Canadian Science Publishing

ARTICLE

Cold tolerance varies among invasive populations of the Asian clam (*Corbicula fluminea*)

E. Cvetanovska, R.A. Castañeda, A.P. Hendry, D.B. Conn, and A. Ricciardi

Abstract: The distribution of the subtropical Asian clam (*Corbicula fluminea* (O.F. Müller, 1774)), one of the world's most invasive freshwater molluscs, is reportedly constrained by a lower thermal tolerance limit of 2 °C. Although its occurrence in north temperate regions is typically restricted to artificially heated waterbodies, the species has been found to overwinter in unheated lakes and rivers. In laboratory experiments, we compared the cold tolerance of populations from several geographically distinct sites spanning 35°N to 46°N in eastern North America. Each population contained individuals that fully recovered from 2 months of continuous exposure to near-freezing (1 °C) conditions, contrary to published accounts of *C. fluminea*'s thermal ecology. Survivorship increased with body size and was enhanced by prior acclimation to a low temperature (10 °C) compared with a higher one (18 °C). When acclimated to 10 °C, clams from northern populations exhibited greater survivorship (55.0% ± 16.1%) than those from southern populations (26.7% ± 19.2%). However, one southern population demonstrated survivorship as great as that of the most tolerant northern population, suggesting that its clams could overwinter in unheated northern waterbodies. Differences among populations indicate either that contemporary evolution has occurred or that developmental plasticity shapes future acclimation responses.

Key words: Asian clam, *Corbicula fluminea*, cold tolerance, adaptation, latitudinal variation, interpopulation comparison.

Résumé : Il a été rapporté que la répartition de la petite corbeille d'Asie (*Corbicula fluminea* (O.F. Müller, 1774)), une espèce subtropicale et l'un des mollusques d'eau douce les plus envahissants du monde, serait limitée par un seuil de tolérance thermique inférieur de 2 °C. Bien que sa présence dans des régions tempérées nordiques soit typiquement limitée à des plans d'eau chauffés artificiellement, l'espèce a été observée hivernant dans des lacs et rivières non chauffés. Dans le cadre d'expériences en laboratoire, nous avons comparé la tolérance au froid de populations de plusieurs sites allant de 35°N à 46°N dans l'est de l'Amérique du Nord. Chaque population renfermait des individus qui se sont complètement remis après une exposition continue de 2 mois à des conditions près du point de congélation (1 °C), ce qui est contraire aux descriptions publiées de l'écologie thermique des petites corbeilles d'Asie. La survie augmentait avec la taille du corps et était rehaussée par l'acclimatation préalable à faible température (10 °C), comparativement à l'acclimatation à plus haute température (18 °C). Une fois acclimatées à 10 °C, les petites corbeilles de populations nordiques présentaient un taux de survie (55,0 % ± 16,1 %) plus important que les petites corbeilles de populations méridionales (26,7 % ± 19,2 %). Cependant, une population méridionale présentait un taux survie aussi grand que la plus tolérante des populations nordiques, ce qui indiquerait que les individus de cette population pourraient passer l'hiver dans des plans d'eau nordiques non chauffés. Les différences entre populations indiquent soit qu'une évolution parallèle s'est produite ou qu'une plasticité développementale modèle les réactions d'acclimatation. [Traduit par la Rédaction]

Mots-clés : petite corbeille d'Asie, *Corbicula fluminea*, tolérance au froid, adaptation, variation latitudinale, comparaison entre populations.

Introduction

Species are spreading into regions outside their indigenous range at unprecedented rates (Ricciardi 2007; Seebens et al. 2017), yet many introduced non-indigenous species fail to establish persistent populations (Zenni and Nuñez 2013). Why some introduced species are more successful invaders than others, and why the invasion success of a species can vary greatly across space and time, are two central questions in invasion ecology (Lockwood et al. 2013). Invasion success has been linked to species traits such as environmental tolerance (Kolar and Lodge 2001; Lenz et al. 2011; Früh et al. 2012; Grabowska and Przybylski 2015), phenotypic plasticity (Chown et al. 2007; Nyamukondiwa et al. 2010; Valiente et al. 2010; Davidson et al. 2011; Tepolt and Somero 2014), and contemporary evolution (Shine 2012; Colautti and Lau 2015). The latter two mechanisms could increase the probability of establishment and persistence of an introduced population by enhancing survivorship of individuals under an environmental

Received 23 September 2020. Accepted 5 April 2021.

E. Cvetanovska and A.P. Hendry. Redpath Museum, McGill University, 859 Sherbrooke Street West, Montréal, QC H3A 0C4, Canada; Department of Biology, McGill University, 1205 Docteur Penfield, Montréal, QC H3A 1B1, Canada.
R.A. Castañeda. Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, ON L7S 1A1, Canada.
D.B. Conn. Berry College, 2277 Martha Berry Hwy NW, Mt Berry, GA 30149, USA.
A. Ricciardi. Redpath Museum, McGill University, 859 Sherbrooke Street West, Montréal, QC H3A 0C4, Canada.
Corresponding author: A. Ricciardi (email: tony.ricciardi@mcgill.ca).

stress, such as extreme temperature (Ayrinhac et al. 2004; Chown et al. 2007; Valiente et al. 2010; Tepolt and Somero 2014). Temperature is a major constraint on the geographic distribution of ectotherms (Buckley et al. 2012). Indeed, thermal tolerance and climate variables are critical in determining distributions of introduced aquatic animals (Somero 2010; McDowell et al. 2014) and can mediate interactions between indigenous and non-indigenous species (Ferreira-Rodriguez et al. 2018; Lopez et al. 2018). In addition to providing insights into the influence of climate warming on species distributions and biotic interactions, non-indigenous species offer valuable opportunities to assess latitudinal variation in thermal tolerance and acclimatization potential across conspecific populations (Elderkin and Klerks 2005; Fangue et al. 2006; Sorte et al. 2011; Tepolt and Somero 2014; Wallace et al. 2014).

One of the world's most invasive and widely distributed aquatic animals is the Asian clam (*Corbicula fluminea* (O.F. Müller, 1774)). Indigenous to southeast Asia, Australia, and Africa, *C. fluminea* has expanded its range within three other continents (North America, South America, and Europe) over the past several decades, aided by ballast water transport, food markets, bait bucket dumping from anglers, and aquarium release (Sousa et al. 2008; Crespo et al. 2015). Given its apparent affinity for high temperatures (with a long-term upper thermal tolerance of 36 °C in the field; McMahon 1999) and its predominantly subtropical distribution (Crespo et al. 2015; Penk and Williams 2019), *C. fluminea* was assumed to be unable to invade unheated lakes and rivers in north temperate regions — an assumption promoted by a widely cited laboratory study that tested the response of clams from a single population and concluded that the lower lethal temperature of the species is 2 °C (Mattice and Dye 1975). Several field studies appear to support this conclusion (Horning and Keup 1964; French and Schloesser 1991, 1996; Morgan et al. 2003; Werner and Rothhaupt 2008). In the Connecticut River (USA), for example, survivorship of *C. fluminea* was positively correlated with the mean winter water temperature and negatively correlated with the frequency of daily mean water temperatures of ≤1 °C (Morgan et al. 2003). An Ohio River (USA) population whose abundance varied from 215 to 2390 clams/m^2 was reduced by >96% after an anomalously severe winter season caused the river to freeze over for several days (Horning and Keup 1964). In outdoor mesocosms under natural winter conditions, 99.9% of a *C. fluminea* population died after being exposed to temperatures below 2 °C for 2 months (Werner and Rothhaupt 2008).

Consistent with the view that cold winter temperatures constrain the distribution of *C. fluminea*, almost all populations found in northeastern USA and in the Great Lakes region are restricted to the heated water discharge plumes of power plants (e.g., French and Schloesser 1991, 1996; Ward and Hodgson 1997; Smith et al. 2018). A population of *C. fluminea* became established in the St. Lawrence River circa 2009, the most northernmost occurrence of the species in North America to date, but it remained confined within the thermal plume generated by the Gentilly-2 nuclear power plant in Bécancour, Quebec (Simard et al. 2012). In this environment, water temperatures close to the discharge source were 10–13 °C higher than ambient. Although this temperature difference attenuated downstream and varied throughout the year, it prevented winter freezing in a ~4 km downstream section of the river (Castañeda et al. 2018). Within a few years of its discovery, the St. Lawrence River population consisted of hundreds to thousands of clams per square kilometre inside the thermal plume. The population was abruptly extirpated over the winter months of 2012–2013, after the power plant was shut down permanently in December 2012 (Castañeda et al. 2018).

However, since at least the 1990s, populations of *C. fluminea* have been observed in areas subject to snow and ice cover, prompting speculation of evolving cold tolerance (Kreiser and Milton 1995; Janech and Hunter 1995; Minchin 2014; Natale et al. 2014; Richardson and Selby 2020). In a bay in Lough Derg, Ireland, individuals of *C. fluminea* survived 0.9 °C for almost 2 weeks below ice (Minchin 2014). Janech and Hunter (1995) noted the presence of a persistent population in the upper Clinton River, Michigan, USA, that was exposed to water temperatures of 0–2 °C for over 2 months. Similarly, Müller and Baur (2011) reported that 18% of Asian clams from a population in the River Altrhein, Germany, could tolerate water temperatures near 0 °C for 9 weeks in the laboratory. Overwintering populations of *C. fluminea* continue to be discovered in unheated waterbodies in northeastern North America. One such population was found in Lake George, New York, USA, in 2010, where it has persisted for over a decade, in spite of freezing winter temperatures and repeated eradication attempts by lake managers (Lake George Park Commission 2018; M. Modley, Lake Champlain Basin Program, personal communication). Overwintering populations have also been found in the Seneca River and Owasco Lake in New York State (Natale et al. 2014; E. Cvetanovska, personal observation). Each of these waterbodies occurs in a region where mean winter air temperatures are below freezing from December to February inclusive (https://www.weather-us.com). These discoveries suggest that previous assessments of the thermal tolerance of *C. fluminea* and its potential to expand its northern range are inaccurate, or at least very incomplete.

