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Research Article

Population structure and demography of non-indigenous Japanese mystery snails in freshwater habitats of Virginia and Washington, D.C., USA

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Abstract

Non-indigenous species may drive declines in global freshwater biodiversity. Japanese mystery snails, *Heterogen japonica* (previously *Cipangopaludina / Bellamya / Viviparus japonica*), have invaded numerous freshwater systems in North America. To resolve questions about its population demography and genetics, we surveyed ponds and rivers from six Mid-Atlantic USA locations (Richmond, Virginia to Washington, D.C.) in 2018 and 2019 for mystery snails and co-occurring indigenous snails. A random subset of each snail species (max N = 80) per location was assessed for population demographics (size, sex), and brooding embryos were counted in mystery snails. Because morphological identification can be difficult to discern from its congener, the Chinese mystery snail (*Cipangopaludina chinensis*), we used a mitochondrial barcoding gene (COI) to confirm identity of *H. japonica* and also serve as a population genetics marker. Our barcoding confirmed that the mystery snails detected in our surveys were *H. japonica*, and that, compared to the indigenous range, its populations have low genetic diversity and limited genetic structure. Even so, *H. japonica* had the highest overall catch-per-unit-effort among all snails and sites. In demographic analyses, *H. japonica* populations skewed towards females, and females brooding live young were the largest across all sites. The number of live young ranged from 14 to 101/female (average: 52 live young/female). Further, a linear relationship existed between brooding female shell length and the number of live young for all sites, except one. Possible explanations could include site-level differences in abiotic or biotic parameters, but this requires further research. Altogether, the snail's reproductive capacity documented here suggests that *H. japonica* has the potential to undergo additional population growth, especially if large females spread to new locations. Moreover, it highlights another example of an invasive species with high population abundance, demographic performance, and distributional range even with depauperate genetic diversity.

Key words: *Heterogen/Cipangopaludina japonica*, gastropod, genetic paradox, invasion, North America

Introduction

Globally, freshwater biodiversity is at risk due to a number of leading anthropogenic concerns, including climate change, water pollution, flow modification, degradation of habitats, and invasion by exotic species (Dudgeon et al. 2006; Reid et al. 2019). Invasive species, in particular, rank

as the second leading cause of freshwater biodiversity declines around the world because they may compete with, predate on, or displace indigenous flora and fauna (Parker et al. 1999; Dudgeon et al. 2006; Reid et al. 2019). Exotic freshwater gastropods, specifically, have invaded numerous indigenous ecosystems either through deliberate introductions or accidental anthropogenic transfer (Padilla and Williams 2004).

Two invasive freshwater snail species, the Japanese mystery snail (*Heterogen / Cipangopaludina / Bellamya / Viviparus japonica)* and the Chinese mystery snail (*C. chinensis*), were intentionally transported from Japan to North America in ~ 1911 and ~ 1892, respectively, to be cultivated for human consumption (Prashad 1928; Clench and Fuller 1965). Both species now occur in tributaries throughout North America (Jokinen 1982; United States Geological Survey 2021). The exact species identities of the mystery snails detected in many North American locations remain unclear because identification via traditional visual morphology is challenging due to their morphological similarities (Smith 2000; Van Bocxlaer and Strong 2016). The presence of two morphs of *C. chinensis* in North America further hinders correct identification (Jokinen 1982, 1992; Van Bocxlaer and Strong 2016). In addition, their taxonomies remain in flux as they have each been assigned multiple scientific names, and debate still surrounds the phylogenetic structure of these species and related congeners (Perez et al. 2016). Indeed, recent evidence suggests that Japanese mystery snails belong to the genus *Heterogen*, and thus in this paper, we refer to them as *Heterogen japonica* (Saito and Kagawa 2020).

