 mm), like Plover Cove, Hong Kong (Morton 1977). While subtropical lakes usually have water temperatures >15 °C (maximum 29 °C) for 9 months of the year, and tropical lakes are >20 °C (maximum 32–34 °C) for 9 months of the year, Lake Ohshio is only >15 °C for 6 months of the year. In Lake Ohshio the breeding season is about 3 months (with peak breeding activity in late summer), while in subtropical lakes the breeding season extends 9 months of the year (with peak breeding activity in early summer and late autumn), and for 10 months in tropical lakes (with peak breeding activity in late summer). Choi and Kim (1985) and Choi and Shin (1985) report that golden mussel can survive a surface water temperature at 0 °C in Lake Paldang, South Korea, but the surface freezes for only 1-2 months every winter (Park et al. 2013; Karatayev et al. 2015). Although Lake Paldang freezes in the winter, it reaches ~30 °C in the summer. Karatayev et al. (2015) suggest that the magnitude and duration of warm summer temperatures determine whether selfsustaining populations are possible, rather than minimum winter values. Unfortunately, there is little information on survival rates of golden mussel in Lake Paldang at 0 °C, particularly because the lake is only 6.4 m deep and may mix to the bottom in winter.

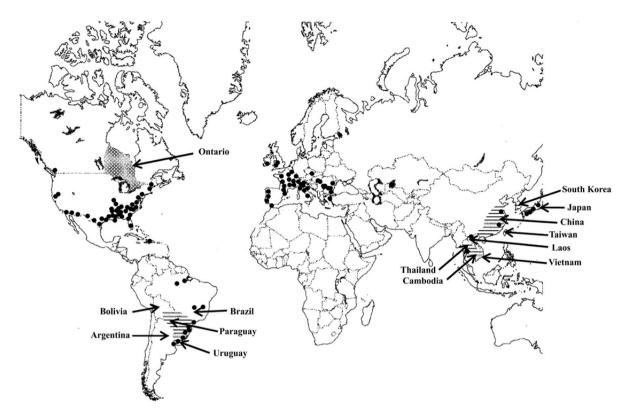


Figure 4. Global distribution of the Asian clam, *Corbicula fluminea* (solid circles), which is often associated with golden mussel (shown with labelled hatched areas). Asian clam is also established in isolated areas of Ontario in Lake Erie and Lake St. Clair. Native ranges of golden mussel are China, Taiwan, and Vietnam; although the Taiwan and Vietnam populations are considered by many to be introduced (see text). Asian clam distribution is from Gama et al. (2016) and their map has been modified to include countries with golden mussel.

In Ontario, water temperatures of 16 °C to 17 °C, which triggers reproduction, do not occur in Lake Ontario and Lake Erie until mid-June (Table 3). Based on this thermal regime, the window for recruitment for Ontario lakes (mainly Lake Erie) is about four to five months, which is probably sufficient for one generation per year. However, water temperatures usually drop below 8 °C in November, below 4 °C in December and between 0 °C and 1 °C from January through March, and from 4 °C to 10 °C from April to early May.

Oliveira et al. (2010b) found population densities of *L. fortunei* decreasing in winter when the water temperature drops below 5 °C. Survival at 5–7 °C was found to be 50% during the first 20 days in their thermal tolerance assay. Their thermal tolerance assays using adult specimens from the tropical Paraguay River showed mussels survived up to 15 days at water temperatures of 0–1 °C, although they were inactive during that time. Survival at 5–7 °C was <50% during the first 20 days, with a maximum survival of about 38 days. At temperatures above 10 °C mussels were

Table 3. Monthly mean, minimum and maximum temperatures recorded by NOAA (2015) for Lake Erie to show the first and last occurrences of 16 °C between 1981 and 2010.

