

Research Article

Temperature and salinity tolerances of juvenile invasive Japanese mystery snails

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Academic editor: Charles Martin

Received: 3 October 2022

Accepted: 15 February 2023

Published: 28 June 2023

Editors' Note: This is an additional contribution to the Proceedings of the 22nd International Conference on Aquatic Invasive Species held in Oostende, Belgium, April 18–22, 2022 (<https://icais.org>). 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.

Citation: Jaishanker P, Hall-Stratton D, Fowler AE (2023) Temperature and salinity tolerances of juvenile invasive Japanese mystery snails. *Aquatic Invasions* 18(2): 263–276. <https://doi.org/10.3391/ai.2023.18.2.104203>

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Abstract

The freshwater Japanese mystery snail (*Heterogen japonica*) was introduced to the United States in the early 1900s and has since established populations throughout the continent. The species has ovoviviparous reproduction (i.e., eggs hatch within the mother and develop inside before being released as juveniles), which is one reason it has been successful. Despite its wide geographic range, little is known about its physiological tolerances. For example, high salinities and temperatures may limit its spread, and determining the species' tolerance to these environmental factors is crucial to predict its possible range expansion. To test this, 600 juvenile *H. japonica* (average shell length: 6.0mm, range: 4.5–8.3mm) were collected from 28 females from a lake in Virginia, USA and placed in a fully crossed design to test the interaction between salinity (0.2 and 2 PSU) and temperature (25 °C, 34 °C and 38 °C). Juveniles were monitored for mortality over two weeks. Kaplan–Meier survival analyses determined median survival probabilities, and generalized linear models compared differences in mean survival. All juveniles in 25 °C (except one in 0.2 PSU) survived (N=199/200), and all juveniles in 38 °C died by the end of 14 days (N=200), irrespective of salinity. However, juveniles kept at 38 °C showed higher early (≤ 4 days) mortality in 0.2 PSU, but lower early mortality in 2 PSU. Importantly, juveniles in 2 PSU survived for ≥ 2 days (N=294/300) across all temperatures, indicating that there may be scope for expansion through estuaries. Future work should examine temperatures between 34 and 38 °C and salinities above 2 PSU to understand the extent of covariance between salinity and temperature and create mathematical models to estimate survivability and spread.

Key words: *Heterogen japonica*, survival, invasive, climate change, freshwater

Introduction

Predicting the geographic range of an invasive species is crucial in its management, especially given the possibility of range expansions of invasive organisms due, in part, to climate change (Stohlgren and Schnase 2006). For aquatic invaders in particular, temperature and salinity tolerance can be major drivers of species distributions, as osmoregulation can be metabolically and physiologically costly (Peña-Villalobos et al. 2016). Increasing temperatures can create new pathways for

invasion, such as changes in streamflow regimes or range shifts for temperature-dependent species (Rahel and Olden 2008). Additionally, higher temperatures result in higher metabolic rates in aquatic organisms, including invaders, which may increase their movement and reproduction (Christensen et al. 2021). For freshwater organisms, extended exposure to saltwater can be a barrier to transport or migration, but some organisms can tolerate it for short periods of time (Venâncio 2018). In the context of freshwater invasive species, the ability of a population to survive short periods of saltwater inundation may allow individuals to colonize novel areas. In some cases, organisms that colonize freshwater systems connected by brackish water may need to move through waters of varying salinities to access new habitats. For example, the short-term survival of invasive freshwater blue catfish in higher salinities (>10 PSU) of the Chesapeake Bay has allowed it to spread between tidal rivers (Nepal and Fabrizio 2019).

Freshwater gastropods are successful invaders in ecosystems throughout the world (Spyra and Strzelec 2014; Preston et al. 2022). In particular, mystery snails in the Viviparidae family have successfully colonized North America and are now found on both coasts of the United States as well as the Great Lakes and Canada (Perez et al. 2016). There are two species of mystery snails in North America - the Japanese mystery snail (*Heterogen / Cipangopaludina / Bellamya / Viviparus japonica*) and the Chinese mystery snail (*C. chinensis*). While originally introduced to be cultivated for human consumption in the early 1900s (Prashad 1928; Clench and Fuller 1965), individuals have been continually released from aquariums or garden centers in the past few decades (Matthews et al. 2017). Additionally, their spread may be aided by recreational boating since they can survive out of water for at least four weeks (Havel 2011).