Of the two species, snails morphologically identified as *C. chinensis* have been studied more extensively in introduced populations. As non-selective detritivores, *Cipangopaludina chinensis* consume benthic and epiphytic algae. In their introduced range, they compete with indigenous 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). They have ovoviviparous reproduction (i.e., eggs hatch within the mother and develop inside before being expelled as juveniles), and females can live five years (Jokinen 1982, 1992; Stephen et al. 2013). Similarly, the Japanese mystery snail, *H. japonica*, acts as a filter-feeder and detritivore and can improve overall water quality and measures of lake productivity (Solomon et al. 2010; Van Bocxlaer and Strong 2016). However, this species can also negatively impact indigenous snail populations, likely due in part to exponential population growth stemming from their ovoviviparous reproductive strategy (Wolfert and Hiltunen 1968). Overall, little information exists about the life history 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*. Moreover, few genetic studies have been used to identify the two mystery snail species in North America (but see Perez et al. 2016 and David and Cote 2019).

Here, we collected mystery snails and co-occurring indigenous snails from six freshwater populations in Virginia and Washington, D.C. over two years. Our study provides a detailed understanding of the demographic features of indigenous and non-indigenous snails in these communities and provides further insight into the demographic success and geographic spread of mystery snails in these systems.

Materials and methods

Study design

Field collections and demographic assessments

We surveyed locations from Richmond to Washington D.C. for freshwater snails in July, August, and September 2018 and June and July 2019. We chose sites based on previous reports of collected "mystery snails" (i.e., species either named as Chinese mystery snails or Japanese mystery snails) to the United States Geological Surveys' Invasive Species Program (https://www.usgs.gov/ecosystems/invasive-species-program), and ultimately we sampled six locations to encompass different Virginia/D.C. rivers or lakes (Table 1). Apple snails (*Pomacea maculata*) are also sometimes referred to as "mystery snails"; however, we specifically chose sites known to host Chinese / Japanese mystery snails, and apple snails are easily distinguished from *C. chinensis* and *H. japonica*. We used hand and visual surveys to collect indigenous and non-indigenous snails using a timed collection method (1 hour) along a ~ 30 m transect at a maximum water depth of 1 m. This provided a catch-per-unit effort (CPUE) method of assessing relative species abundance across all encountered snail species.

We brought a random subset of putatively adult individuals of each snail species (max N = 80) to the laboratory for demographic analyses (sex, size). We used published keys and expert identification (R. Dillon, *pers. comm.*) to visually identify indigenous snails. We used the *World Registry of Marine Species* (WORMS, https://www.marinespecies.org/) as our primary taxonomic source. In mystery snails, the size at maturity for these populations is unknown, so we assessed all encountered snails > 15 mm shell length. We measured all snails for shell length (mm) and dissected them to determine sex (i.e., male, female, hermaphroditic). We collectively enumerated all brooding embryos (e.g., freshly encapsulated, angulated eggs, developing eggs, and juveniles *sensu* Van Bocxlaer and Strong 2016) in mystery snails. We also froze a sample of foot tissue from each mystery snail for species confirmation and population genetics analyses.

DNA isolation, amplification, and sequencing of mystery snails

We used foot tissue from mystery snails from each location to determine species identity (N = 10, Anacostia; N = 14, Lake Barcroft; N = 7, Gunston;

N = 12, Bryan Park; N = 12, Leesylvania; N = 27, Rappahannock). We extracted DNA using a standard cetyl trimethyl ammonium bromide (CTAB)-ethanol precipitation protocol (France et al. 1996). We used the Geller et al. (2013) Universal COI invertebrate primers and newly designed *Heterogen*/*Cipangopaludina* COI primers (COI-F: CCACCTGCAGGATC AAAGAA; COI-R: TCCGGATTAGTTGGAACTGG) based on a *H. japonica* sequence (accession #: MK308324.1) to identify mystery snail species and perform population genetics analyses. We used the following PCR profile: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s; and 72 °C for 5 min (Steinberg et al. 2008). PCR products were purified using EXOSAP-IT (Applied Biosystems) and submitted for sequencing at Psomagen, USA (Rockville, MD). These sequences were deposited to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as accession numbers ON323055–ON323149. We compiled new Virginia/D.C. sequences with Japanese and Chinese mystery snail sequences from Asian and North American populations that were harvested from GenBank and BOLD (http://www.boldsystems.org/) (Supplementary material Table S1).