Month	Mean °C	Minimum °C	Maximum °C
May	11.1	8.3	13.9
June	16.6	13.9	18.3
July	22.2	20.0	23.3
August	23.3	22.8	23.8
September	21.1	19.4	22.8
October	16.1	13.3	18.9
November	10.6	7.8	12.8

active, and about 80% of them survived for the entire 38 days of the experiment. Their data lend support to a threshold of \sim 5 °C for protracted exposure of *L. fortunei* to low winter temperatures (i.e., weeks to months). However, it is not known if golden mussels acclimated to lower temperature could survive at temperatures lower than 5 °C or 10 °C.

The temperatures required for 50% (LT $_{50}$) and 100% (SM100) mussel mortality, and the mean upper death temperature (MUDT) varied between 42.2 °C

and 51 °C over 54 experiments (Perepelizin and Boltovskov (2011a), which is not an issue for Ontario waters where maximum surface water temperatures are well below 30 °C. Perepelizin and Boltovskoy (2011a, b) found heating rates and acclimation temperatures significantly affected mortality rates of mussels between 34 and 36 °C but not between 38 and 43 °C. At Bagliardi Beach and Punta Indio, Argentina, Darrigran et al. (1999, 2011) found water temperature varied according to the season, reaching maxima of 27.9 °C and 28.6 °C in December and passing through minima of 10.7 °C in July and 8.5 °C in June, with average values of 20.1 °C \pm 5.3 °C and 19.8 °C \pm 6.6 °C, respectively. If these are typical seasonal variations in temperature, the critical limiting factor in Ontario will be the species' tolerance or intolerance to 0 to 5 °C for 6 to 7 months of the year, a typical thermal range for most waters in Ontario during winter months. Based on the thermal regimes in Ontario waters, the average number of cohorts would likely be one, with most water bodies not sustaining water temperatures of 25-27 °C required for two or more cohorts.

In addition, the literature does not report populations of golden mussel established in waters with ice cover for more than two months in the winter in Asia or South America. Based on the current distribution of golden mussel limited to warmer waters, the probability for survival in Ontario waters is estimated to be low, and the level of uncertainty is estimated to be moderate because of the preponderance of evidence for preferences for warm climates (Table 4). However, empirical evidence, such as low thermal threshold bioassays that show 100% mortality between 0 °C and 4 °C of different life stages, are still required. At present we only know the mussel occurs mostly in waters >10 °C in Asia and South America. If empirical evidence demonstrates that all life stages of golden mussel cannot live at 0 to 4 °C, the probability of survival would be very low and the level of uncertainty would be very low.

Probability of establishment

Probability of establishment of golden mussel is assessed here by examining: (1) the latitude, which in part determines regional water temperatures; (2) the similarity in climates between donor and recipient regions; (3) the degree days required for reproduction to occur between 16 and 17 °C; and (4) the degree days required for development to occur to allow settlement and growth of juveniles and adults. It is appreciated that there are other ecological factors that affect establishment. These include man's extensive modification of landscapes, in particular construction of reservoirs,

Table 4. Overall estimated probability of survival, establishment, and spread of golden mussel in Ontario.

Probability of:	Level of risk	Level of certainty
Survival	Low	Moderate
Establishment	Low	Moderate
Spread	Low	Moderate

large inter-basin connections, and r-selected traits of invasive species (e.g. rapid reproduction, prolific progeny, short lives, quick maturation, habitat generalists, and diverse dispersal mechanisms). These have all been discussed by others (e.g. Mackie 2002, 2004; Boltovskoy 2015; Karatayev et al. 2015; Uliano-Silva et al. 2013) and no further discussion is warranted here.