Here, we compared low-temperature survivorship of six eastern North American populations of Asian clams, all identifiable as the same clonal morphotype, Form A (Lee et al. 2005). Although exposure to extreme environmental conditions can produce compromising non-lethal effects on feeding, growth, and reproduction (e.g., Morey et al. 2019), survivorship of such conditions can indicate the capacity for a species to establish in suboptimal habitats (McDowell et al. 2014). Recognizing that the persistence of a population requires its individuals to resume normal activities following a major stress, we also assessed the ability of clams to resume normal feeding at non-stressful temperatures after prolonged cold temperature exposure.

We hypothesized that survivorship would vary with population (site), acclimation temperature, and body size. Thus, we tested the following predictions:

(P1) Owing to their acclimation history in the field, individuals from the overwintering population in Lake George would have a higher survivorship under cold conditions than would individuals collected from the artificial thermal plume in the St. Lawrence River prior to the shutdown of the Gentilly-2 nuclear power plant.

(P2) Individuals from St. Lawrence River sites located farther away from the thermal discharge source, where water temperatures are relatively cooler in winter, would exhibit higher survivorship under cold conditions than those located closer to the discharge source.

(P3) Northern populations in general would exhibit a higher survivorship under cold conditions than would southern populations (cf. Addo-Bediako et al. 2000).

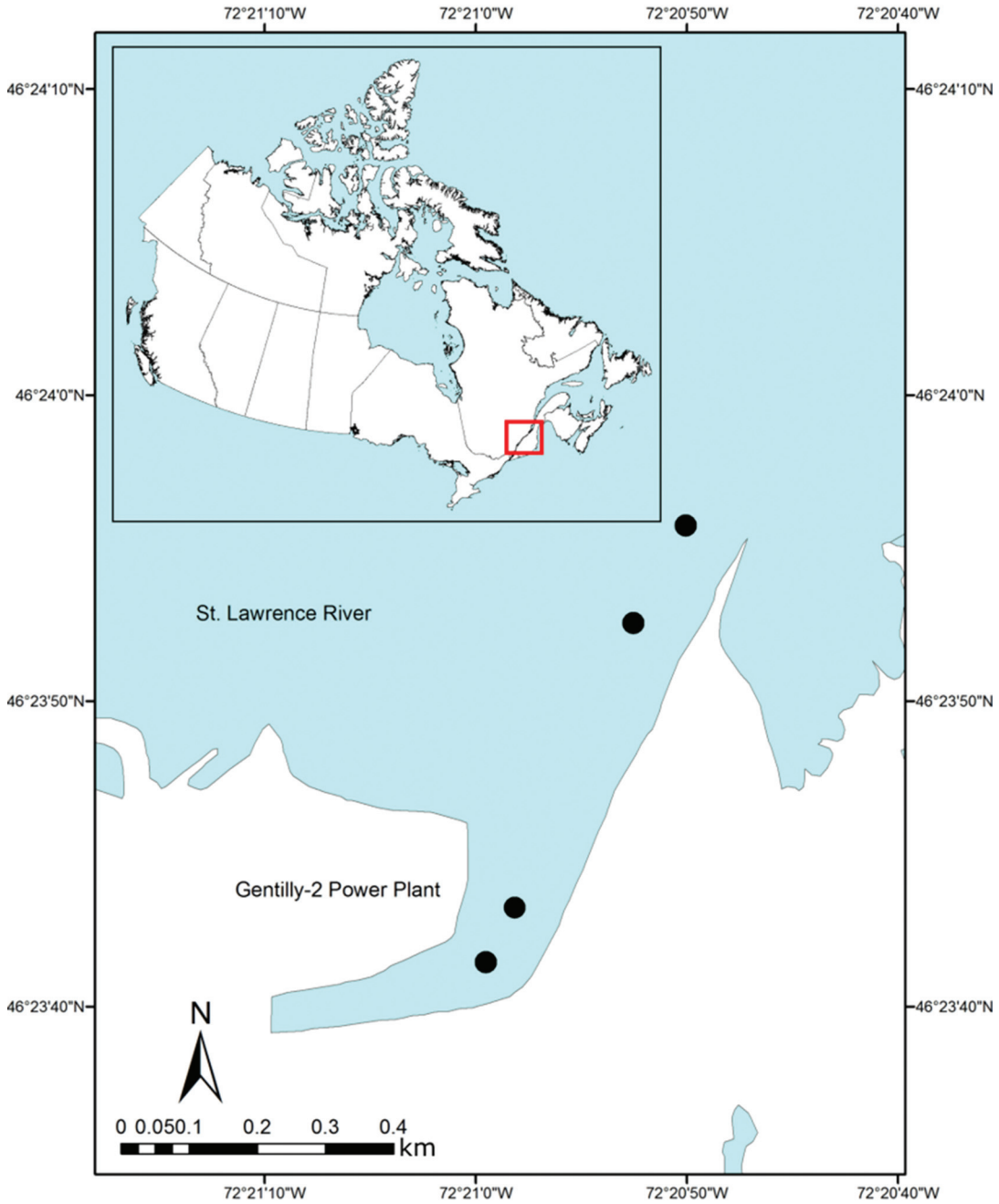
(P4) Initial acclimation to a lower temperature prior to exposure to cold conditions would enhance the survivorship of individuals from all populations (cf. Hoffmann and Watson 1993; Leroi et al. 1994).

(P5) Larger Asian clams would have higher survivorship than smaller clams, consistent with observations of various species of bivalves under thermal stress (Ansart and Vernon 2003; Rajagopal et al. 2005; Karatayev et al. 2011; see also Müller and Baur 2011), and this size-dependent survival could differentiate the performances of conspecific populations.

Materials and methods

Specimens were collected using either a Petite Ponar grab at depths 2.0–3.5 m (from St. Lawrence River, Seneca River, and Owasco Lake) or a hand-held sieve at depths of 0.3–1.0 m (from all other sites). All clams were active and appeared healthy at the

Fig. 1. Locations of St. Lawrence River Asian clam (*Corbicula fluminea*) populations along the discharge plume of the Gentilly-2 nuclear power plant at Bécancour, Quebec, Canada. Black dots: sites 1 to 4, moving downstream from the warmwater discharge source. Figure was created using ArcGIS release 10.8.1 (Esri, Inc. 2020) with Government of Canada "Open Government" data; St. Lawrence River outline was mapped using "Water File – Coastal Waters (polygons) – 2011 Census" and the inset of Canada was mapped using "Province and Territory Digital Boundary Files – 2011 Census" (https://open.canada.ca/data). Colour version online.



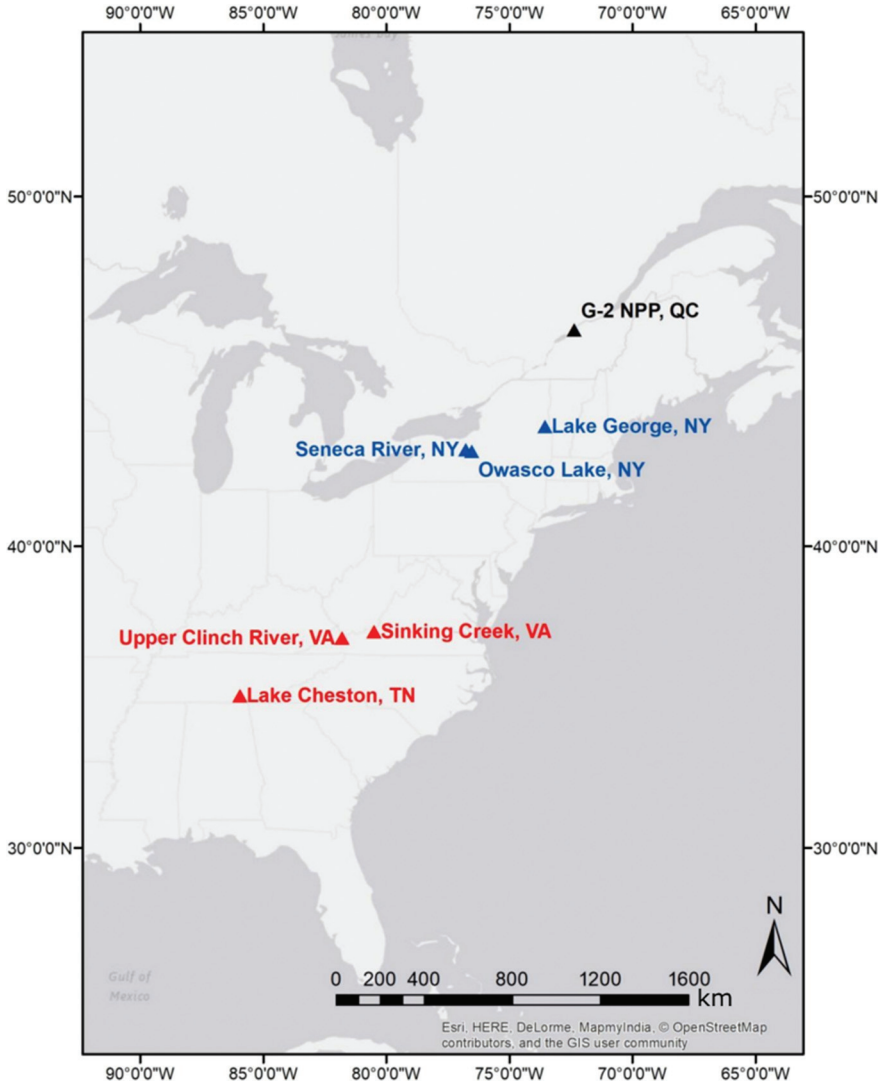
time of collection. Samples were placed in plastic containers filled with water from the site and transported to our laboratory in coolers within 3 to 24 h of collection. Clams from the three southern sites were shipped by overnight courier in sealed plastic containers with water and sediment. During transport in all cases, mortality was minimal (<2%).

