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# Thermal tolerance limits of the Chinese mystery snail (*Bellamya chinensis*): Implications for management

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# RESEARCH NOTE

## Thermal tolerance limits of the Chinese mystery snail (*Bellamya chinensis*): implications for management

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**Abstract:** The Chinese mystery snail, *Bellamya chinensis* (Gray, 1834) is a gastropod native to East Asia and is considered an invasive species in North America where its impacts on native species and ecosystems are not well understood. Scientific literature describing its biology and life history are sparse. Thermal tolerance limits, or the maximum and minimum temperature under which a species can survive, are key to identifying the potential geographical range of a species. The ability of managers to control invasive species is directly impacted by the thermal tolerance limits of a species. We attempted to identify the thermal tolerance limits of *B. chinensis* in a laboratory setting. Using a random sampling design, we exposed groups of wild-caught *B. chinensis to* either extreme high or low temperature treatments. We identified the upper temperature tolerance limit as between 40 and 45 °C. This result indicates some hot water management techniques may successfully prevent spread of *B. chinensis* among waterways. Despite exposing *B. chinensis to* freezing temperatures for extended periods of time we did not identify a lower temperature limit. Identifying the thermal tolerance limits of this and other invasive species informs predictions of range expansion and identification of potential prevention efforts.

**Key words:** invasive, non-native, temperature tolerance, *Cipangopaludina*

Global warming is a major driver of range expansion and distributional shifts in flora and fauna throughout the world (Lenoir and Svenning 2015, Pauchard *et al.* 2016) and it may impact changes in local, seasonal temperatures and contribute to an increase in the frequency of extreme weather events (IPCC 2014). Changes in local climatic characteristics (*e.g.*, seasonal temperature extremes) may prove conducive or detrimental to different species (Ummenhofer and Meehl, 2017). The abilities of non-native species to tolerate changing conditions, including changes in extreme seasonal temperatures, can increase or decrease their invasive potential and management effectiveness in preventing, eradicating, or controlling them (Hellmann *et al.* 2008, Mainka and Howard 2010, Canning-Clode *et al*. 2011, Gallardo and Aldridge 2013).

Thermal tolerance, or the range of temperatures a species can survive without mortality, constrains the fundamental and realized geographic ranges of terrestrial and aquatic species (Brett 1956). Thermal tolerance also limits the depths at which some aquatic species operate within the water column. Identifying the thermal limits of aquatic invasive species is important for both managing and preventing their introduction, establishment, and spread (Rahel and Olden 2008, Gallardo and Aldridge 2013, Kelley 2014). Once identified, the upper and lower thermal tolerance temperatures can be used to inform the development and refinement of invasive species distribution models (Uden *et al.* 2015), which can be used to explain variability and predict changes in invasive species' geographic distributions.

The Chinese mystery snail, *Bellamya chinensis* (Gray, 1834) is native to freshwater systems of eastern Asia (Jokinen 1982), and is a popular food item in the local Chinese markets of North America (Mills *et al.* 1993). First known introductions of *B. chinensis* to North America were in the early 1890s (Clench and Fuller 1965, Clarke 1981, Jokinen 1982). Since, its North American range spans from the east to west coasts, extending as far north as southern Canada (Jokinen 1982, Therriault and Kott 2002, Karatayev *et al.* 2009). Like many aquatic species the primary vector for the spread of *B. chinensis* in North America is direct or indirect introduction by humans (Jokinen 1982, Mackie and Claudi 2009). Although considered an invasive species in North America, the ecological impacts of *B. chinensis* on native species and ecosystems are not well-understood (Solomon *et al.* 2010).

Unlike other poikilotherms, gastropods are less able to behaviorally adapt to changing temperatures, thus, primarily rely on periods of slow acclimation (Segal 1961). However, *Bellamya chinensis* might escape potentially lethal weather by retreating to deeper water. As such, extreme temperature events may not be a reliable predictor of die off of *B. chinensis* in lakes and ponds. Management techniques with potential to influence the probability of survival and spread of this species include drawdowns of water bodies, and the cleaning, draining and drying of watercrafts and related equipment. Identifying the thermal tolerance limits and capacity to withstand desiccation is required to determine the efficacy of using these temperature-dependent management techniques for controlling *B. chinensis*.