1). Latitude: The most northern and southern latitudes above and below the equator were considered the most northern limits of golden mussel in Japan and South Korea and southern limits in South America. The mean annual isotherms were then determined for the range in distribution of the golden mussel (based on IUCN (2014); see Appendix II in Mackie (2015), which is a map of global mean annual isotherms). It is highly unlikely that golden mussel will survive the year in climates <16 °C (61.8 °F), which is the temperature needed for reproduction to occur. The 16 °C isotherm corresponds roughly to 37°30'N latitude and includes South Korea, where the most northern range of the golden mussel in Asia occurs. The 16 °C isotherm swings up to about the 43°N latitude east of the Rocky Mountains in the western USA and then back down to 28°N to 32°N latitude on the Pacific coast, extending the potential range of the golden mussel in USA. Interestingly, the 16 °C isotherm closely approximates the southern extent of snow cover in North America (Figure 5). While Figure 5 shows the southern extent of snow cover, it varies from one year to the next; snow is present until March 1 north of 41°N latitude, the most southern limit of Ontario, every year except 2012.

Perhaps coincidentally, the prediction by Oliveira et al. (2010b) is that the golden mussel will establish itself in the United States in the Mississippi River system up to the confluence of the Ohio River, based on GARP and Maxtent analyses. This confluence is near Cairo, Illinois (not Thebes, Illinois as suggested by Oliveira et al. (2010b), which is at 36°58.848′N latitude, close to the southerly limit of snow cover and the maximum northerly extent of the range of the golden mussel at 37°30′N. On these premises, the estimated probability of establishment of golden mussel in Ontario is low and the estimated level of uncertainty is moderate (Table 4).

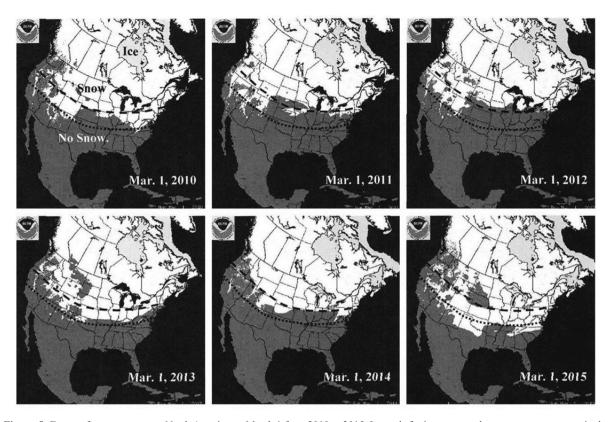


Figure 5. Extent of snow cover over North America on March 1 from 2010 to 2015. Legends for ice, snow and no snow coverages are in the March 1, 2010 image. All images were downloaded from the database of the U.S. National Ice Center (NIC), a multi-agency operational center operated by the United States Navy, the National Oceanic and Atmospheric Administration, and the United States Coast Guard. The dashed line is at 41°N and the dotted line is at 37°30°N.

2). Climatch: The climate matching model, Climatch (Australian Bureau of Rural Sciences 2008), allows the prediction of the potential range of a species by matching climate data from weather stations in donor and recipient regions. The first source region used was Japan. The northern-most and southern-most latitudes, and the eastern-most and western-most longitudes were selected for 16 source stations representing the distribution of golden mussel in Japan and were compared to 64 target stations in Ontario (Figure 6A). The higher the match class (e.g. 10) the closer the match of climates between the source and target regions. The analyses support the contention that climates are very dissimilar and there is a low risk of the mussel establishing itself in Ontario (Figure 6A). Figure 6B shows the relative influence of each station using Japan as a source region. The area of each circle is proportional to the number of matched cells that a data point contributes to the target region, as shown in the legend on the right of the map. Square points represent stations selected in the source region, which were not used in the match (that is, their distance to all cells in the target map (Figure 6A) was evaluated but other stations had a closer match. One data point in the Japan source map contributed 23 matched cells in the target region. Another Asian donor Climatch analysis was done for South Korea (Figure 6C), where Lake Paldang is the most northern lake reported with golden mussel, using 12 source stations and the same 64 target stations. 10 of 12 cells matched the target region using South Korea as a source (Figure 6D)