Upon arrival in the laboratory, clams were placed in 50 L aquaria supplied with ~3 cm of pre-washed gravel and a charcoal filter in water maintained at 17–18 °C in a room with a 12 h dark : 12 h light cycle — except when under cold exposure, in which the clams were held in complete darkness in the temperature chamber. They were subjected to 20% water exchanges (using conditioned tap water) and fed with ground spirulina flakes (Nutrafin Max Spirulina Flakes; 5 mg/100 clams) every 2 days. During acclimation and exposure treatments, water temperature in each aquarium was monitored with an electronic logger and checked daily.

Experiment 1: St. Lawrence River versus Lake George populations

For this first experiment, Asian clams from the St. Lawrence River were collected from the thermal plume originating at the outflow of the Gentilly-2 nuclear power plant in Bécancour,

Fig. 2. Locations of Asian clam (*Corbicula fluminea*) populations used as sources for experimental animals. Northern populations are indicated in blue (G-2 NPP is the Gentilly-2 nuclear power plant); southern populations are indicated in red. Figure was created using ArcGIS release 10.8.1 (Esri, Inc. 2020). Light Gray Canvas basemap from Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community. Colour version online.



Quebec (46°23′42.5″N, 72°21′23.5″W). Four sites along a 1 km section of the plume (Fig. 1) were sampled repeatedly during other monitoring studies (Hamelin et al. 2016; Castañeda et al. 2018). Throughout the year, a 6 °C difference in mean water temperature existed between the two most widely spaced sites (Castañeda et al. 2018). In September 2012, we collected live clams at each site from 1.9 to 2.8 m depths using a Petite Ponar grab. In the laboratory, the clams were kept in 50 L aquaria separated according to their site of origin and maintained at 18 °C. Three times per week they were subjected with 20% water exchanges and fed with ground spirulina flakes. Clams were collected from Lake George (43°37′20″N, 73°32′48″W) (Fig. 2) in October 2012, shipped to the laboratory within 24 h, and were maintained in two additional tanks at 18 °C using the same protocol.

In January 2013, we began an 8-week experiment using 20 clams from each of the four St. Lawrence River sites and 20 clams from Lake George. Groups of 10 clams were separated by site into 10 different 16 L aquaria and acclimated by ramping down at 1 °C/h to a final temperature of 1 °C. The aquaria with the clams then were kept in a temperature-controlled chamber at 1 °C for 8 weeks. Clams were not fed during this period. To minimize disturbance to individuals, mortality was checked every 3 days and 20% of the water was replaced with conditioned water weekly. Mortality was checked by gently forcing the tip of a dissection needle between posterior valve margins in the region of the siphons (McMahon et al. 1995; Müller and Baur 2011); living clams resisted needle entry by firmly closing their valves, whereas dead clams failed to react after needle insertion. This test causes no apparent damage to live clams (McMahon et al. 1995; E. Cvetanovska, personal observation). After 8 weeks, temperatures were ramped up at the rate of 1 °C/h to 18 °C, and recovery (i.e., ability of mussels to survive exposure and return to normal feeding) was tracked for an additional 8 weeks using the same procedure as above. Every 2 days during recovery, water was replaced and clams were fed with spirulina flakes (5 mg/100 clams).

Experiment 2: northern versus southern populations

The results of the first experiment prompted a larger interpopulation comparison in which experimental animals were collected from six unheated waterbodies during autumn 2013

Table 1. Air temperature data for sites of populations tested in experiment 2 in 2013 (USGS 2014).

Location	Daily temperature (°C), December–March		Total number of days below 0 °C
	Grand mean	Mean minimum	
Lake George, New York. USA	–2.7	–8.1	152
Seneca River, New York, USA	–0.7	–4.0	121
Owasco Lake, New York, USA	–0.6	–4.7	103
Sinking Creek, Virginia, USA	5.2	1.0	54
Upper Clinch River, Virginia, USA	2.6	–1.5	93
Lake Cheston, Tennessee, USA	5.8	0.8	75

Note: The grand mean daily temperature and mean minimum daily temperature is shown for the period covering late autumn to early spring (1 December 2012 to 31 March 2013), encompassing the bulk of negative degree-days. Also shown is the total number of days of below-freezing temperatures from autumn 2012 to spring 2013. Data for Sinking Creek was based on a proxy site (Roanoke, Virginia, USA) and obtained from https://www.wunderground.com/history/monthly/us/va/roanoke/KROA/date/2013-4.

(late August to early-November). The three northern populations were located in New York State: Lake George (43°37′20″N, 73°32′48″W), Seneca River (42°56′08″N, 76°46′01″W), and Owasco Lake (42°53′35″N, 76°31′51″W).

The three southern populations were located at Sinking Creek, Virginia, USA (37°18′11″N, 80°29′09″W); Upper Clinch River, Virginia, USA (37°05′38.5″N, 81°47′08″W); and Lake Cheston, Tennessee, USA (35°12′36″N, 85°55′49″W) (Fig. 2). These populations were from sites that differed in exposure to low temperatures. Lake Cheston is a very small reservoir (area 0.03 km^2, depth 7 m) formed by the impoundment of a small stream; its winter surface water temperatures typically exceed 9 °C (D.B. Conn, unpublished data). Owing to their higher elevation and greater depths, the other two sites periodically experienced minimum water temperatures that approached our experimental treatment; surface water temperatures typically range from 0.5 to 29.5 °C for Sinking Creek and from 1.5 to 25 °C for Upper Clinch River (D. Hua, Tennessee Wildlife Resource Agency, personal communication). However, among the southern sites, Upper Clinch River had experienced the greatest number of days in which the mean daily temperature was below 0 °C and was the only site whose mean minimum winter temperature was below 0 °C (Table 1).

The 10 °C acclimation trial

Two replicate groups of ten clams from each of the six sites were kept separately in twelve 2.5 L aquaria. The ambient water temperature (17–18 °C) was ramped down to 10 °C at a rate of 2 °C/day. The aquaria were then maintained in a temperature-controlled chamber at 10 °C for a 30-day acclimation period; during this time, clams were fed with ground spirulina flakes. Following acclimation, the ambient water temperature was ramped down to 1 °C at a rate of 2 °C/day and the aquaria were subsequently maintained in a temperature-controlled chamber at 1 °C for the 8-week experimental period; during this time, clams were not fed and they were checked twice per week for mortality, using the same procedure as in experiment 1. As a control for the experiments, clams from all 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 were maintained for >6 months; mortality was negligible (<2%) during this time. At the end of the 8-week exposure period, temperatures were raised at the rate of 1 °C/h to 18 °C, after which tanks were moved to a temperature-controlled wet laboratory and maintained at 16–18 °C for an 8-week recovery period. Water was changed every 2 days during the acclimation, exposure, and recovery periods; clams were fed every 2 days during the acclimation and recovery periods. Mortality was checked daily and dead clams were removed immediately.