Currently, “mystery snails” are found in several tributaries of the Chesapeake Bay, USA, including the Potomac River, Anacostia River, and James River (United States Geological Survey 2022). However, the species identity remains unknown in many cases due to difficulties in comparing *H. japonica* with *C. chinensis*. Previous work has genetically confirmed the presence of only *H. japonica* throughout northern Virginia and Maryland in tributaries that lead to the Chesapeake Bay (Fowler et al. 2022a, Hall-Stratton, unpublished). Overall, little is known about the life history or environmental tolerances of *H. japonica* in its invasive ranges, but this may be partially because of the difficulty in distinguishing between the two species, which could limit the association of characteristics to *H. japonica* (David and Cote 2019). The two species are virtually morphologically identical, and it is difficult to differentiate the two without molecular barcoding (David and Cote 2019). Indeed, few genetic studies have been used to identify the two mystery snail species in North America (Perez et al. 2016; David and Cote 2019; Fowler et al. 2022a).

Both mystery snail species have ovoviviparous reproduction (i.e., eggs hatch within the mother and develop inside before being released as juveniles) and females can live up to five years (Jokinen 1982; Jokinen 1992; Stephen et al. 2013). Importantly, given that they both have a reproductive strategy of crawl-away juveniles (Fowler et al. 2022a), establishment in new locations could happen even if only a small number of adults or juveniles are introduced. For example, the annual production in a 6.47-ha reservoir in Nebraska, USA was ~2.2 – 3.7 million juveniles from an estimated 250,000 adult *C. chinensis* (Chaine et al. 2012; Stephen et al. 2013). In Long Island, New York, USA, a *C. chinensis* population increased from 150 snails to 950 snails in only two years (McCann 2014). We are not aware of any density estimates of *H. japonica* in either their native or invasive range. Both species of mystery snails can also attain greater shell lengths in comparison

to native snails, increasing the likelihood of a competitive advantage for the mystery snails. For example, previous work in Virginia, USA found an average size of 49mm shell length for *H. japonica* across several sites (n=581, maximum size of 78mm) (Fowler et al. 2002a). The maximum size of native snails (*Elimia virginica*) collected at these same sites was 38.8mm shell length (n=319, average 20.7mm) (Fowler, unpublished data); all other native snails were smaller in size. However, the majority of work in introduced populations has been completed on *C. chinensis* (e.g., Johnson et al. 2009; Solomon et al. 2010). Apart from possibly competing with native snails for resources (Wolfert and Hiltunen 1968), *H. japonica* also carries parasites (aspidogastrea and digenean trematodes) which may infect native snails (Perez et al. 2016; reviewed in Fowler et al. 2022b).

Due to the rise in sea levels from climate change, inland freshwater bodies including lakes and rivers may become flooded, providing an avenue for species expansion during periods of higher-than-normal water levels (Tully et al. 2019). However, to move between different river systems throughout an estuarine system, like the Chesapeake Bay, Japanese mystery snails would have to tolerate salinity for at least part of that journey, as well as the increased sea temperatures that global warming is projected to bring. Using satellite data, Urquhart et al. (2012) calculated that, on average, salinities in the northern Chesapeake Bay ranged from 0 to 5 PSU, with areas near the mouth of the Potomac River (i.e., where the river meets the bay) approaching 10 PSU. Throughout the Chesapeake Bay, salinity varies between 0 and 28 PSU, with the salinity in tidal rivers decreasing drastically in wet months due to a high volume of freshwater discharge (Nepal and Fabrizio 2019). Najjar et al. (2010) used mathematical models to estimate water temperature rise in the bay as between 2 to 6 °C by 2100. With a maximum water temperature of 32 °C in 2020 in the Washington, D.C. area (NOAA 2022), this would mean a maximum water temperature between 34 and 38 °C in 2100.

We are not aware of any studies on the temperature or salinity tolerance of Japanese mystery snails. However, previous studies have examined the salinity or temperature tolerances of similar freshwater snails. Abiotic tolerance studies indicated that adult *C. chinensis* could survive a wide variation of temperature, from to <0 to 45 °C (Burnett et al. 2018). A recent study by Fraser (2020) showed that juvenile *C. chinensis* could not survive two weeks in 10 PSU and suggested that juveniles could not tolerate salinities above 5 PSU. The adults and juveniles of a similar freshwater invasive species, Island apple snails (*Pomacea maculata*), could survive salinities between 0 and 16 PSU (Bernatis et al. 2016; Underwood et al. 2019).