The same Climatch analysis was performed using South America as the source map (Figure 6E). Distribution data of *L. fortunei* in South America were taken from Crosier et al. (2007), Oliveira et al. (2010a, b), and Bogan (2012). A total of 81 source stations were selected within the range of *L. fortunei* in South America and 64 target stations (Ontario). The results are very similar to Climatch using Japan and South Korea, indicating very different climates in Ontario compared to South America. The analysis supports the contention that climates are very dissimilar

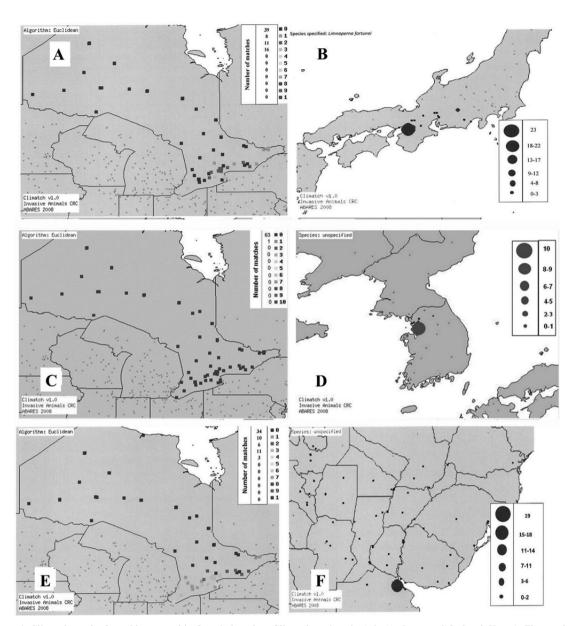


Figure 6. Climatch results for golden mussel in Ontario based on Climatch stations in Asia (**A**, Japan and **C**, South Korea). The number of matches for each climate match bin (the bins are 0 to 10, with 10 being the most similar) are provided. (**B**, **D**) Maps showing relative influence of each station in the source region (**B**, Japan; and **D**, South Korea) used in matches with the target region (Ontario). (**E**) Climatch results for golden mussel in Ontario based on Climatch stations in South America. (**F**) Map showing relative influence of each station in the source region (South America) used in the match with the target region (Ontario).

and there is a low risk of the mussel establishing itself in Ontario (Table 4). Figure 6F shows the relative influence of each station in the source map of South America. As for Figures 6B and 6D, the area of each circle is proportional to the number of matched cells that a data point contributes in the target region. One data point in the source map contributed 19 matched cells in the target region.

Using Climatch as evidence the estimated probability of establishment of golden mussel in Ontario is low and the estimated level of uncertainty is moderate (Table 4).

3). Degree days for reproduction in the Great Lakes: When days above 16.5 °C for lakes with golden mussel were plotted against their latitude, as in Figure 7A, the lowest value was for Lake Ohshio (150 days, see

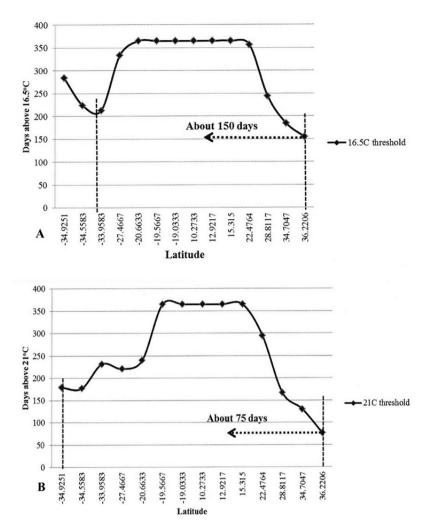


Figure 7. (A) Degree days at the 16.5 °C threshold for the onset of reproduction for golden mussel in relation to latitude. The lowest value is at 150 degree days. **(B)** Degree days at the 21 °C threshold for the settlement and establishment of golden mussel in relation to latitude.