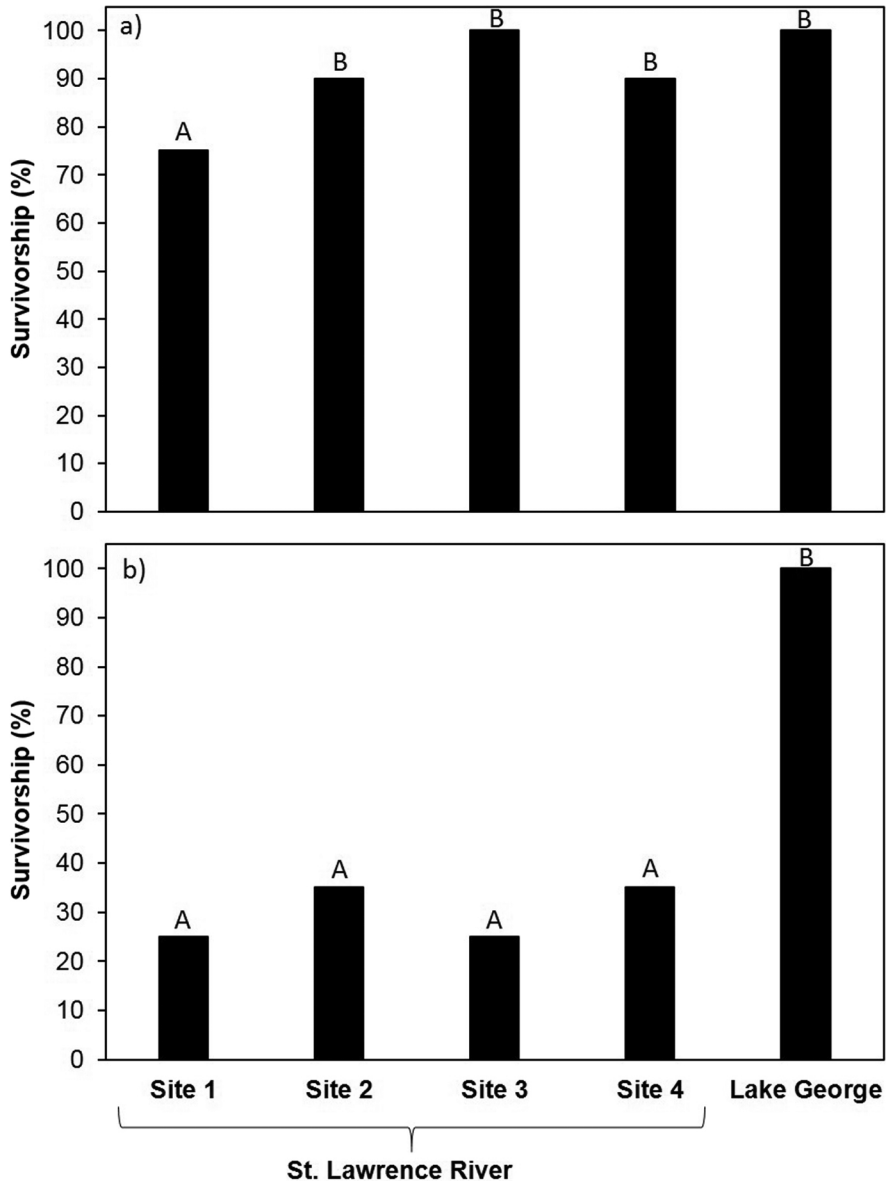
The 18 °C acclimation trial

In this experimental trial, four of the six populations were used, because clams from Seneca River and Lake Cheston populations were depleted from the previous experimental trial more rapidly than anticipated and there were insufficient numbers for this second trial. Two groups of 10 clams from each of the remaining four sites were separated into eight 2.5 L aquaria. The aquaria were kept in a temperature-controlled room at 18 °C for a 30-day acclimation. Immediately following acclimation, clams were moved to an experimental chamber and the water temperature was ramped down to 1 °C at a rate of 2 °C/day. The aquaria were maintained at 1 °C for 8 weeks, as in the previous experiment. At the end of the exposure period, water temperatures were raised to 12 °C at 2 °C/day and all tanks were moved to the temperature-controlled laboratory maintained at 16–18 °C for an 8-week recovery period, following the protocol for the previous experiment. In all trials for this and the previous experiment, water temperatures were monitored using electronic temperature loggers.

Statistical analysis

All statistical analyses were conducted using R (version 3.6.1 2019; R Foundation for Statistical Computing, Vienna, Austria, available from https://www.r-project.org). For data from experiment 1, the assumption of normality was not met; therefore, a χ^2 test was used to examine differences in survival among all five sites and the post hoc χ^2 test package was used to identify population differences. For experiment 2, survivorship results were analyzed using a generalized linear mixed model (GLMM) with a binomial error distribution and logit link, which is appropriate for non-normal data containing random effects and a binary response variable (Bolker et al. 2009). The binary response variable was clam survivorship (alive or dead) after the recovery period, with fixed factors of site and clam size (maximum length, millimetre) and a random effect of tank nested within population. Owing to the difference in the number of populations tested at each acclimation temperature, and because these two treatments were conducted separately, separate GLMMs were run for the 10 °C and 18 °C acclimations. Interpopulation differences were compared using a multiple comparisons Tukey's HSD post hoc test. The GLMM-predicted probabilities of survivorship were plotted against body size (length).

Fig. 3. Experiment 1: survivorship (%) of Asian clams (*Corbicula fluminea*) (*a*) after 8 weeks of exposure at 1 °C and (*b*) after an additional 8 weeks of recovery at 18 °C at four sites along the St. Lawrence River (sites 1–4) and at one site from Lake George. Different letters above the bars indicate $p < 0.05$ (χ^2 post hoc test).



For experiment 2, daily variation in survivorship following the 10 °C acclimation experiment was explored further using the Kaplan–Meier method to generate survivorship curves. The relationship between location and survivorship was tested using a Cox model (Cox and Oakes 1984), and survivorship curves were compared among populations using the log-rank (Mantel–Cox) test. To determine exactly which populations differed in survival, a multiple comparisons Tukey's HSD post hoc test was used.

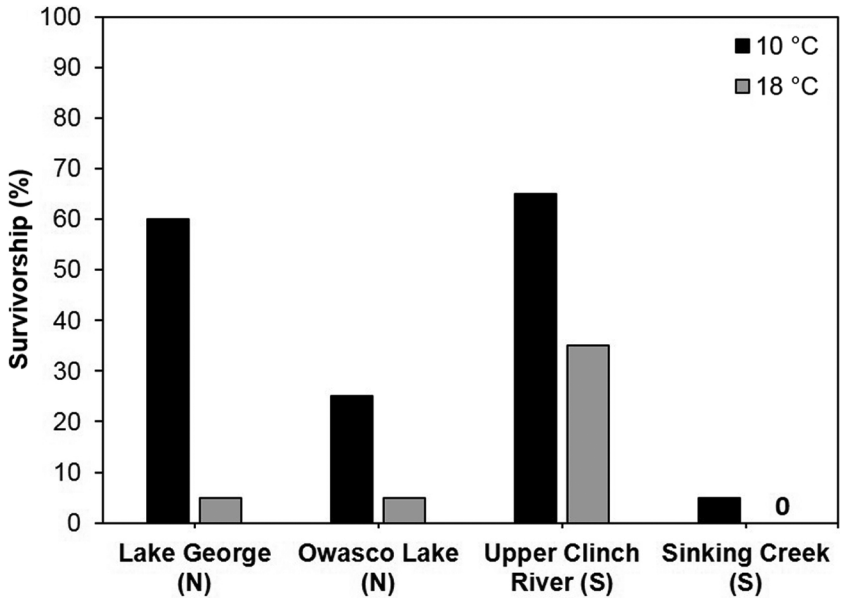
Results

Experiment 1: St. Lawrence River and Lake George populations

After 8 weeks of exposure at 1 °C, all clams (100%) from the Lake George population survived, whereas St. Lawrence River clams had 75%–100% survivorship across all four sites (Fig. 3*a*). Survivorship during exposure was statistically lower for clams from the St. Lawrence River site-1 population ($\chi^2_{[4]} = 11.69$, $p = 0.02$, $n = 100$; χ^2 post hoc, $p = 0.02$), partially supporting our prediction (P2) that survivorship would increase with distance from the discharge point. During the subsequent recovery period, the survival of clams from Lake George (again 100%) was significantly higher than that of clams from the St. Lawrence River, which ranged between 25% and 35% across all four sites (Fig. 3*b*; $\chi^2_{[4]} = 28.94$, $p < 0.001$, $n = 100$; χ^2 post hoc (Lake George, $p < 0.001$)), supporting our first prediction (P1). There was no tank effect on the survivorship of the clams ($\chi^2_{[4]} = 2.21$, $p = 0.7$, $n = 100$). Survivorship of the St. Lawrence River populations did not differ from each other after recovery (Figs. 3*a* and 3*b*); however, the lower survivorship of clams from the site closest to the thermal discharge is consistent with our prediction (P2).

During exposure, all clams closed their valves tightly and sealed the valve edges with hardened mucus; this behaviour was exhibited in each experimental trial in our study.

Fig. 4. Experiment 2: full model comparison of variation in survivorship (%) of Asian clams (*Corbicula fluminea*) after exposure and recovery at experimental acclimation temperatures of 10 °C (black bars; n = 20) and 18 °C (grey bars; n = 20). Sample sites are organized from left to right to reflect the distribution of populations from north to south.



Experiment 2: northern versus southern populations

Survivorship of clams from the Lake George and Upper Clinch River populations differed from each other, although not significantly (p = 0.056), and both of these populations had higher survivorship than each of the other populations (Fig. 4; Supplementary Table S1[1]). Survivorship was affected by acclimation temperature (GLMM, $z = -3.77$, $p = 0.0002$) such that it was higher for clams acclimated at 10 °C (mean (±1 SE) survivorship = 38.75% ± 14.3%) than at 18 °C (11.25% ± 8.0%), supporting our prediction (P4). Clam body size (Table 2) had a positive effect on survivorship at both acclimation temperatures (GLMM, $z = -0.054$, $p = 0.04$; Fig. 5), supporting our final prediction (P5).

The 10 °C acclimation trial

After 10 °C acclimation, 40.8% of all experimental individuals (n = 120, pooled across all populations) survived the full duration of the experiment. Although some individuals from each of the six populations remained alive, clams from northern populations generally had a higher survivorship (55.0% ± 16.1%) than those from southern populations (26.7% ± 19.2%) (GLM, $z = -3.597$, $p = 0.0003$), supporting our prediction (P3). Seneca River had the highest survivorship (80.0%), followed by the Upper Clinch River (65.0%) and Lake George (60.0%) populations (Supplementary Table S2[1]). By contrast, the majority (75%–95%) of individuals in the remaining three populations (Owasco Lake, Lake Cheston, and Sinking Creek) died by the end of the experiment (Fig. 6). In addition, larger body size had a positive effect on survivorship (GLMM, $z = -2.415$, $p = 0.016$).

The survival curves for the six populations in the 10 °C acclimation experiment differed significantly over the duration of the exposure and recovery period (log-rank test, $\chi^2_{[6]} = 45.68$, $p < 0.005$) (Fig. 7). Survivorship of clams from Lake George, Seneca River, and Upper Clinch River populations did not differ from each other, but was higher over time compared with those from Owasco Lake, Sinking Creek, and Lake Cheston, which also did not differ from each other (Supplementary Table S3[1]).

Table 2. Size range and mean length (measured anterior to posterior) of experimental clams from six populations of Asian clams (*Corbicula fluminea*).

Location	Size range (length, mm)	Mean length (mm)
St. Lawrence River, Quebec, Canada	9.7–37.4	18.8
Lake George, New York, USA	14.1–18.7	16.6
Seneca River, New York, USA	22.4–28.1	25.5
Owasco Lake, New York, USA	10.8–14.1	12.2
Sinking Creek, Virginia, USA	10.7–16.7	12.7
Upper Clinch River, Virginia, USA	19.9–25.6	22.9
Lake Cheston, Tennessee, USA	18.4–28.9	23.8

The 18 °C acclimation trial

Only 11.3% of the 80 clams acclimated at 18 °C survived the duration of the experiment. Survivorship was highest for the Upper Clinch River population (35.0%), which had the largest individuals among the four populations tested (mean 22.9 mm, range 19.9–25.6 mm). Survivorship did not differ among the remaining populations: 5.0% of clams from Lake George and Owasco Lake survived, whereas no clams from Sinking Creek survived the experiment. In contrast to the results at 10 °C acclimation, neither site nor clam size was found to have a significant effect on survivorship ($p > 0.05$).