Here, we examined how the interaction of temperature and salinity stress affected the survival of juvenile *H. japonica* to help understand the possible future spread of this species under a changing climate. As juveniles can be the propagules driving new invasions, their ability to survive salinity and temperature stress may be the most informative in terms of determining the risk of new introductions or the spread or range expansion of current populations (Farani et al. 2015). Live juveniles were collected from female *H. japonica* and placed into six fully-crossed salinity (0.2 and 2 PSU) and temperature (25 °C, 34 °C and 38 °C) combinations and monitored for mortality for two weeks. Salinities were chosen based on known salinity tolerances of *C. chinensis* and projected temperature changes for the region. We predicted highest survival in the least stressful conditions (i.e., low salinity and low temperature) and lowest survival in the most stressful conditions (i.e., high salinity and high temperature). This study represents the first salinity and temperature challenge for invasive populations of *H. japonica*.

Methods

Field collections and juvenile collections

Adult Japanese mystery snails (>50cm shell length) were collected by hand from Lake Royal in Fairfax, Virginia, USA (38°48'14.5"N, 77°17'27.3"W) over two days (September 15 and October 8, 2021). Lake Royal is an artificial lake that was fully dredged and restored in 2016, so the population of *H. japonica* could be, at most, five years old. While we did not take salinity measurements from Lake Royal, similar lakes in the area had salinities between 0.1 and 0.3 ppt. Using a mitochondrial barcoding gene (COI) per Fowler et al. 2022a, we genetically confirmed that the mystery snails in this lake were *H. japonica*. After collection, snails were placed in a standard 5-gallon bucket filled halfway with 50% dechlorinated tap water and 50% lake water and kept at room temperature and natural sunlight exposure at the Potomac Science Center, Woodbridge, Virginia, USA. Snails were not fed.

Fully formed juveniles were removed from the female's brood-pouch, and only juveniles ≥ 4.5 mm shell length were used in experiments (N = 600, average shell length: 6.0 mm, range: 4.5–8.3 mm). If a brood contained less than 18 juveniles of appropriate size, the brood was not used in the trial as there would not be three individuals per brood in each of the six treatments. Twenty-eight broods (total N = 600 juveniles) from individual females (N = 28, average female shell length: 56.2 mm, range: 51.3–66.7 mm) were used in the experiments. Due to space and time limitations, experiments were conducted in the following three time periods: October 11–22, November 1–15, and November 8–22.

Experimental design and treatment groups

Juveniles from each brood were divided among each treatment group, allowing for at least three individuals per brood for each treatment. Treatment groups were fully crossed to test the interaction between salinity (0.2 and 2 PSU) and temperature (25 °C, 34 °C and 38 °C) and monitored for mortality over two weeks. Each juvenile snail was kept in approximately 60 ml of water (i.e., to the top of the container to ensure continuous exposure to the treatment as well as to provide good water quality) in one well of an 18-well plastic parts box. Experimental salinities were made using deionized water with Instant Ocean brand mix at a concentration of 0.2 PSU or 2.0 PSU. Salinities were tested and monitored using a hand-held YSI (Yellow Springs, OH, USA).

Boxes in the 25 °C treatment group were placed at room temperature in a sun-lit room, while the boxes at 34 °C and 38 °C were placed in Percival Scientific I-36LL incubators set to the respective temperatures and a 08:00 a.m.:08:00 p.m. light:dark cycle. The sun-lit room had the same light:dark regime as the other treatments, and 25 °C was maintained by room temperature. Temperatures were monitored for stable conditions using HOBO temperature loggers submerged within separate containers of water. No food was given, and no water changes were conducted over the course of the experiment. Juvenile survival was assessed on days 2, 4, 7, 10, 12, and 14 after the beginning of the treatment, and, if dead or experiment completed, the size of the individual was recorded. Mortality was discerned by gently pushing the operculum using a tweezer; the snail was recorded as deceased if the operculum was not pulled in, no other movement was discerned, and/or a murky fluid was released.

Data analyses

Because each juvenile was held separately in one well of the parts box, all juveniles were treated as independent replicates, allowing for examination of differences in survival among individual juveniles as well as combining data to obtain overall percent mortality from individuals from the same brood. Data analysis was conducted using the survival and survminer packages in R version 4.1.0 (Kassambara et al. 2021; Therneau 2022). Kaplan-Meier survival curves were plotted with lines for each treatment group, and pairwise comparisons using Bonferroni corrections were performed to determine significance ($p < 0.05$) between the treatment groups. A Cox-proportional hazards model was run to determine whether juvenile shell size was a significant covariate in mortality data.