Figure 3A for its estimation). Lake Ohshio is among the most northern lakes in Japan containing golden mussel, but water temperature data was not available for other northern lakes.

To determine which of the Great Lakes fell within the 16.5 °C threshold value, the means and ranges of degree days present at or above 16.5 °C from 2010 to 2015 were plotted against latitudes of the lakes, as in Figure 7A. For the averages, the 16.5 °C threshold of 150 degree days was not met for any of the Great Lakes for the 6 years of data (Figure 8). However, in 2010 Lake Erie had 151 degree days at 16.5 °C, which met the threshold of 150 degree days. Using degree days to represent the likelihood of reproduction the estimated probability of establishment of golden mussel in Ontario is low and the estimated level of uncertainty is moderate (Table 4).

4). Degree days for settlement and establishment in the Great Lakes: A similar approach was used to determine the minimum number of degree days at the minimum temperature reported for lakes with golden mussel in Japan, South America, and the Great Lakes. As best as could be determined from the literature, Lake Ohshio, Japan had the lowest mean summer water temperature of the known established populations of golden mussel (Figure 7B).

The number of degree days at 21 °C was plotted against the latitude of each body of water, as in Figure 7B. In the dataset used, Lake Ohshio, Japan, had the lowest number of degree days, with 75 days above 21 °C (see Figure 3A for its estimation). The number of degree days is not known for the Lake Paldang (South Korea) mussels.

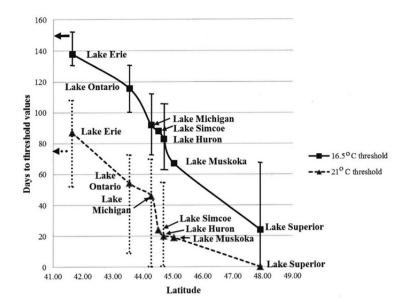


Figure 8. Days required to meet the threshold values for golden mussel in the Great Lakes at 16.5 °C (150 days required) and 21 °C (75 days required). The thresholds for reproduction (solid line and squares at 16.5 °C) and establishment (dashed line and triangles at 21 °C) are shown by heavy arrows on the y-axis.

For the average degree days, the 21 °C threshold of 75 days was met for only Lake Erie (Figure 8), with the means for all other Great Lakes (on the Ontario side) falling well below the threshold value. For the 6 years of data for Lake Erie, the threshold of 75 days was met in all years except 2010 (51 days) (Figure 8). For the other Great Lakes, the threshold of 75 degree days was met only once over the 6 years of data, that being in 2012 for Lake Ontario. Lake Superior and Huron never met the threshold of 75 degree days over the 6 years of analysis. Using degree days to estimate the probability of establishment, the risk is estimated to be moderate for Lake Erie but low in other Great Lakes with an estimated level of uncertainty of moderate in all of the Great Lakes because the analyses do not consider southern ports in the USA, like Lake Michigan (e.g. at Chicago). One may argue that shallow waters in sheltered bays of Lake Erie, as at Sandusky, Ohio, may have the 21 °C threshold. Although Sandusky is not a harbour it is only about 80 km from ports in Toledo and Cleveland. While the golden mussel may arrive and survive the summer, it would perish in the winter because the winters are as harsh on the southern shores of Lake Erie and Lake Michigan as the northern shores. Overall, on the basis of degree days, the estimated probability for establishment of golden mussel in Ontario is low and the estimated level of uncertainty is moderate (Table 4).

Oliveira et al. (2010a) also concluded that the lower thermal threshold for golden mussel will determine its survival and establishment in North

America. She forecasted the potential distributions in the Mississippi, Colorado, and Rio Grande river systems based mainly on water temperature because buffer variables (pH, calcium, alkalinity, etc.) would not be limiting to golden mussel establishment in those waters. According to Oliveira et al. (2010a) golden mussel could become established in the Mississippi, Colorado and Rio Grande drainage systems, although the northern Mississippi River system including the Missouri River may be too cool in the winter to support golden mussel as the minimum winter temperature is about 0 °C, with around 3 to 4 months of temperatures lower than 5 °C. The present study supports their conclusions that the probability of survival and establishment is greater in the Mississippi River below its confluence with Ohio River, as well as in the Colorado River and Rio Grande, where the minimum winter water temperatures are above 5 °C.