Discussion

Variation in cold tolerance across populations

Risk assessments, in general, have not considered *C. fluminea* a major invasive threat to mid- to high-latitude waterbodies, owing to its presumed intolerance to temperatures below 2 °C (e.g., Foster et al. 2021). However, this view has been challenged by observations of populations persisting in northern waterbodies and in controlled experiments (Müller and Baur 2011; Minchin 2014; Natale et al. 2014; Lake George Park Commission 2018; Richardson and Selby 2020). Our results suggest the potential for clams from

[1]Supplementary tables are available with the article at https://doi.org/10.1139/cjz-2020-0226.

Fig. 5. Experiment 2: probability of survivorship of Asian clams (*Corbicula fluminea*) from the full model, including both 10 °C acclimation and 18 °C acclimation experiments, as a function of body size (length). Line fitted by a binary logistic regression (p = 0.04). Shaded grey area represents the 95% confidence intervals. Colour version online.

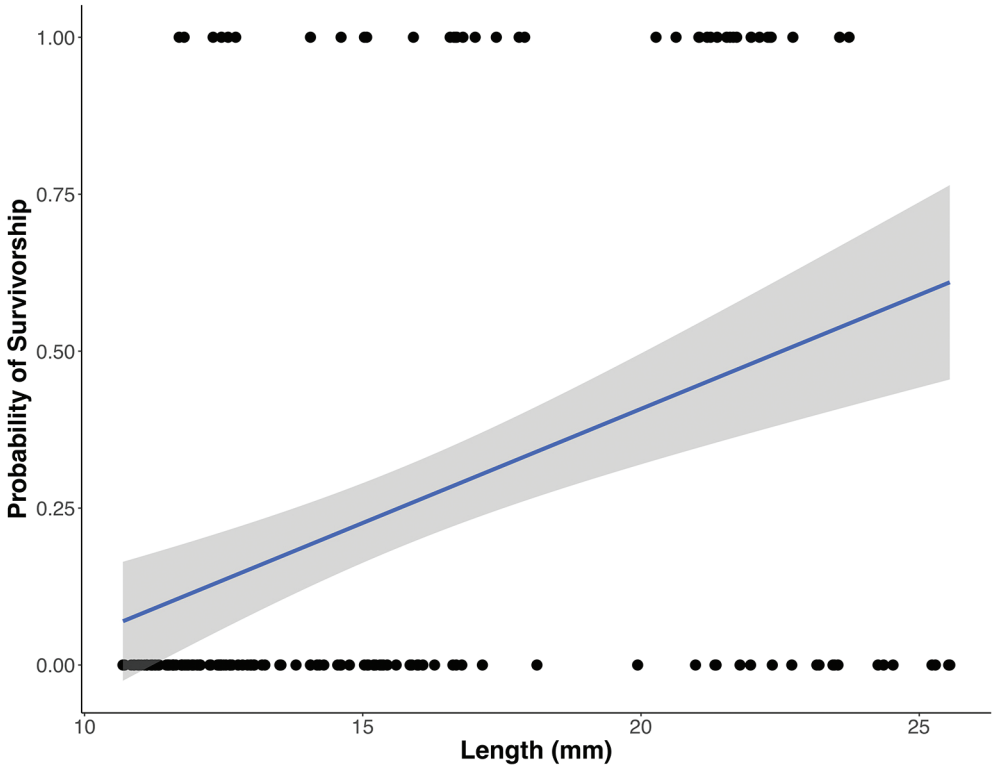


Fig. 6. Experiment 2: variation in survivorship (%) of Asian clams (*Corbicula fluminea*) after exposure and recovery from 10 °C acclimation experiment. Each bar (black, northern populations; grey, southern populations) represents the mean survivorship from each site (n = 20). Different letters indicate differences at $p < 0.05$.

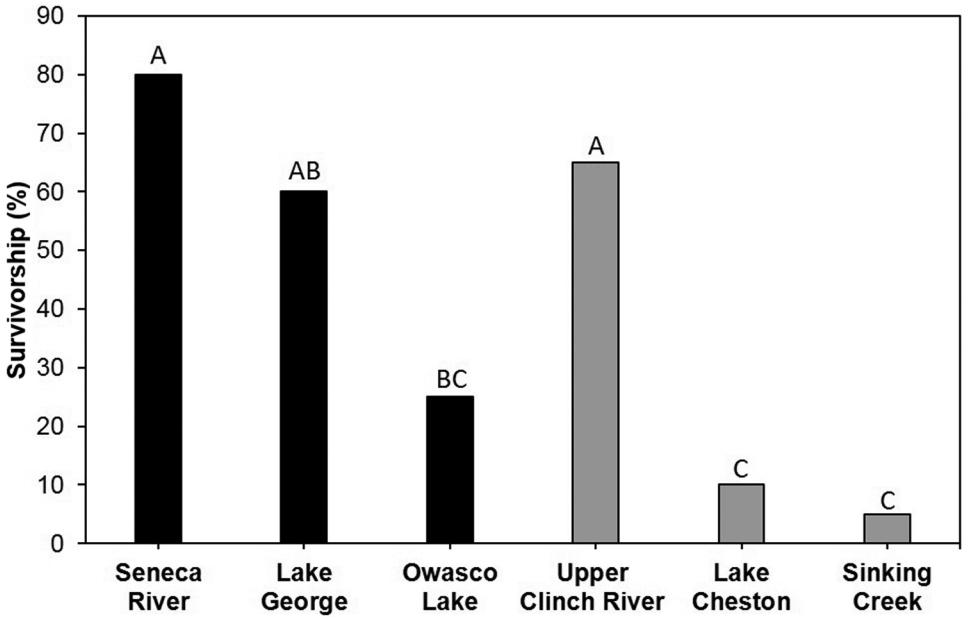
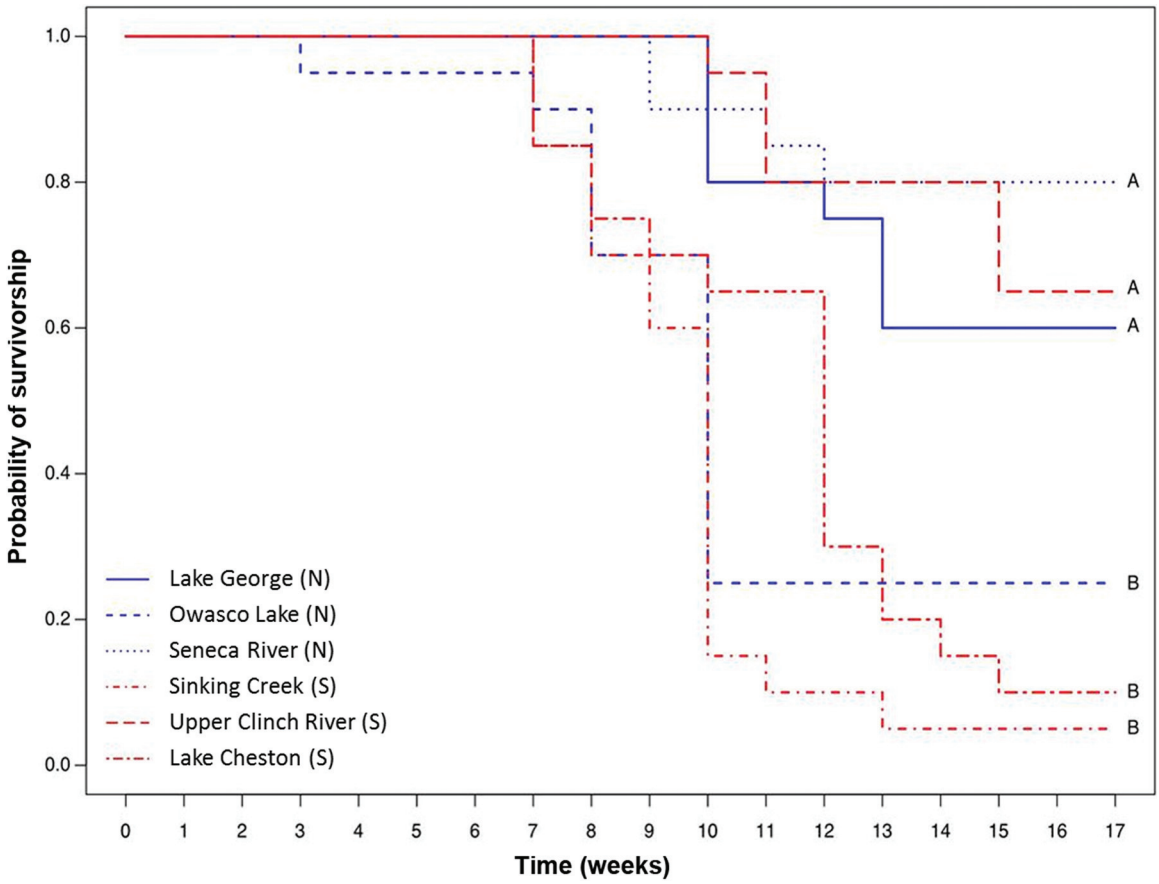


Fig. 7. Experiment 2: Kaplan–Meier survivorship curves for Asian clams (*Corbicula fluminea*) from northern (N) and southern (S) populations over the duration of the 16-week, 10 °C acclimation trial. In the first 8 weeks, clams were exposed to an experimental temperature of 1 °C; in the next 8 weeks, clams were held at 18 °C for the recovery period. Survivorship curves represent the mean survivorship from each site (n = 20). Curves with shared letters (A or B) do not differ significantly.



different source populations to survive near-freezing conditions, but the capacity to do so varies across locations. In the first experiment, clams from the thermal plume population in the St Lawrence River exhibited lower tolerance to near-freezing conditions than those from the overwintering population in Lake George (Fig. 3*b*), supporting our first prediction. However, it is noteworthy that differences in survivorship became pronounced during the recovery period, which suggested that some individuals who endured the exposure treatment were too stressed to persist. In this case, the recovery period is perhaps analogous (although much accelerated by comparison) to the early-spring transition from reduced activity to regular feeding and growth as water temperatures warm in a north temperate climate.