Based on published observations and information we hypothesize the lower and upper thermal limits of *Bellamya chinensis* are approximately 1 °C (Soes *et al.* 2011) and 24 °C (benthic) to 30 °C (surface water), respectively (Wolfert and Hiltunen 1968). Testing the conservativeness of these estimates is critical in identifying the realized thermal tolerance limits of *B. chinensis*. In this study, we use wild-caught *B. chinensis* to form a baseline range of temperatures to hypothesize the limits of their temperature tolerances and discuss the implications of these tolerances for managing and predicting the ultimate distribution of *B. chinensis* in North America.

## MATERIALS AND METHODS

### Capture, maintenance, acclimation, and marking of snails

To identify the thermal tolerance limits of *Bellamya chinensis* we subjected wild-caught snails to ambient temperature manipulation in a laboratory setting. We collected adult snails with shell length > 30 mm from Wild Plum Lake in Southeast Nebraska (40° 36' 52" N, 96° 54' 09" W) during the summer of 2012. We housed live specimens in a 1500 L aquarium filled with aerated, de-chlorinated tap water, maintained at an ambient temperature of approximately 22 °C. Water exchange occurred weekly at a rate of 25%. We fed snails a diet of romaine lettuce, TetraVeggie algae wafers, and goldfish flake food *en masse* three times a week.

Prior to experimentation, snails were acclimated inside one of two 80 L aquariums for a period of 14 days at one of two temperatures: 18 °C or 25 °C. We used an infrared thermometer to quickly obtain water temperatures. After the acclimation period, each individual was removed from its aquarium and marked with a unique number using Rust-oleum™ industrial appliance touch-up paint (Wong *et al.* 2013), during which time each snail was exposed to room temperature (approximately 22 °C) for less than one hour. After marking and prior to experimentation, snails were given a period of 48 hours to recover from handling. Specimens were handled using the Guidelines for Use of Fishes in Research (Use of Fishes in Research Committee 2004).

### Study design

Our original study design was a critical thermal minimum and maximum approach (see Fry 1947, Beitinger *et al.* 2000), with the use of small pilot studies to hone in on the temperatures that should be assessed. During those pilot studies, we determined that (1) snails became unresponsive at temperatures above 40 °C and (2) more than 50% of snails were able to withstand temperatures down to 0 °C. Given results of these pilot studies and our desire to better understand *Bellamya chinensis* thermal tolerance from an ecological perspective, we modified our approach and used two experimental designs to provide new insights about the thermal tolerances of *B. chinensis*. Thus, we attempted to determine the approximate upper temperature at which *B. chinensis* can survive using critical thermal maximum (CTM; Huntsman and Sparks 1924, Beitinger et al. 2000), and the approximate time that *B. chinensis* can survive in 0 °C water using the incipient lower lethal temperature (ILLT; Fry 1947, Beitinger *et al.* 2000) technique for snails acclimated to 18 °C or 25 °C (Fig. 1).

### Critical Thermal Maximum (CTM)

To estimate the warmest water temperatures at which either 18 °C or 25 °C - acclimated *Bellamya chinensis* can survive, we randomly placed snails into one of 10, 80-L glass aquaria located in one of two thermal chambers (*i.e.*, acclimation group) resulting in 20 snails in each observation group (Fig. 1). Pilot studies revealed death did not occur when temperature was cooler than 40 °C (Wong, unpublished). As such, we began observations for mortality at this temperature and made additional observations at 5 °C intervals until water temperature reached 60 °C. Temperatures in aquaria were raised at a rate of 1 °C · $hour^{-1}$. When water heated to the predetermined observation temperature (*e.g.*, 45 °C), a randomly selected aquarium was removed from the thermal chamber and allowed to equilibrate to room temperature (approximately 22 °C), and sat for a recovery period of 48 hours that began once water temperature equilibrated with room temperature. We assessed snails for mortality at the end of this recovery period.