Data analyses

Genetic sequence alignment, analysis, and barcoding

We aligned and manually inspected genetic sequences for ambiguities using Geneious 10.1.2 (Biomatters Ltd). Sequences were aligned without gaps using the ClustalW algorithm (Larkin et al. 2007). Following alignment and curation, our COI sequence fragment was 545 base-pairs (bp). We aligned these sequences with those available in Genbank/BOLD (per above), and the overlapping sequence fragment among all sequences was 404 bp (i.e., we had to further truncate the overall sequence length to match our sequences with these published sequences). We collapsed sequences into haplotypes using TCS v.1.21 (Clement et al. 2000). The optimal nucleotide substitution model was selected based on the Akaike Information Criterion in jModelTest2 (Darriba et al. 2012) and used in Bayesian phylogeny reconstructions with MrBayes (Huelsenbeck and Ronquist 2001). The substitution model was set to GTR with four gamma categories; the rate variation set to gamma; and MCMC settings were default.

Along with all available GenBank or BOLD mystery snail sequences overlapping with our *H. japonica* and *C. chinensis* sequences, we also included nine additional sequences of *Elimia virginica* and four sequences of *Campeloma decisum* from Virginia/D.C. for comparative purposes (Table S1). We used a *Nodilittorina pyramidalis* (GenBank: MN389024) sequence as our outgroup and to root the tree. We calculated pairwise FSTs using Arlequin311, constructed a resemblance matrix of populations from Table S1 using Primer7 and used an nMDS plot and boxplots to compare population differences in haplotype identities and frequencies.

While our phylogenetic tree included all published sequences from the indigenous and non-indigenous ranges of *H. japonica* and *C. chinensis* (Table S1), our nMDS analysis only included populations that had population sizes of ≥ 3 individuals per population. For these populations, we also calculated haplotype (*h*) and nucleotide (π) diversity values using Arlequin311, and also compared the total *h* and π for all indigenous versus non-indigenous sequences. Because there were too few samples from *C. chinensis*' non-indigenous range, this latter analysis only examined comparative population level indices in *H. japonica*. In Arlequin, we also calculated Tajima's *D* and Fu's *Fs* for *H. japonica* in its indigenous and non-indigenous ranges (all Japanese and all Virginia/DC populations, respectively) to look for evidence of population bottlenecks or recent population expansions in the two regions.

Population demography

We calculated a catch-per-unit-effort for each species at each site by dividing the total number of snails found, by species, by the total search time. We first tested whether there were differences in *H. japonica* snail shell length due to sampling year. Finding no differences, we combined all data and used a general linear model to examine the influence of site and snail sex (male, brooding female, non-brooding female) on snail shell length, followed by pairwise comparisons using Tukey's tests. A generalized linear model using a negative binomial distribution and log-link function compared whether the number of live young found in *H. japonica* females differed by site, using shell length as a covariate. Pairwise comparisons between sites were made using sequential Bonferroni corrections. A general linear model compared the relationships between shell length of brooding *H. japonica* females and the number of live young across sites, followed by pairwise comparisons using Tukey's tests.

Results

Genetic analyses

We included 193 sequences in our dataset (Table S1), including *Heterogen japonica* from indigenous (N = 20) and non-indigenous (N = 90) ranges (82 new sequences from Virginia/D.C.), *C. chinensis* from indigenous (N = 85) and non-indigenous (N = 4) ranges and *Elimia virginica* (N = 9) and *Campeloma decisum* (N = 4) from Virginia. Sequences were collapsed into 37 haplotypes: 11 haplotypes were *H. japonica* (8 only in the indigenous range, 2 only in the non-indigenous range, and 1 shared between ranges), 20 were *C. chinensis* (18 only in the indigenous range, 1 only in the non-indigenous range, and 1 shared between ranges), 5 were *E. virginica* from Virginia, and 1 was *C. decisum* from Virginia (Figure 1A).