The mortality rates across broods, modeled as counts, were non-normally distributed. Therefore, to compare the average mortality rates across broods, a generalized linear model (GLM) with a Poisson distribution and log function was performed using salinity, temperature, and an interaction of the two as factors. Because day 4 was the time at which 50% of the population died in the 38 °C group (no other treatment group experienced 50% mortality), two models were performed – one at day 4 and the other at day 14. Because the interaction term was not significant, it was removed from both models, and the GLMs were run without the interaction term. Pairwise Wilcoxon comparison tests were also performed to determine differences in brood mortality between treatment groups on both day 4 and 14.

Results

Juvenile *H. japonica* were placed in six fully-crossed salinity (0.2 and 2 PSU) and temperature (25 °C, 34 °C and 38 °C) combinations and monitored for mortality for 14 days. Fourteen-day survival probabilities ranged from 0% to 100% (Table 1, Fig. 1). All juveniles in 25 °C (except one in 0.2 PSU) survived ($N = 199$), and all juveniles in 38 °C died by the end of 14 days ($N = 200$), irrespective of salinity (Table 1). Importantly, juveniles in 2 PSU survived for \geq two days ($N=294$) across all temperatures. Therefore, there was a significant impact of treatment on juvenile survival (Kaplan-Meier $p < 0.0001$). All groups had significant differences in survival except 0.2 PSU 25 °C with 2.0 PSU 25 °C ($p = 1.00$), 0.2 PSU 25 °C with 0.2 PSU 34 °C ($p = 0.86$), and 2.0 PSU 25 °C with 0.2 PSU 34 °C ($p = 0.20$) (Table 2). The Cox proportional-hazard model found that the covariate of juvenile shell size did not result in a significant hazard on survival ($p = 0.763$), so it was not used as a covariate in any of the models.

All juveniles in both salinities at 38 °C experienced $<50\%$ survival after four days, while no other groups reached 50% mortality during the entirety of the experiment (14 days). While there were some mortalities recorded by day 4 in the 34 °C treatment, all juveniles at 25 °C were still alive. However, juveniles kept at 38 °C and 0.2 PSU had higher mortality after day 4 than at 38 °C and 2 PSU, opposite the result of the other two temperatures (Fig. 1).

Both salinity and temperature significantly affected brood mortality at day 14, but only temperature affected brood mortality at day 4 (Table 3, Fig. 2). On day 14, broods at 2 PSU had a significantly higher mortality than those at 0.2 PSU ($p=0.01$; Table 3, Fig. 2), irrespective of temperature.

Table 1. Number of Japanese mystery snail (*Heterogen japonica*) juveniles that died on each observation day of the 14-day trial, along with the survival probability, error, and confidence interval of the probability.

Salinity (PSU)	Temperature (°C)	No. Days	No. Juveniles	No. Deaths	Survival Probability	Standard Error	Lower 95% CI	Upper 95% CI
0.2	25	7	100	1	0.99	0.01	0.97	1
Total number of juvenile mortalities after 14 days = 1								
0.2	34	4	100	1	0.99	0.01	0.97	1
		14	99	5	0.94	0.02	0.90	0.99
Total number of juvenile mortalities after 14 days = 6								
2	34	2	100	1	0.99	0.01	0.97	1
		4	99	1	0.98	0.01	0.95	1
		7	98	12	0.86	0.03	0.80	0.93
		9	86	13	0.73	0.04	0.65	0.82
		11	73	7	0.66	0.05	0.57	0.76
		14	66	7	0.59	0.05	0.50	0.70
Total number of juvenile mortalities after 14 days = 59								
0.2	38	2	100	41	0.59	0.05	0.50	0.69
		4	59	44	0.15	0.04	0.09	0.24
		7	15	13	0.02	0.01	0.01	0.08
		9	2	2	0	NA	NA	NA
Total number of juvenile mortalities after 14 days = 100								
2	38	2	100	5	0.95	0.02	0.91	0.99
		4	95	56	0.39	0.05	0.31	0.50
		7	39	35	0.04	0.02	0.02	0.10
		9	4	4	0	NA	NA	NA
Total number of juvenile mortalities after 14 days = 100								

Data not shown for 25 °C, 2 PSU group because no deaths occurred in the group over 14 days.