Probability of spread

The pathways of spread of golden mussel are similar to those of quagga and zebra mussel, including human-mediated (e.g. unauthorized introductions, accidental introductions, trailered boats) and natural dispersal mechanisms through physical connections, such as canals and downstream dispersal. However, the natural physical barrier to their spread will be cold, winter temperatures, implying a low probability of spread (Table 4) and an estimated moderate level of uncertainty.

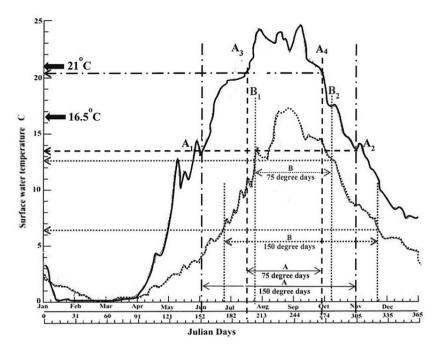


Figure 9. Estimating the predicted times (in years) to reach the 16.5 °C and 21 °C thresholds for golden mussel reproduction and establishment using the rates of increasing temperatures from Trumpickas et al. (2008) for the Ontario Great Lakes (lakes Erie, Ontario, Huron, and Superior). For example, 150 degree days occurs at 6.5 °C for hypothetical Lake B, 10 °C below the 16.5 °C threshold for reproduction; 75 degree days occurs at 20.5 °C for hypothetical Lake A, 0.5 °C below the 21 °C threshold for establishment. The estimated values were inserted into Table 5.

Table 5. Predicted times (years) to reach the 16.5 °C and 21 °C thresholds for reproduction and establishment using the rates of increase over three time periods from Trumpickas et al. (2008) relative to the 1971–2000 baseline data for the Ontario Great Lakes (Erie, Ontario, Huron, and Superior). Means and ranges for each lake are based on data from 2010–2015.

Lake	Mean °C difference from 16.5 °C	Range °C difference from 16.5 °C	2011-2040 Mean yrs (range)	2041-2070 Mean yrs (range)	2071-2100 Mean yrs (range)
Erie	1.8	0.7-2.8	38 (14-58)	33 (13-51)	18 (7-27)
Ontario	4.1	2.0-5.9	108 (52-154)	59 (29-84)	34 (17-49)
Huron	5.9	3.5-6.7	130 (78-149)	78 (47-89)	49 (29-56)
Superior	8.6	4.5-10.7	197 (100-238)	118 (60-143)	70 (36-84)
	Mean °C difference from 21 °C	Range °C difference from 21 °C	2011-2040 Mean (range)	2041-2070	2071-2100
Erie	-0.3	-1.2-0.5	5.1 (0-21.8)	2.1 (0-9.1)	1.1 (0-4.8)
Ontario	2.4	1.3-3.5	61.3 (33.9-91.3)	33.6 (18.6-50.0)	19.6 (10.8-29.2)
Huron	3.8	1.9-5.8	84.4 (42.2-128.9)	50.7 (25.3-77.3)	31.7 (15.8-48.3)
Superior	8.7	5.2-12.6	193.7 (115.6-280.0)	111.3 (66.4-160.9)	68.8 (42.1-99.5)