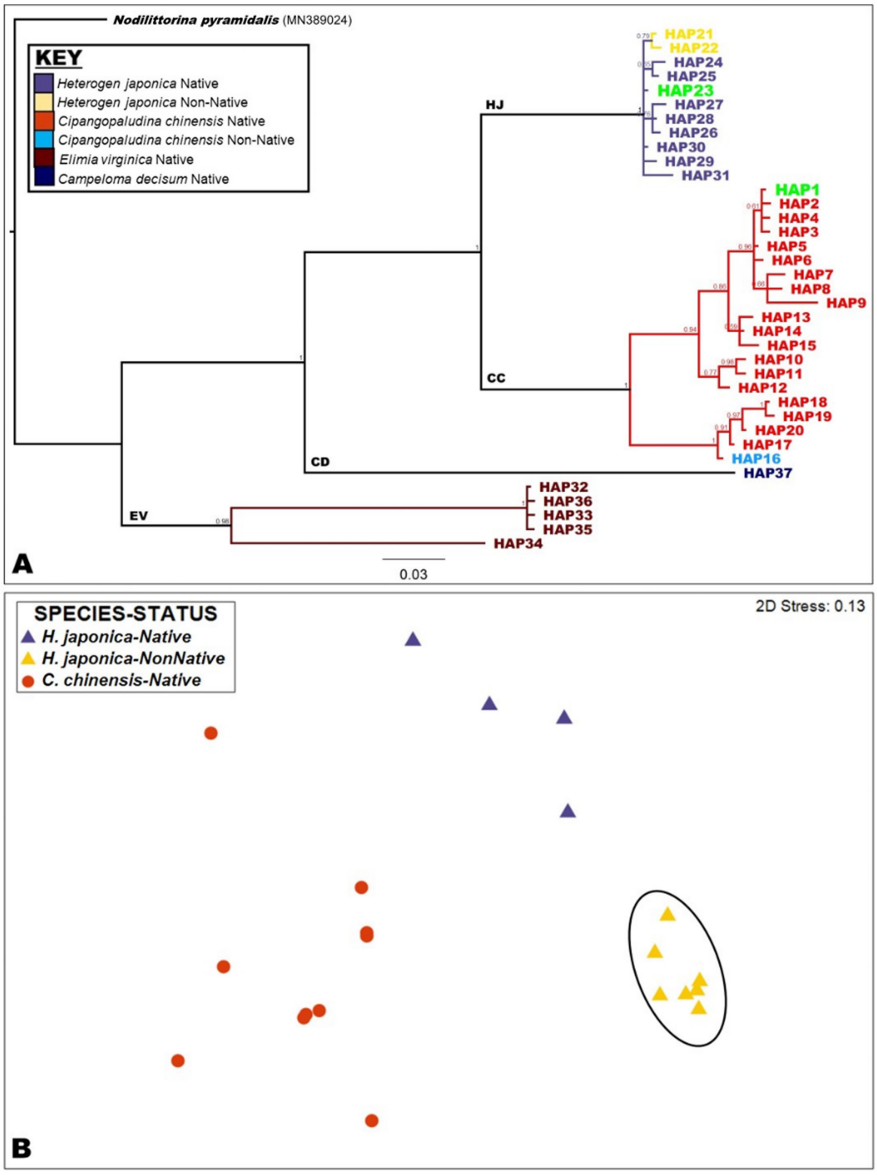

Figure 1. A) Phylogenetic reconstruction of the COI-fragment of indigenous and non-indigenous snails. Posterior probabilities for each clade are noted on the nodes. Two major clades are represented for the two mystery snail species: *H. japonica* (HJ) (haplotypes 21–31) and *C. chinensis* (CC) (haplotypes 1–20) from indigenous Asia and non-indigenous North America. North American indigenous snails, *Campeloma decisum* (CD) and *Elimia virginica* (EV), are represented by haplotype 37 and haplotypes 32–26, respectively. The tree is rooted using an outgroup sequence from *Nodilittorina pyramidalis* (MN389024). The green coloring on the tree signifies shared haplotypes between the indigenous and non-indigenous regions for each mystery snail species. (B) nMDS plot of pairwise FSTs from *H. japonica* and *C. chinensis* populations in the indigenous and non-indigenous ranges, based on previous records and newly sampled Virginia/D.C. locations in our study (represented by the oval surrounding these sites).