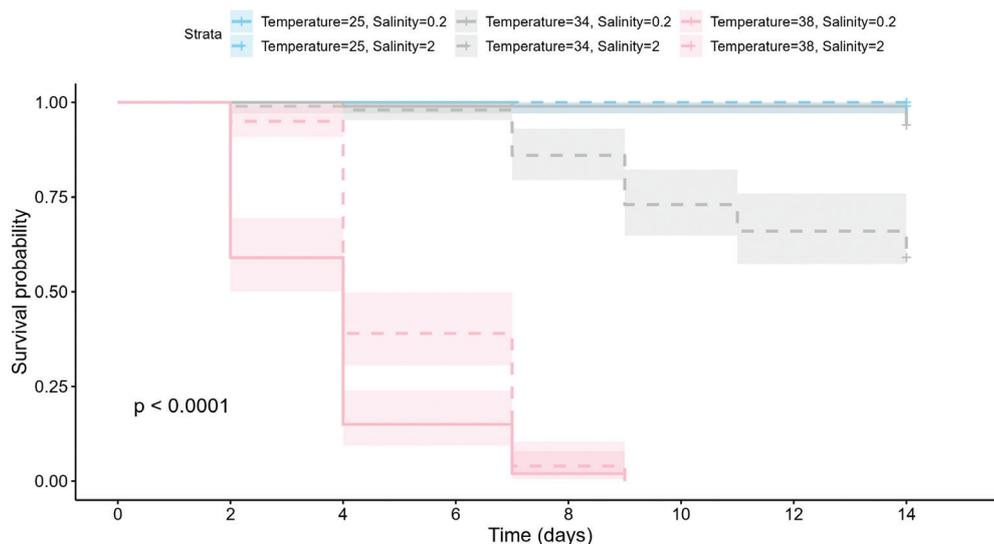


Figure 1. Kaplan-Meier survival curves of Japanese mystery snail (*Heterogen japonica*) juveniles over the 14-day experiment, plotting survival time in days with the associated survival probability. Different colors denote the different experimental temperatures (°C), and different line styles are associated with the two tested salinities (PSU), with the shaded area indicating the 95% confidence interval. The p-value calculated via the log-rank test on the Kaplan-Meier survival curves indicates an overall significant difference in survival between the crossed temperature and salinity factors.

Table 2. Pairwise comparison of individual juvenile survival during the 14-day experiment between treatment groups of Japanese mystery snail (*Heterogen japonica*) juveniles from the Kaplan-Meier survival curve analyses using the Bonferroni correction. Values in each box correspond to the p-value of survival between the corresponding groups. Significant differences ($p < 0.05$) between experimental temperatures ($^{\circ}\text{C}$) and salinities (PSU) are denoted with an asterisk.

	Salinity=0.2, Temperature=25	Salinity=0.2, Temperature=34	Salinity=0.2, Temperature=38	Salinity=2, Temperature=25	Salinity=2, Temperature=34
Salinity=0.2, Temperature=34	0.86	–	–	–	–
Salinity=0.2, Temperature=38	<0.0001*	<0.0001*	–	–	–
Salinity=2, Temperature=25	1.00	0.20	<0.0001*	–	–
Salinity=2, Temperature=34	<0.0001*	<0.0001*	<0.0001*	<0.0001*	–
Salinity=2, Temperature=38	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Table 3. Results of the generalized linear model for day 4 and day 14 on brood survival (%) of Japanese mystery snail (*Heterogen japonica*) juveniles. Estimate, standard error, t-value, and p-value results are shown for each factor and the intercept. Significant differences ($p < 0.05$) are denoted with an asterisk.

	Day 4				Day 14			
	Estimate	Std. Err	T value	Pr(> t)	Estimate	Std. Err	T value	Pr(> t)
(Intercept)	-0.882761	0.109087	-8.092	<0.0001*	-1.843860	0.128982	-14.295	<0.0001*
Salinity	0.019180	0.019697	0.974	0.332	0.060847	0.023290	2.613	0.00982*
Temperature	0.031865	0.003261	9.772	<0.0001*	0.067647	0.003856	17.545	<0.0001*