Fortunately, we can compare these results to observations of the St. Lawrence River population's response to natural winter temperatures in the field. In December 2012, 4 months after clams were collected from the thermal plume for our experiments, the Gentilly-2 nuclear power plant was shut down permanently, thereby re-establishing winter temperatures in that section of the river for the first time since the 1970s. A mass die-off of the *C. fluminea* population occurred over the following winter and spring, as evidenced by dense aggregations of fresh empty shells observed during two intensive sampling periods that failed to locate any live clams in summer 2013 (Castañeda et al. 2018). The entire St. Lawrence River population was apparently extirpated following exposure to near-freezing water temperatures, whereas the overwintering population in Lake George has persisted to date. Although we would not generalize from this single pair of observations, we note that the difference in survivorship measured in our experiment is consistent with the differential performance of these two populations under winter conditions in the field.

The second series of experiments examined cold tolerance across populations from unheated waterbodies in eastern North America (Fig. 2). These include three southern populations (Upper Clinch River, Sinking Creek, and Lake Cheston) that have been established for at least a few decades and that experience relatively mild climatic conditions, and three northern populations (Lake George, Seneca River, and Owasco Lake) that became established more recently and experience extended periods of prolonged exposure to near-freezing conditions every winter. This experiment provided additional evidence that *C. fluminea* has a greater tolerance to low temperatures than commonly reported. All populations, regardless of their location or acclimation history, had some individuals that survived prolonged exposure to 1 °C and resumed feeding and burrowing activities (indicated by their movements and conspicuous filtration activity; E. Cvetanovska, personal observation). After acclimation to 10 °C, the three northern populations exhibited greater cold tolerance than two of the southern ones, whereas one southern population (Upper Clinch River) exhibited tolerance similar to that of the Lake George population (Fig. 7). We suggest that winter conditions at the Upper Clinch River site, whose daily air temperatures are more frequently below 0 °C during winter and spring than those of either Lake Cheston or Sinking Creek (Table 1),

could have enhanced its survivorship at low temperatures through natural acclimation.

Behavioural adaptations for surviving stress

Although our clams were not fed for the duration of the 8-week exposure period, food limitation can perhaps be dismissed as having had a major influence on observed survivorship, given evidence that at least some populations can survive 5 months of starvation at room temperatures of 22–24 °C (Thorp and Covich 2009). Starvation is likely to be more stressful to clams under warm summer temperatures, when energetic demands are typically great (Dillon et al. 2010). At low temperatures, clams close their valves to reduce metabolic activity (Guppy and Withers 1999; Ortmann and Grieshaber 2003). Indeed, during the cold temperature treatments in both our experiments, clams sealed the valves with hardened mucus, thereby preventing direct exposure to the external environment. Similar behaviour was observed by Matthews and McMahon (1999), who noted that *C. fluminea* can remain anaerobic with the valves shut for at least 3 to 4 days at high temperatures and for several weeks at low temperatures. They suggested that clams can remain in this state until anaerobic end products (such as acetate, propionate, and succinate) accumulate to an extreme level, ultimately forcing them to open their valves and bathe their gills to eliminate the toxins. Ortmann and Grieshaber (2003) also studied valve movement of *C. fluminea* and found that clams can shut their valves for periods of several days, during which time they reduce their metabolism to less than 10% of the standard rate measured when valves are open. Such a response has been documented in other bivalves (Kramer et al. 1989; Holopainen and Penttinen 1993; Borcherding and Jantz 1997) and is beneficial during limited periods of starvation and other unfavorable conditions (McMahon 1999; Ortmann and Grieshaber 2003; Sousa et al. 2008). Similar metabolic reductions have been described for various invertebrates facing severe stress (Guppy and Withers 1999).

Size-dependent survivorship

In support of our prediction (P5), the probability of survivorship in our experiments increased with clam size. Size-dependent survivorship of cold temperatures by *C. fluminea* has been reported elsewhere, both in the laboratory (Müller and Baur 2011) and in the field (French and Schloesser 1991; Werner and Rothhaupt 2008). In a population inhabiting the thermal plume of a power plant on the St. Clair River (Michigan), increased winter mortality of *C. fluminea* was observed at the most remote downstream sites where water temperatures were less buffered by thermal discharge; first-year clams were disproportionately affected, suggesting greater sensitivity in smaller individuals (French and Schloesser 1991). Size-dependent survivorship of exposure to extreme temperatures where small individuals suffer higher and more rapid mortality has been reported for other bivalves (Ansart and Vernon 2003; Rajagopal et al. 2005; Karatayev et al. 2011). A common cause of mortality in organisms subjected to overwintering stress is energy deficiency (Shuter et al. 2012; Fernandes and McMeans 2019), and if starvation is an issue, then larger animals could be more resistant because of their greater capacity to store energy (Finstad et al. 2004; Eckmann 2004). In addition, smaller molluscs are at greater risk of tissue freezing (Ansart and Vernon 2003).

Mechanisms of developing cold tolerance in the wild

Three non-exclusive mechanisms exist for the variance in cold tolerance among conspecific populations in their invaded range: plasticity, selection within a generation, and contemporary evolution. Each of these contributes to the invasion success of species in general (Terblanche and Chown 2006; Ghalambor et al. 2007; Früh et al. 2012; Colautti and Lau 2015). Formally distinguishing between these alternatives generally requires common-garden experiments spanning at least an entire generation (Merilä and Hendry 2014; Hendry 2017). We worked only with adult clams captured from the wild — a typical first step in studies of interpopulation variation — and therefore cannot clearly parse the relative contributions of plasticity and contemporary evolution. However, it is nevertheless useful to leverage existing data and information to address the potential importance of each mechanism.

Plasticity clearly has the potential to shape interpopulation variation in cold tolerance for *C. fluminea* in North America. We found that acclimating clams at cold temperatures had dramatically improved their survival after subsequent exposure to even colder temperatures, but variation in survivorship was pronounced across populations (Figs. 4 and 6). Those individuals from northern populations had a better acclimation potential than two of the three southern populations; the exception was the Upper Clinch River population, which exhibited survivorship similar to that of the overwintering population in Lake George and thus indicated that even individuals from some southern locations can survive winter in a temperate region. The early-life winter exposure of clams from the northern populations might have prepared them for exposure to near-freezing temperatures in our experiments. However, plasticity seems unlikely to be the sole reason for the observed variation in survivorship, given that removal of the thermal effluent plume in the St. Lawrence River apparently caused the extinction of that entire population (Castañeda et al. 2018), contrary to our prediction (P2).

Selection within a generation could contribute to interpopulation variation by removing individuals from northern populations that had the weakest cold tolerance (cf. Johnson et al. 2014). That is, a wide range of individual cold tolerance might be present in all populations at the start of each generation, but mortality could winnow out all but the most tolerant individuals from the northern but not the southern populations. We suggest that this mechanism is not the sole reason for variation among populations, given the striking differences observed: for example, after acclimation to 10 °C, only 5% of Sinking Creek individuals survived prolonged exposure to 1 °C, whereas 60% of Lake George individuals survived the same conditions. Contemporary evolution could also play a role, as a number of studies have documented the evolution of cold tolerance in many species on relatively short time frames of years to decades (Barrett et al. 2011; Diamond et al. 2017; Campbell-Staton et al. 2017). Indeed, such changes have been observed in other bivalves (e.g., Thyrring et al. 2019). Given the apparent insufficiency of plasticity and selection within a generation (as explained above), contemporary evolution seems likely to have contributed to the remarkable cold tolerance of some *C. fluminea* populations in North America. It was believed that the much of the plasticity in life-history traits exhibited by *C. flumina* is environmentally induced, owing to its low genetic diversity in North America (McMahon 1999; Pigneur et al. 2014). However, androgenetic reproduction in *Corbicula* (as well as in other highly invasive species; Schwander and Oldroyd 2016) not only facilitates rapid colonization but could also allow for increased, potentially adaptive genetic variation (Hedtke et al. 2011). Further focused experiments are necessary to measure this genetic variation and establish its potential role in the future spread of *C. fluminea*.

Implications for risk assessment

Our experiments demonstrate the capacity for acquired cold tolerance by *C. fluminea* following acclimation, as well as strong interpopulation variation in ability to survive near-freezing temperatures. Geographically dispersed conspecific populations that have developed under different thermal conditions can differ greatly in thermal tolerance (see also Hoffmann and Watson 1993; Elderkin and Klerks 2005; Wallace et al. 2014), highlighting the need for risk assessments to incorporate physiological data from multiple populations. Furthermore, our results suggest that a series of failed establishments, or an extirpation of a

population following establishment, is no indication of invulnerability against subsequent invasion if propagules are re-introduced from a different source population.

Corbicula fluminea populations subject to seasonal near-freezing temperatures in Lake George, Owasco Lake, and Seneca River in New York State (and in other areas, such as the Clinton River in Michigan; Janech and Hunter 1995), seem to be persisting at the edge of their lower lethal limit. However, farther northward expansion might not be limited solely by cold tolerance. Studies suggest that *C. fluminea* requires temperatures of 14–15 °C for reproduction (McMahon 1999); low spring temperatures could thus restrict reproduction and farther northward expansion (McMahon 1999; Werner and Rothhaupt 2008) until such barriers are lifted as a result of climate warming.