While we detected no *C. chinensis* in our North American sampling, we included North American *C. chinensis* sequences (i.e., Hawaii, New York, and Ontario, Canada) that had been extracted from GenBank (Table S1). The New York (David and Cote 2019) and Hawaiian (Hayes et al. 2019) sequences shared haplotype h16 and were subsumed within a *C. chinensis* clade that contained multiple sequences (h17–h20) from the indigenous range (Figure 1A). The Ontario sequence (Center for Biodiversity Genomics) shared a frequent hapolotype (h1) that was part of a *C. chinensis* clade (h1–

h4) that only included individuals from Japan. Given the limited number of non-indigenous *C. chinensis* samples available for comparison, we did not further analyze the *C. chinensis* data for indigenous/non-indigenous comparisons, except for the nMDS plot where we included *C. chinensis* from the indigenous range (Figure 1B).

Within the *H. japonica* clade, all non-indigenous samples, including Virginian/D.C. samples, fell within a clade containing Japanese records (Figure 1A). However, Japan was the only indigenous region where published sequences existed. All non-indigenous North American records (N = 90) were assigned to three haplotypes: haplotype h23 was identified in New York (David and Cote 2019) and Rappahannock and shared with several populations in the indigenous range; haplotype h21 was found at all Virginian/D.C. sites, Texas (Perez et al. 2016) and Florida (Sengupta et al. 2009); haplotype h22 was only found in two Virginian (Gunston Cove and Rappahannock) sites. Haplotypes h21 and h22 did not match any of the indigenous records extracted from GenBank, most likely because the indigenous range is vast and undersampled (Figure 1A). This result can also be seen in the nMDS plot (Figure 1B) where the non-indigenous North American sequences were not subsumed within the Japanese indigenous sequences.

Haplotype (h) and nucleotide diversity (π) analyses of indigenous versus non-indigenous populations found nearly 7x greater h diversity and 48x greater π diversity in the indigenous range. Tajima's D was not significant for either region. Fu's Fs was significant and negative for the non-indigenous range (Table 2), suggesting a recent population expansion of the species in this region following introduction events.

Snail field surveys

Invasive mystery snails were hand-collected at all locations during the timed survey, with highest counts at the Rappahannock site and lowest at Leesylvania State Park (Figure 2). Mystery snails were most often found attached to hard surfaces (e.g., wooden docks, tree limbs and downed tree trunks, cement walls, and rock boulders) and were very rarely found attached to living plant material or on the bare sediment surface (except for Leesylvania, where they were found along the edge of a macrophyte bed). In addition to the invasive species, the following nine indigenous snail species were also encountered and collected: *Amnicola limosa*, *Campeloma decisum*, *Elimia virginica*, *Lymnaea humilis*, *Lyogyrus granum*, *Physella acuta, Physella pomilia*, and *Helisoma trivolvis* (Figure 2). Including the invasive mystery snail, the species richness of snails at our sites ranged from two species in Gunston Cove to seven species in Lake Barcroft. The invasive mystery snails made up between 18% (N = 19/104 from Leesylvania) and 56% (N = 98/174 from Gunston Cove) of the total number of hand-collected snails. *Heterogen japonica* had the highest overall catch-per-unit-effort (CPUE) across all sites at 0.92 individuals hand collected/minute

Table 1. Total number and male to female ratios of *Heterogen japonica* hand collected during a timed 60 min search from each location July–September 2018 and June–July 2019 (*only sampled in 2018). The abundance and percent of brooding female snails and the average number of embryos (± standard error) and maximum and minimum number for those that had embryos is also given.