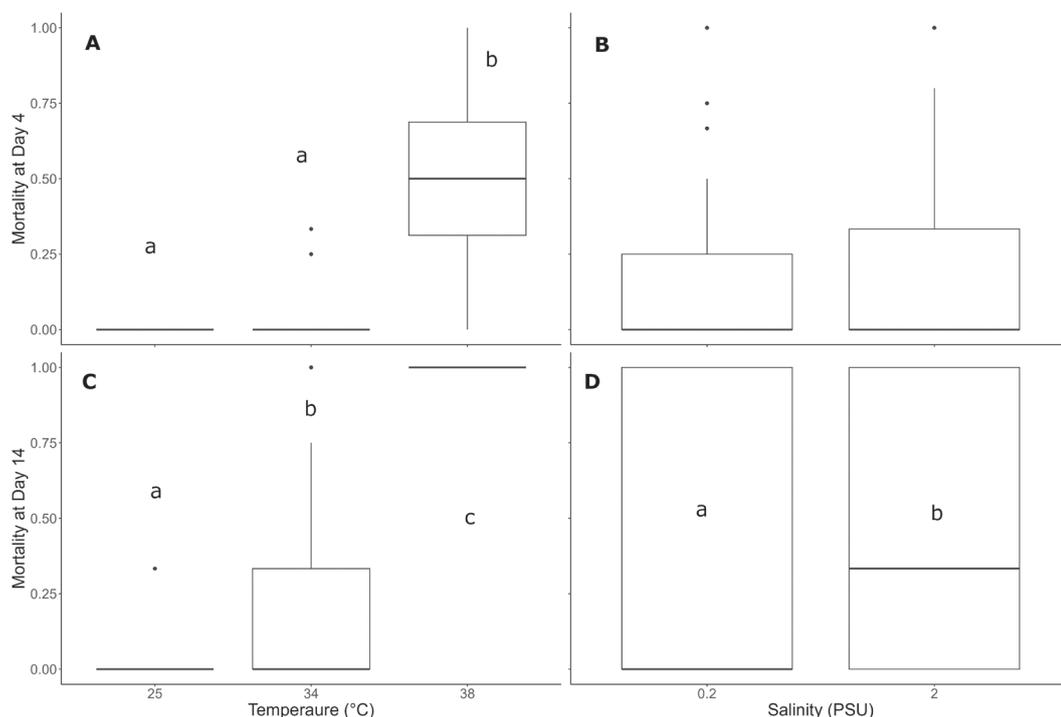


Figure 2. Boxplots of mortality percentage within broods of Japanese mystery snail (*Heterogen japonica*) juveniles at day 4 of the experiment, which was the 50% mortality date for groups in the 38 $^{\circ}\text{C}$ treatment, and the end of the experiment. The mortality measurements taken at day 4 were grouped by temperature – $p < 0.0001$ (A) and salinity – $p = 0.33$ (B). Mortality measurements taken at day 14 were also grouped by temperature – $p < 0.0001$ (C) and salinity – $p = 0.01$ (D). P-values indicate the overall significance of the generalized linear model, and bars with significant differences, as determined by Pairwise Wilcox comparison tests, are denoted by differing lowercase letters. Edges of the boxes indicate the upper quartile, median, and lower quartile, while the whiskers extend a length of 1.5 times the interquartile range. Dots represent outliers.

Discussion

This study is the first to report the temperature and salinity tolerances of Japanese mystery snails, *H. japonica*, from an invasive population. The results of this study indicate that juvenile *H. japonica* have high potential to spread even in high water temperatures and up to 2 PSU salinity, as all groups showed at least multi-day survival in all treatments. Results suggest a possible temperature threshold between 34 °C and 38 °C for this species from this location. The results for juvenile survival at 38 °C were peculiar as survival was initially higher in the 2 PSU salinity group than in the 0.2 PSU group. This differed from the 34 °C group and seems counterintuitive to the expectation that higher salinity should result in expedited mortality. Potentially, the juvenile snails could have closed their operculum primarily in response to salinity, allowing them to survive for longer in the elevated temperatures than their lower salinity counterparts. Given that juvenile *H. japonica* could tolerate at least 2 PSU, it is possible that they could float on the water surface and travel to new tributaries of the Chesapeake Bay (AEF, personal observation). Indeed, Hoy et al. (2012) found that invasive freshwater New Zealand mud snails (*Potamopyrgus antipodarum*) tolerated salinities of up to 38 PSU by closing their operculum. We did not document behavior, so cannot confirm this to be the case. However, given the results of these trials, their propensity to spread in brackish conditions should not be underestimated. Adult *H. japonica* can survive four weeks out of water via this method, while adult *C. chinensis* can tolerate nine weeks out of water (Unstad et al. 2013). In a similar study, Havel et al. (2011) found that adult *C. chinensis* survived approximately 6 weeks out of water, while only 75% of juvenile *C. chinensis* (<10mm shell length) survived air exposure after four weeks. Additional laboratory experiments are needed to challenge juvenile Japanese mystery snails to higher salinities as well as air exposure.