Acknowledgements

We thank D. Hua (Tennessee Wildlife Resource Agency) and R. Neves (Freshwater Mollusk Conservation Center, Virginia Tech) for providing specimens from Virginia, and M. Modley (Lake Champlain Basin Program) and S. Nierzwicki-Bauer (Darrin Fresh Water Institute) for arranging access to Lake George specimens. K. Hamelin and A. Morden provided valuable assistance in the field. Funding to A.R. from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

References

Addo-Bediako, A., Chown, S.L., and Gaston, K.J. 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. B Biol. Sci. **267**: 739–745. doi:10.1098/rspb.2000.1065. PMID:10819141.

Ansart, A., and Vernon, P. 2003. Cold hardiness in molluscs. Acta Oecol. **24**: 95–102. doi:10.1016/S1146-609X(03)00045-6.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B., et al. 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Funct. Ecol. **18**: 700–706. doi:10.1111/j.0269-8463.2004.00904.x.

Barrett, R.D.H., Paccard, A., Healy, T.M., Bergek, S., Schulte, P.M., Schluter, D., and Rogers, S.M. 2011. Rapid evolution in cold tolerance in stickleback. Proc. R Soc. B Biol. Sci. **278**: 233–238. doi:10.1098/rspb.2010.0923. PMID:20685715.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., and White, J.-S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. **24**: 127–135. doi:10.1016/j.tree.2008.10.008. PMID:19185386.

Borcherding, J., and Jantz, B. 1997. Valve movement response of the mussel *Dreissena polymorpha* — the influence of pH and turbidity on the acute toxicity of pentachlorophenol under laboratory and field conditions. Ecotoxicology, **6**: 153–165. doi:10.1023/A:1018666525205.

Buckley, L.B., Hurlbert, A.H., and Jetz, W. 2012. Broad-scale ecological implications of ectothermy and endothermy in changing environments. Global Ecol. Biogeogr. **21**: 873–885. doi:10.1111/j.1466-8238.2011.00737.x.

Campbell-Staton, S.C., Cheviron, Z.A., Rochette, N., Catchen, J., Losos, J.B., and Edwards, S.V. 2017. Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. Science, **357**: 495–498. doi:10.1126/science.aam5512. PMID:28774927.

Castañeda, R., Cvetanovska, E., Hamelin, K., Simard, A., and Ricciardi, A. 2018. Distribution, abundance and condition of an invasive bivalve (*Corbicula fluminea*) along an artificial thermal gradients in the St. Lawrence River. Aquat. Invasions, **13**: 379–392. doi:10.3391/ai.2018.13.3.06.

Chown, S.L., Slabber, S., McGeoch, M.A., Janion, C., and Leinaas, H.P. 2007. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proc. R. Soc. B Biol. Sci. **274**: 2661–2667. doi:10.1098/rspb.2007.0772. PMID:17686728.

Colautti, R.I., and Lau, J.A. 2015. Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Mol. Ecol. **24**: 1999–2017. doi:10.1111/mec.13162. PMID:25891044.

Cox, D.R., and Oakes, D. 1984. Analysis of survival data. CRC Press, Boca Raton, Fla.

Crespo, D., Dolbeth, M., Leston, S., Sousa, R., and Pardal, M. 2015. Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. Biol. Invasions, **17**: 2087–2101. doi:10.1007/s10530-015-0862-y.

Davidson, A.M., Jennions, M., and Nicotra, A.B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol. Lett. **14**: 419–431. doi:10.1111/j.1461-0248.2011.01596.x. PMID:21314880.

Diamond, S.E., Chick, L., Perez, A., Strickler, S.A., and Martin, R.A. 2017. Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. Biol. J. Linn. Soc. **121**: 248–257. doi:10.1093/biolinnean/blw047.

Dillon, M.E., Wang, G., and Huey, R.B. 2010. Global metabolic impacts of recent climate warming. Nature, **467**: 704–706. doi:10.1038/nature09407. PMID:20930843.

Eckmann, R. 2004. Overwinter changes in mass and lipid content of *Perca fluviatilis* and *Gymnocephalus cernuus*. J. Fish Biol. **65**: 1498–1511. doi:10.1111/j.0022-1112.2004.00553.x.

Elderkin, C.L., and Klerks, P.L. 2005. Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, *Dreissena polymorpha*. J. Shellfish Res. **24**: 221–226. doi:10.2983/0730-8000(2005)24[221:VITTAT]2.0.CO;2.

Esri, Inc. 2020. ArcGIS Desktop. Release 10.8.1. Environmental Systems Research Institute (Esri), Inc., Redlands, Calif.

Fangue, N.A., Hofmeister, M., and Schulte, P.M. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. J. Exp. Biol. **209**: 2859–2872. doi:10.1242/jeb.02260. PMID:16857869.

Fernandes, T., and McMeans, B.C. 2019. Coping with the cold: energy storage strategies for surviving winter in freshwater fish. Ecography, **42**: 2037–2052. doi:10.1111/ecog.04386.

Ferreira-Rodriguez, N., Fandiño, L., Pedreira, A., and Pardo, I. 2018. First evidence of asymmetric competition between the non-native clam *Corbicula fluminea* and the native freshwater mussel *Unio delphinus* during a summer heat wave. Aquat. Conserv. Mar. Freshw. Ecosyst. **28**: 1105–1113. doi:10.1002/aqc.2964.

Finstad, A.G., Ugedal, O., Forseth, T., and Næsje, T.F. 2004. Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **61**(12): 2358–2368. doi:10.1139/f04-213.

Foster, A.M., Fuller, P., Benson, A., Constant, S., Raikow, D., Larson, J., and Fusaro, A. 2021. *Corbicula fluminea* (O. F. Müller, 1774). U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, Fla., and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, Mich. Available from https://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx?SpeciesID=92 [revision date: 13 September 2019; accessed 18 March 2021].

French, J.R.P., and Schloesser, D.W. 1991. Growth and overwinter survival of the Asiatic clam, *Corbicula fluminea*, in the St. Clair River, Michigan. Hydrobiologia, **219**: 165–170. doi:10.1007/BF00024753.

French, J.R.P., and Schloesser, D.W. 1996. Distribution and winter survival health of Asian clams, *Corbicula fluminea*, in the St. Clair River, Michigan. J. Freshw. Ecol. **11**: 183–192. doi:10.1080/02705060.1996.9663477.

Früh, D., Stoll, S., and Haase, P. 2012. Physico-chemical variables determining the invasion risk of freshwater habitats by alien mollusks and crustaceans. Ecol. Evol. **2**: 2843–2853. doi:10.1002/ece3.382. PMID:23170218.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., and Reznick, D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. **21**: 394–407. doi:10.1111/j.1365-2435.2007.01283.x.

Grabowska, J., and Przybylski, M. 2015. Life-history traits of non-native freshwater fish invaders differentiate them from natives in the Central European bioregion. Rev. Fish Biol. Fish. **25**: 165–178. doi:10.1007/s11160-014-9375-5.

Guppy, M., and Withers, P. 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol. Rev. **74**: 1–40. doi:10.1017/S0006323198005258. PMID:10396183.

Hamelin, K.M., Castañeda, R., and Ricciardi, A. 2016. Cryptic invaders: Non-indigenous and cryptogenic freshwater Bryozoa and Entoprocta in the St. Lawrence River. Biol. Invasions, **18**: 1737–1744. doi:10.1007/s10530-016-1116-3.

Hedtke, S.M., Glaubrecht, M., and Hillis, D.M. 2011. Rare gene capture in predominantly androgenetic species. Proc. Natl. Acad. Sci. U.S.A. **108**: 9520–9524. doi:10.1073/pnas.1106742108. PMID:21606355.

Hendry, A.P. 2017. Eco-evolutionary dynamics. Princeton University Press, Princeton, N.J.

Hoffmann, A.A., and Watson, M. 1993. Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. Am. Nat. **142**: S93–S113. doi:10.1086/285525. PMID:19425954.

Holopainen, I., and Penttinen, O.-P. 1993. Normoxic and anoxic heat output of the freshwater bivalves *Pisidium* and *Sphaerium*. Oecologia, **93**: 215–223. doi:10.1007/BF00317674. PMID:28313610.

Horning, W.B., and Keup, L. 1964. Decline of Asiatic clam in Ohio River. Nautilus, **78**: 29–30.

Janech, M., and Hunter, R. 1995. *Corbicula fluminea* in a Michigan river: Implications for low temperature tolerance. Malacol. Rev. **28**: 119–124.

Johnson, D.W., Grorud-Colvert, K., Sponaugle, S., and Semmens, B.X. 2014. Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecol. Lett. **17**: 743–755. doi:10.1111/ele.12273. PMID:24674603.

Karatayev, A.Y., Mastitsky, S.E., Padilla, D.K., Burlakova, L.E., and Hajduk, M.M. 2011. Differences in growth and survivorship of zebra and quagga mussels: size matters. Hydrobiologia, **668**: 183–194. doi:10.1007/s10750-010-0533-z.

Kolar, C.S., and Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. Trends Ecol. Evol. **16**: 199–204. doi:10.1016/S0169-5347(01)02101-2. PMID:11245943.

Kramer, K.J., Jenner, H.A., and de Zwart, D. 1989. The valve movement response of mussels: a tool in biological monitoring. Hydrobiologia, **188–189**: 433–443. doi:10.1007/BF00027811.

Kreiser, B.R., and Milton, J.B. 1995. The evolution of cold tolerance in *Corbicula fluminea* (Bivalvia: Corbiculidae). Nautilus, **109**(4): 111–112.