Site	Latitude, Longitude	Total # collected	M:F ratio	% Female	No. (%) Brooding	Average Brood No. ± SE (Min–Max)
Anacostia*	38°52′16.2″N; 76°59′19.4″W	59	1: 1.68	63%	32 (86%)	63 ± 3.6 (1–97)
Gunston Cove	38°46′40.7″N; 77°02′55.7″W	98	1: 2.06	67%	52 (79%)	101 ± 5.6 (1–188)
Bryan Park	37°35′48.1″N; 77°28′14.3″W	136	1: 1.49	60%	63 (83%)	47 ± 2.6 (2–95)
Lake Barcroft	38°50′48.6″N; 77°10′04.3″W	113	1: 1.51	60%	49 (72%)	55 ± 7.1 (2–197)
Leesylvania*	38°35′06.2″N; 77°15′47.2″W	19	1: 2.8	74%	14 (100%)	56 ± 4.2 (24–78)
Rappahannock	38°14′39.4″N; 77°13′33.6″W	156	1: 1.5	60%	77 (86%)	14 ± 0.9 (1–42)

Table 2. Regional and population-level diversity indices and neutrality tests for *Heterogen japonica* using newly contributed sequences and published sequences (see Methods). Population-level indices include populations with > 3 individuals. Regional analyses include all sequences from Table S1. Per population or region, N = number of individuals; a = number of alleles; h = haplotype diversity; π = nucleotide diversity. For regional analyses, Tajima's *D* and Fu *Fs* statistics, along with their respective *p* values, are denoted. Bolded values represent significant deviation from neutrality.

Site	N	a	*h* ± S.E.	π ± S.E.	Tajima's *D*	*p* (Dsim < Dobs)	Fu's *Fs*	*p* (Fssim < Fsobs)
INDIGENOUS								
Aichi, Japan	4	2	0.6667 ± 0.2041	0.00179 ± 0.00166				
Hyogo, Okayama, Japan	3	1	0.0000 ± 0.0000	0.00000 ± 0.00000				
Osaka, Nara, Wakayama, Japan	4	2	0.5000 ± 0.2652	0.00124 ± 0.00153				
Shiga, Japan	5	4	0.8000 ± 0.1721	0.00941 ± 0.00635				
All Japanese Populations	20	9	0.8579 ± 0.0611	0.00771 ± 0.00466	−0.770	0.245	−1.619	0.193
NON-INDIGENOUS								
Anacostia, Washington, D.C.	10	1	0.0000 ± 0.0000	0.00000 ± 0.00000				
Belle Haven, Virginia	7	2	0.2857 ± 0.1964	0.00000 ± 0.00000				
Bryan Park, Virginia	12	1	0.0000 ± 0.0000	0.00000 ± 0.00000				
Potomac, Virginia	3	1	0.0000 ± 0.0000	0.00000 ± 0.00000				
Lake Barcroft, Virginia	14	2	0.2637 ± 0.1360	0.00065 ± 0.00086				
Leesylvania, Virginia	12	1	0.0000 ± 0.0000	0.00000 ± 0.00000				
Rappahannock, Virginia	17	2	0.2206 ± 0.1208	0.00000 ± 0.00000				
All Virginian/D.C. Populations	90	3	0.1281 ± 0.0474	0.00016 ± 0.00038	−0.784	0.214	**−3.159**	**0.000**

(Figure 2). However, there were some indigenous snails with higher CPUEs at particular sites (i.e., *C. decisum* at Anacostia – 1.33 and *E. virginica* at Leesylvania – 1.33 and Rappahannock – 1.36) (Figure 2).