Impact of climate change

Climate change will certainly affect the probability of invasion, especially in more northern latitudes. Winter ice cover in the middle of Lake Erie and the other Great Lakes has been diminishing by 1.3 percent a year for three decades. That is more than a 30 percent decline since the 1970s (Wang et al. 2012). Trumpickas et al. (2008) predicted temperature increases in lakes Superior, Huron, Erie and Ontario over three time periods, 2011–2040, 2041–2070, and 2071–2100, using 1971–2000 as a baseline and developed two climate change scenarios. The average of

their two scenarios was used here. The differences in mean degree days above 16.5 °C for a 150 day interval (Figure 9) and above 21 °C for a 75 day interval (Figure 9) were calculated for each year and lake. The impact of climate change was calculated as years to attain the threshold surface water temperatures for reproduction (16.5 °C) and for establishment (21 °C) for each of the four Ontario Great Lakes (Table 5) using rate increase values from Trumpickas et al. (2008) for each of three terms, 2011–2040, 2041–2070, and 2071–2100. Table 5 shows that golden mussel would be able to reproduce and settle in Lake Erie in less than 40 years (i.e. between 2011 and 2040), Lake Ontario in about 108

years, Lake Huron in 130 years, and lake Superior in about 200 years assuming minimum temperatures are >4 °C. There is considerable variation among years and among terms for the four lakes (Table 5).

Although Nakano et al. (2011) found 4.2 °C to be the lowest observed temperature only once in Lake Ohshio he concluded the lower temperature limit for growth to be between 5 and 10 °C. On this basis establishment is presumed to occur only when minimum temperatures exceed 5 °C for more than 270 days. Kobayashi et al. (2010) found that half the mussels died after 309 days of rearing at 5 °C using mussels from two reservoirs, Lake Ohshio and Lake Takenuma. The lowest temperature in Lake Ohshio is usually 5 °C and they observed decreases in density during winter. Oliveira et al. (2010b) observed 50% mortality after 20-38 days in thermal bioassays suggesting that 5 °C must be exceeded all year. However, as discussed earlier, it is not known if golden mussels acclimated to lower temperature could survive at temperatures lower than 5 °C or 10 °C. Using Table 14 in Trumpickas et al. (2008) it would take 90 years for an average of 303 days to exceed 4 °C in Lake Erie. Thus, it is estimated that this threshold will be exceeded in Lake Erie sometime beyond the year 2100, or possibly even beyond 150 years if a warming rate of 17 days per 30 years is used. For 5 °C, it is conceivable that it would take much longer than 200 years. Surface waters in lakes Superior, Huron, and Ontario are projected to take even longer than Lake Erie according to Trumpickas et al. (2008) and estimates made herein.

Conclusions

The final probability of invasion was determined to be low, with levels of uncertainty being high for arrival but moderate for survival, establishment and spread. The final probability of invasion as determined through using the lowest of the scores for the four stages of invasion was a low probability for all four stages but with high uncertainty for arrival and moderate uncertainty for survival, establishment, and spread. A summary of the probability scores for four stages of invasion by golden mussel is given in Table 6. Based on the current distribution of golden mussel being limited to waters with minimum temperatures of 10 °C to 12 °C at 36° latitude, the Climatch analysis, and the number of degree days required for reproduction and establishment, the level of risk for invasion in Ontario waters is low and the level of uncertainty is moderate. However, with additional empirical evidence, such as low thermal threshold bioassays that show 100% mortality between 0 °C and 4 °C, and using additional

Table 6. Summary of estimated probability scores for the four stages of invasion for golden mussel in Ontario.

Stage	Probability	Uncertainty
Arrival	low	high
Survival	low	moderate
Establishment	low	moderate
Spread	low	moderate

evidence such as its occurrence mostly in waters >10 °C in Asia and South America, the probability of survival would be very low and the level of uncertainty would be moderate. Indeed, if snow and ice cover is a suitable surrogate for the distribution of golden mussel in North America, all of Canada and most of the USA, especially above the 36th parallel, will not support survival during the winter under current climate conditions. On the basis of a lower thermal threshold of 5 °C for at least 270 days per year, and recent rates of surface water temperature increases, it is estimated that it would take more than 200 years for Lake Erie to reach that threshold.

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