Further expansion of the mystery snails could be anthropogenic (i.e., human-mediated movement of adults into new river systems) via attachment to boats and other aquatic equipment or macrophytes, which can also be transported in the same way (Johnstone et al. 1985). The occurrence of *C. chinensis* in Wisconsin, USA lakes is correlated with human dispersal means such as the number of boat ramps and population densities (Solomon et al. 2010). Expansion could also be “natural” via dispersion of the juveniles, animal-borne dispersal via birds or mammals, or flooding events (Van Leeuwen et al. 2013). Due to the reproductive strategy of crawl-away juveniles (Fowler et al. 2022a), higher brood sizes may aid *H. japonica* expansion. In their native range in Japan, female *H. japonica* release between 10 and 120 live young between April and October (Taki 1981). Two earlier surveys of female *H. japonica* from Virginia, USA collected found averages of 108 (N = 7; range 46–220) and 52 live young (N = 287; range 1–197) per female during the boreal summer months (Van Bocxlaer and Strong 2016; Fowler et al. 2022a). Stephen et al. (2013) hypothesized that this reproductive strategy produced between ~ 2.2–3.7 million live young per year in a Nebraska reservoir and was the main contributor to *C. chinensis*’ success there. Given the naturally high numbers of juveniles produced, juvenile survival in various environmental conditions remains an intriguing area of study.

The spread of *H. japonica* is concerning for several larger ecological reasons. Both species can be found in a variety of freshwater habitats, including lentic ponds and urban lakes, with sandy or muddy substrates where they are generalist consumers of benthic organic matter via filter feeding and unselective deposit feeding but feed predominately on epiphytic benthic algae, especially diatoms (Jokinen 1982; van Bocxlaer and Strong 2016). *Heterogen japonica* can also grow rapidly in individual size (i.e., from 5 mm at birth to 35 mm after one year) (Clench and Fuller

1965; Jokinen 1982), and both *H. japonica* and *C. chinensis*, can reach high densities (Wolfert and Hiltunen 1968; Chainé et al. 2012). Fishermen in Lake Erie, USA reported that they could catch two tons of *H. japonica* in a single seine haul (Wolfert and Hiltunen 1968). In many cases, ecological impacts are exacerbated due to their exponential population growth stemming from the ovoviviparous reproductive strategy and fast individual growth (Wolfert and Hiltunen 1968). We are unaware of any work that has identified predators on *H. japonica* in its invasive range; however, crayfish and a variety of fish species consume *C. chinensis* (Olden et al. 2009; Twardochleb and Olden 2016; reviewed in Kingsbury et al. 2021). The physical advantages of mystery snails compared to native counterparts could also increase their resistance to predation. For instance, the larger and thicker shell and hard operculum of *C. chinensis* protects it from native predators such as the yellow perch, *Perca flavescens* (Olden et al. 2009). Mystery snails (specifically *C. chinensis*) also compete for food with native molluscs, as well as alter nutrient cycling, algal biomass, and microbial communities (Jokinen 1982; Bury et al. 2007; Clark 2009; Johnson et al. 2009; Olden et al. 2013). For example, Johnson et al. (2009) showed that *C. chinensis* reduced algal biomass, leading to increased competition with native snails, and increased the ratio of nitrogen to phosphorous as a sink for phosphorous in mesocosm experiments. The combined presence of *C. chinensis* with another common invader in similar habitats, the rusty crayfish (*Orconectes rusticus*), drove one native snail species to local extinction and reduced another species to only 5% of the original population size (Johnson et al. 2009). While mystery snails and native snails both feed in the same manner on similar items, the larger size of individual mystery snails in addition to their high densities and biomass suggests increased competition for food and/or negative impacts on the feeding behavior of native snails (Johnson et al. 2009; Sura and Mahon 2011). As both mystery snail species are similar in ecological requirements and functional ecology, we hypothesize similar impacts of *H. japonica* in invaded habitats; however, we are unaware of published work that documents the impacts of *H. japonica* in introduction locations.