Heterogen japonica *population demographics*

The largest snails (shell length) across all snail species and sites were brooding *H. japonica* females (average shell length 48.93 ± 9.76 mm standard error). Non-brooding females from Gunston Cove measured significantly smaller (average 34.79 ± 4.69 mm) than all other site and sex combinations (average 44.81 ± 11.16 mm) (Figure 3), resulting in a significant interaction between site and sex on *H. japonica* shell length (N = 566, LM, $F_{(10,555)}$ = 20.86, p < 0.001).

In the majority of locations, male *H. japonica* were less abundant than females, with an average male to female sex ratio of 1:1.63 (Table 1). In addition, of those females collected, the majority (between 72% and 100% depending on the site) were brooding live young. The average number of brooding young in a single female across sites was 52 live young. After accounting for shell length, there was a significant difference in the number of embryos between sites (N = 286, GLM, df = 5, Wald Chi-Square

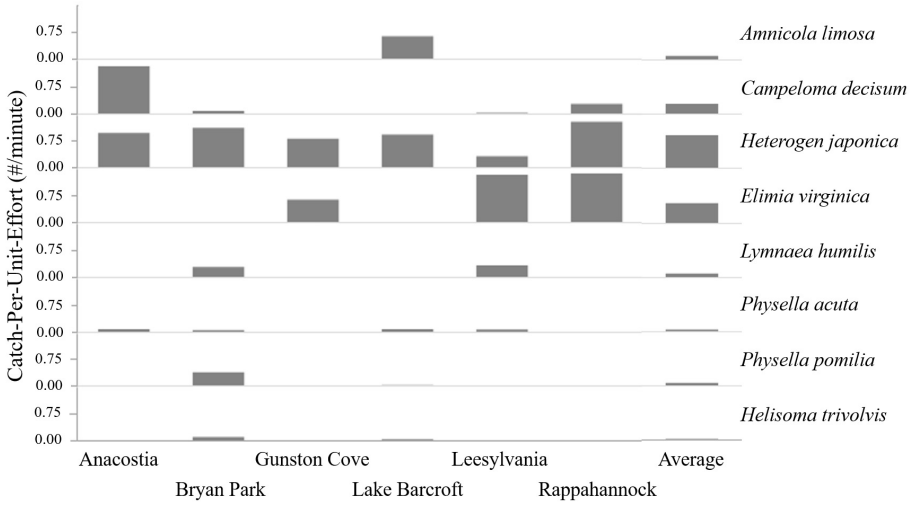

Figure 2. Catch-per-unit-effort for each indigenous and non-indigenous snail species hand collected during a timed 60 min search from each location July–September 2018 and June–July 2019 (except Anacostia and Leesylvania, which were only sampled in 2018). Values represent how many snails could be hand collected per minute.