### Incipient Lower Lethal Temperature (ILLT)

To estimate the duration that *Bellamya chinensis* acclimated to either 18 °C or 25 °C can survive in water that is

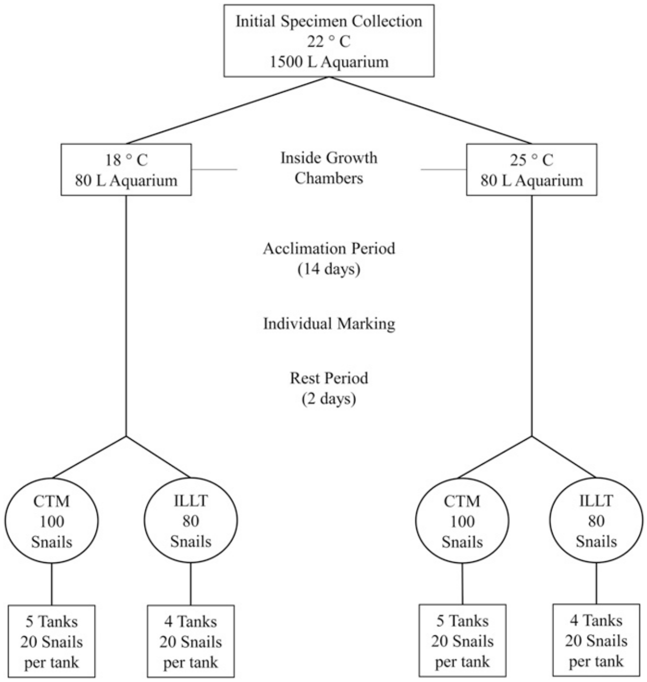


**Figure 1.** Collection, acclimation, and experimental treatment selection scheme for experimental estimation of Critical Thermal Maximum (CTM) and Incipient Lower Lethal Temperature (ILLT) for wild-caught *Bellamya chinensis* (Chinese mystery snail).

0 °C, we randomly placed snails into one of eight 19-L plastic aquaria located in the respective thermal chamber (*i.e.*, acclimation group) such that there were 20 snails in each aquarium. Water was cooled at a rate of 1 °C · hour$^{-1}$ until water temperature was 0 °C. To ensure complete freezing of the water, the ambient temperature in the chamber was maintained at -1 °C. After the pre-determined exposure period to 0 °C (0 hours, 23 hours, 28 hours, or 33 hours), a randomly selected aquarium was removed from the thermal chamber, allowed to equilibrate to room temperature (approximately 22 °C), and sat for a recovery period of 48 hours that began once water temperature equilibrated with room temperature. We assessed snails for mortality at the end of this recovery period.

### Determining death of individuals

Observing mortality in the *Bellamya chinensis* is difficult; dormancy (retracted into shell with operculum closed) is a common reaction of *B. chinensis* when exposed to stress. Thus, after each trial, snails were given a recovery period to resume normal activity. For each test, we assumed mortality if the snail met one or more of the following criteria: snail mantle body mass had blue coloration; corporal mass separated from the snail shell; the snail did not move during the recovery period (we used photographs to assess displacement of an individual over the 48 hours) and the snail was unresponsive to gentle prodding. For the ILLT experiment, we also assumed a lack of net movement of individual snails to be indicative of death.

## RESULTS

### Critical Thermal Maximum (CTM)

We observed slight differences among survival of each of the acclimation groups at each temperature increment. All snails (N = 20) in the cooler (18 °C) acclimation group survived and 75% (15 of 20 individuals) in the warmer (25 °C) acclimation survived the 40 °C treatment. Most snails (95%) in the cooler acclimation group, and only 10% in the warmer group died by the 45 °C measurement. No snails survived temperatures at or above 50 °C.

### Incipient Lower Lethal Temperature (ILLT)

We observed slight differences among survival of each of the acclimation groups at each duration increment. We observed no mortality of snails belonging to the 18 °C acclimation group. Within the 25 °C acclimation group snails, we observed 30% mortality at the first observation (0 hours at 0 °C), 5% mortality at the second (23 hours at 0 °C), and no mortality at or beyond 28 hours at 0 °C.