Lake George Park Commission. 2018. Lake George Asian Clam lake-wide survey: final report. Lake George Park Commission, Lake George, N.Y. Available from https://lgpc.ny.gov/system/files/documents/2018/09/2018-asian-clam-lakewide-survey-final-report.pdf.

Lee, T., Siripattrawan, S., Ituarte, C.F., and Foighil, D.Ó. 2005. Invasion of the clonal clams: *Corbicula* lineages in the New World. Am. Malacol. Bull. **20**: 113–122.

Lenz, M., da Gama, B.A.P., Gerner, N.V., Gobin, J., Gröner, F., Harry, A., et al. 2011. Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. Environ. Res. **111**: 943–952. doi:10.1016/j.envres.2011.05.001. PMID:21632049.

Leroi, A.M., Bennett, A.F., and Lenski, R.E. 1994. Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. Proc. Natl. Acad. Sci. U.S.A. **91**: 1917–1921. doi:10.1073/pnas.91.5.1917. PMID:8127906.

Lockwood, J.L., Marchetti, M.P., and Hoopes, M.F. 2013. Invasion ecology. Wiley-Blackwell.

Lopez, L.K., Davis, A.R., and Wong, M.Y.L. 2018. Behavioral interactions under multiple stressors: temperature and salinity mediate aggression between an invasive and a native fish. Biol. Invasions, **20**: 487–499. doi:10.1007/s10530-017-1552-8.

Matthews, M.A., and McMahon, R.F. 1999. Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia. J. Mollusc. Stud. **65**: 317–325. doi:10.1093/mollus/65.3.317.

Mattice, J.S., and Dye, L.L. 1975. Thermal tolerance of the adult Asiatic clam Corbicula manilensis (Mollusca: Bivalvia). United States Energy Research and Development Administration, NTIS No. CONF-750425-5.

McDowell, W.G., Benson, A.J., and Byers, J.E. 2014. Climate controls the distribution of a widespread invasive species: implications for future range expansion. Freshw. Biol. **59**: 847–857. doi:10.1111/fwb.12308.

McMahon, R.F. 1999. Invasive characteristics of the freshwater bivalve *Corbicula fluminea*. *In* Non-indigenous freshwater organisms: vectors, biology and impacts. *Edited by* R. Claudi and J. Leach. Lewis Publishers. Boca Raton, Fla. pp. 315–343.

McMahon, R.F., Matthews, M.A., Ussery, T.A., Chase, R., and Clarke, M. 1995. Studies of heat tolerance of zebra mussels: effects of temperature acclimation and chronic exposure to lethal temperatures. United States Army Corps of Engineers Technical Report EL-95-9, Washington, D.C.

Merilä, J., and Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Ecol. Appl. **7**: 1–14. doi:10.1111/eva.12137.

Minchin, D. 2014. The distribution of the Asian clam *Corbicula fluminea* and its potential to spread in Ireland. Manage. Biol. Invasions, **5**: 165–177. doi:10.3391/mbi.2014.5.2.10.

Morey, A.C., Venett, R.C., and Hutchison, W.D. 2019. Sublethal effects of subzero temperatures on the light brown apple moth, *Epiphyas postvittana*: fitness costs in response to partial freezing. Insect Sci. **26**: 311–321. doi:10.1111/1744-7917.12560. PMID:29193863.

Morgan, D., Keser, M., Swenarton, J., and Foertch, J. 2003. Population dynamics of the Asiatic clam, *Corbicula fluminea* (Mueller) in the Lower Connecticut River: Establishing a foothold in New England. J. Shellfish Res. **22**: 193–203.

Müller, O., and Baur, B. 2011. Survival of the invasive clam *Corbicula fluminea* (Müller) in response to winter water temperature. Malacologia, **53**: 367–371. doi:10.4002/040.053.0207.

Natale, B., Wunderlich, M., and Resler, S. 2014. Follow-up survey of Asian clams (Corbicula fluminea) in Owasco Lake, Cayuga County, NY, 2013. A Report to the Cayuga County Soil and Water Conservation District, Cayuga County Dept. of Planning and Economic Development, and the Cayuga County Water Quality Management Agency's Owasco Lake Asian Clam Task Force. Cayuga County Department of Planning and Economic Development, Cayuga, N.Y.

Nyamukondiwa, C., Kleynhans, E., and Terblanche, J.S. 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). Ecol. Entomol. **35**: 565–575. doi:10.1111/j.1365-2311.2010.01215.x.

Ortmann, C., and Grieshaber, M.K. 2003. Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. J. Exp. Biol. **206**: 4167–4178. doi:10.1242/jeb.00656. PMID:14555755.

Penk, M.R., and Williams, M.A. 2019. Thermal effluents from power plants boost performance of the invasive clam *Corbicula fluminea* in Ireland's largest river. Sci. Total Environ. **693**: 133546. doi:10.1016/j.scitotenv.2019.07.352. PMID:31369893.

Pigneur, L.M., Etoundi, E., Aldridge, D.C., Marescaux, J., Yasuda, N., and Van Doninck, K. 2014. Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. Mol. Ecol. **23**: 5102–5116. doi:10.1111/mec.12912. PMID:25208249.

Rajagopal, S., van der Velde, G., van der Gaag, M., and Jenner, H.A. 2005. Factors influencing the upper temperature tolerances of three mussel species in a brackish water canal: size, season and laboratory protocols. Biofouling, **21**(2): 87–97. doi:10.1080/08927010500133584. PMID:16167389.

Ricciardi, A. 2007. Are modern biological invasions an unprecedented form of global change? Conserv. Biol. **21**: 329–336. doi:10.1111/j.1523-1739.2006.00615.x. PMID:17391183.

Richardson, T.D., and Selby, J.M. 2020. The nonindigenous Asian Clam, *Corbicula fluminea*, in New Hampshire. Northeast. Nat. **27**(2): 272–280. doi:10.1656/045.027.0209.

Schwander, T., and Oldroyd, B.P. 2016. Androgenesis: where males hijack eggs to clone themselves. Philos. Trans. R. Soc. B Biol. Sci. **371**: 20150534. doi:10.1098/rstb.2015.0534. PMID:27619698.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., et al. 2017. No saturation in the accumulation of alien species worldwide. Nat. Commun. **8**: 14435. doi:10.1038/ncomms14435. PMID:28198420.

Shine, R. 2012. Invasive species as drivers of evolutionary change: cane toads in tropical Australia. Evol. Appl. **5**: 107–116. doi:10.1111/j.1752-4571.2011.00201.x. PMID:25568034.

Shuter, B.J., Finstad, A.G., Helland, I.P., Zweimüller, I., and Hölker, F. 2012. The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change. Aquat. Sci. **74**: 637–657. doi:10.1007/s00027-012-0274-3.

Simard, M., Paquet, A., Jutras, C., Robitaille, Y., Blier, P., Courtois, R., and Martel, A. 2012. North American range extension of the invasive Asian clam in a St. Lawrence River power station thermal plume. Aquat. Invasions, **7**: 81–89. doi:10.3391/ai.2012.7.1.009.

Smith, B.J., Harris, B.S., Harris, T.J., LaBudde, L.A., and Hayer, C.A. 2018. Status and trends of the Asian clam (*Corbicula fluminea*) in the lower Fox River and Green Bay. J. Gt. Lakes Res. **44**: 943–949. doi:10.1016/j.jglr.2018.02.012.

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. **213**: 912–920. doi:10.1242/jeb.037473. PMID:20190116.

Sorte, C.J.B., Jones, S.J., and Miller, L.P. 2011. Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. J. Exp. Mar. Biol. Ecol. **400**: 209–217. doi:10.1016/j.jembe.2011.02.009.

Sousa, R., Antunes, C., and Guilhermino, L. 2008. Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: An overview. Ann. Limnol. Int. J. Limnol. **44**: 85–94. doi:10.1051/limn:2008017.

Tepolt, C.K., and Somero, G.N. 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. J. Exp. Biol. **217**: 1129–1138. doi:10.1242/jeb.093849. PMID:24671964.

Terblanche, J.S., and Chown, S.L. 2006. The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). J. Exp. Biol. **209**: 1064–1073. doi:10.1242/jeb.02129. PMID:16513933.

Thorp, J.H., and Covich, A.P. 2009. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, Calif.

Thyrring, J., Tremblay, R., and Sejr, M.K. 2019. Local cold adaption increases the thermal window of temperate mussels in the Arctic. Conserv. Physiol. **7**: coz098. doi:10.1093/conphys/coz098. PMID:31890211.

USGS. 2014. National Water Information System (Water Data for the Nation). U.S. Department of the Interior and U.S. Geological Survey (USGS). Available from https://waterdata.usgs.gov/nwis.

Valiente, A.G., Juanes, F., Nuñez, P., and Garcia-Vazquez, E. 2010. Brown trout (*Salmo trutta*) invasiveness: plasticity in life-history is more important than genetic variability. Biol. Invasions, **12**: 451–462. doi:10.1007/s10530-009-9450-3.

Wallace, G.T., Kim, T.L., and Neufeld, C.J. 2014. Interpopulational variation in the cold tolerance of a broadly distributed marine copepod. Conserv. Physiol. **2**: cou041. doi:10.1093/conphys/cou041. PMID:27293662.

Ward, J.L., and Hodgson, J.R. 1997. The presence of the Asiatic clam, *Corbicula* sp., from Lake Superior. J. Freshw. Ecol. **12**(1): 167–169. doi:10.1080/02705060.1997.9663520.

Werner, S., and Rothhaupt, K.-O. 2008. Mass mortality of the invasive bivalve *Corbicula fluminea* induced by a severe low-water event and associated low water temperatures. Hydrobiologia, **613**: 143–150. doi:10.1007/s10750-008-9479-9.

Zenni, R.D., and Nuñez, M.A. 2013. The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos, **122**(6): 801–815. doi:10.1111/j.1600-0706.2012.00254.x.