Second, both species of mystery snails are intermediate hosts for several parasites, including trematodes (Fowler et al. 2022b). It is hypothesized that trematode infection negatively affects host reproduction, physiology, and behaviour (Combes 2001; Fowler et al. 2022b), but these impacts have not been explored in *H. japonica*. Currently, introduced populations of *H. japonica* have only been reported to host aspidogastreaan trematode parasites (Michelson 1970; Fowler et al. 2022b). Recent work suggests that female *H. japonica* in introduced populations infected with aspidogastreaan trematodes have lower numbers of live brooding embryos, but the mechanisms behind this and the impacts on populations remain unknown (Fowler et al. 2022b). Specific populations of *H. japonica* from northern Virginian and Maryland populations had a relatively high prevalence of aspidogastreaans (34% of individuals were infected), the highest of which were found where *H. japonica* co-occurred with the indigenous snail *Elimia virginica* (Fowler et al. 2022b, Hall-Stratton unpublished data). Genetic data found at least two cryptic lineages of aspidogastreaans, and one was shared between *H. japonica* and *E. virginica*, suggesting host-switching has occurred between the two snails (Fowler et al. 2022b). The ability of parasites to host switch could further influence community interactions in this system, particularly if *H. japonica* continues to spread in North America.

Third, the salinity and temperature tolerance of *H. japonica* gives it the potential to impact ecosystems other than the rivers and lakes in which it currently occurs. For example, Qin et al. (2020) found that the broad salinity tolerance of the invasive freshwater golden apple snail, *Pomacea canaliculata*, enables the species to expand into new ecosystems such as mangrove forests, as well as Chinese rice paddies that

supply food for large populations. The tidal freshwater marshes of Virginia's inner coast act as a similar habitat that also experiences fluctuations in salinity. In a study comparing salinity tolerance of native and invasive plants, Sutter et al. (2015) observed that seawater intrusion of tidal marshes due to climate change hastened the invasion of *Spartina alterniflora*. Other freshwater habitats may increase in salinity due to contamination such as from road salt runoff (Novotny et al. 2008). Similarly, climate change may unlock further habitats for *H. japonica* expansion, via sea level rise and saltwater intrusion into freshwater habitats (DeMarco et al. 2022), due to its tolerance of higher salinities and temperatures associated with a changing climate.

Determining the tolerances of an invasive species is an important step in identifying at-risk areas and targeting control efforts. Due to *H. japonica*'s wide but fully unknown range, determining environmental tolerances can vastly narrow down areas of interest. This study explored the salinity tolerances of juveniles extracted from the brood pouch of an adult female; further work could compare these values with mortality estimates from juveniles naturally expressed from the female or from adult *H. japonica*. As seen with other freshwater gastropods, there may be ontogenetic differences in salinity tolerances (Bernatis et al. 2016). Additionally, snails may have behavioral mechanisms to tolerate adverse temperature or salinity conditions, such as burrowing (Taki 1981; Jokinen 1982; Burnett et al. 2018), which they would not have been able to do in these laboratory experiments due to lack of substrate. It would also be important to compare the tolerances of native and invasive snails to document if the invasive species has a broader tolerance range to these important abiotic factors. While many other factors, including pH, oxygen content, and nutrient availability, may affect *H. japonica*'s range, salinity and temperature are especially important in tidally influenced freshwater environments. Future work should examine temperatures between 34 and 38 °C and salinities above 2 PSU to understand the extent of covariance between salinity and temperature and create more robust mathematical models to estimate survivability and spread.

Funding declaration

George Mason University (GMU)'s high school research program (ASSIP) supported this work. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author's contribution

PJ designed the sampling and methods, collected the data, completed the data analysis, interpreted the data, wrote the original draft, and reviewed and edited all other drafts. DHS designed the sampling and methods, collected the data, interpreted the data, and reviewed and edited all other drafts. AEF conceptualized the research, designed the sampling and methods, completed the data analysis, interpreted the data, wrote the original draft, and reviewed and edited all other drafts.

Acknowledgements

We thank Thomas Jefferson High School for Science and Technology for providing Pratyush the opportunity to complete his senior thesis in the Fowler lab at George Mason University. We thank the Aspiring Scientists Summer Internship Program at George Mason University for providing the mechanism for hosting high school students. We would also like to thank our reviewers and the thematic editor for their suggestions and feedback.

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