## DISCUSSION

Our study identified the upper thermal tolerance of *Bellamya chinensis* as approximately 45 °C, but did not identify a lower lethal limit, which suggests the lethal limit is < 0 °C. Our estimates are based on slow exposures to low and high temperatures for 24-hour and up to 33-hour periods, respectively. Based on observational studies of wild *B. chinensis,* we hypothesized the lower and upper limits to be 1 °C and between 24 and 30 °C, respectively (Soes *et al.* 2011, Karatayev *et al.* 2009, Wolfert and Hiltunen 1968). This study expanded the published estimates, exceeding also our expectations. In the experimental setting *B. chinensis* withstood temperatures much greater than the estimated 26 – 30 °C and did not experience die off during exposure to freezing water. We determined that *B. chinensis* is a relatively temperature-hardy species; it is therefore unlikely that benthic temperatures in most water bodies of the United States of America and Canada will exceed the upper or lower tolerance limits of *B. chinensis* (Wetzel 2001). Inverse stratification in the winter precludes the event of benthic temperatures reaching less than 0 °C in most temperate lakes (Wetzel 2001). Additionally, *B. chinensis* has been observed to burrow under adverse conditions (Unstad *et al.* 2013), perhaps isolating the animal from extreme temperatures. Based on the current evidence and current climatic conditions of the United States and Canada, we suggest that *B. chinensis* will not be limited geospatially on the basis of current climatic patterns alone.

Drawdowns are a common management technique utilized for kill-off of undesirable aquatic animals (Verill and Berry 1995, Cheng and LeClair 2011). Although desiccation is a primary source of mortality in animals during drawdowns, it is an improbable method for inducing *Bellamya chinensis* mass mortality. *Bellamya chinensis* are capable of closing their operculum extended periods both in and out of water, supporting the ability to withstand dry and hot conditions for at least 8 weeks (Havel 2011, Unstad *et al.* 2013). Additionally, this study identified an in-water, upper temperature limit of approximately 45 °C. Schmidt-Nielson *et al.* (1971) found that the somatic tissue temperature of the land snail *Sphincterochila boissieri* reached approximately 50 °C during a day with a maximum air temperature of 43 °C. Thus, the air temperature required to achieve mortality of *B. chinensis* via desiccation may be less than the in-water upper temperature limit we identified. To be effective, drawdowns would likely need to last for months under extremely high, sustained air temperatures.

Techniques for preventing the spread of aquatic invasive species include the cleaning, draining and drying of watercrafts and equipment between launches. Adult and larval aquatic invasive species can be present in live wells, bilges, bait buckets and engines (Johnson *et al.* 2001) and entrained on boat propellers and trailers (Rothlisberger *et al.* 2010). High-pressure washing, low-pressure washing and hand removal are three forms, with varying successes, of cleaning boats and equipment of aquatic invasive species (Rothlisberger *et al.* 2010). Current practices primarily involve high-pressure washing with 60 °C water for a minimum of 10 seconds to decontaminate watercrafts and equipment from aquatic invasive species. According to our study, *Bellamya chinensis* is intolerant of temperatures above 50 °C; however, we did not test their tolerance to very hot temperatures (>50 °C) for substantially shorter periods (*i.e.*, <60 seconds). Future studies that focus on *B. chinensis* mortality caused by very hot water for short periods could be used to evaluate the efficacy of watercraft decontamination procedures in preventing the spread of *B. chinensis*. Additionally, the effects of *B. chinensis* exposure to chemicals commonly used to decontaminate watercrafts and equipment (*i.e.* quaternary ammonium compounds, vinegar, bleach, etc.) could be evaluated, though expectations are low; neither rotenone nor copper sulfate effectively killed adult Chinese mystery snails in laboratory experiments (Haak *et al.* 2014). Identifying thermal limits of *Bellamya chinensis* may inform our understanding of best practices for managing invasive populations of *B. chinensis*.

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