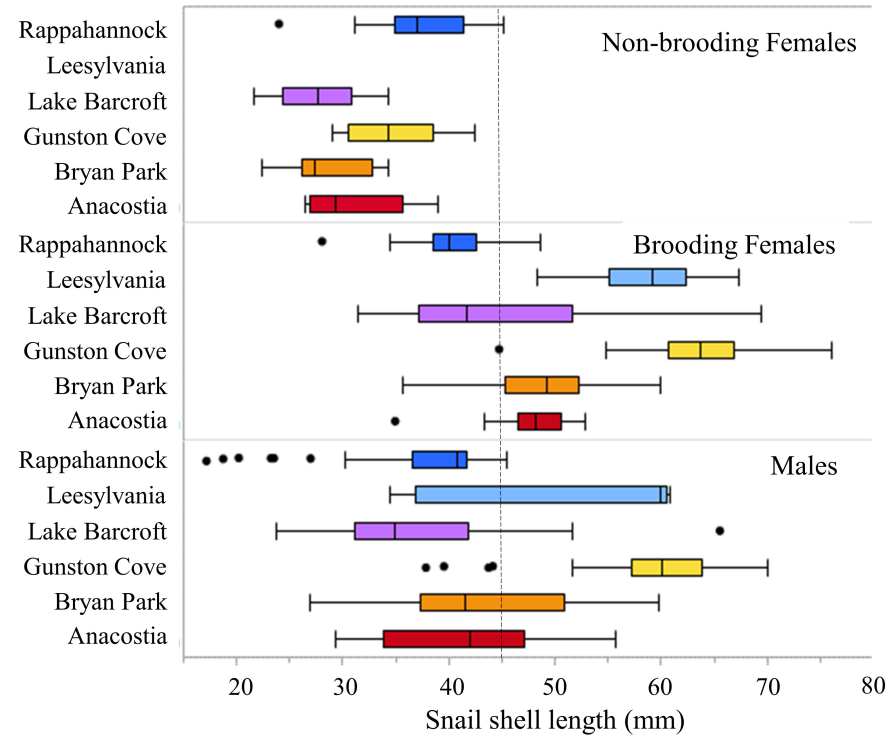

Figure 3. Shell lengths (mm) by site of male, brooding female, and non-brooding female *Heterogen japonica* hand collected during a timed 60 min search from each location July–September 2018 and June–July 2019 (except Anacostia and Leesylvania, which were only sampled in 2018). Box plots represent the interquartile (IQ) range (i.e., the middle 50% of the records), the vertical line is the median, and the whiskers are the highest and lowest values which are no greater than 1.5 times the IQ range. Outliers (i.e., black circles) are values between 1.5 and 3 times the IQ range, beyond the whiskers. The dashed line at 45 mm represents the overall average size of invasive mystery snails across all sexes and sites.

= 134.07, $p < 0.001$), with the highest average number of embryos in females from Gunston Cove (101 embryos) and the lowest in females from the Rappahannock (14 embryos) (Table 1, Figure 4A). With the exception of females from the Rappahannock and Gunston Cove, larger females were

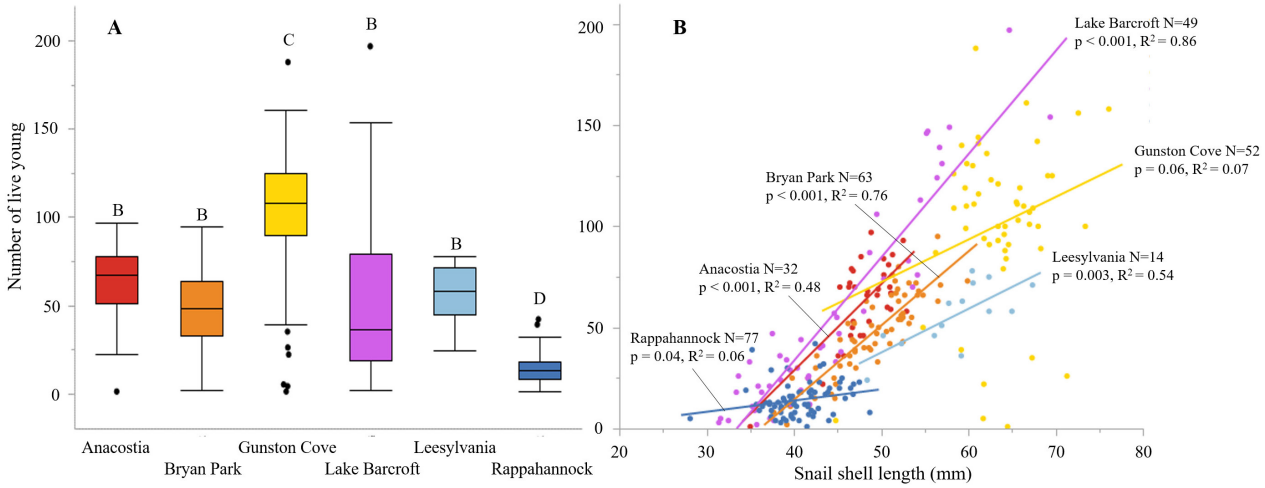

Figure 4. (A) Number of live young found in brooding female *Heterogen japonica* by site. The boxes represent the interquartile (IQ) range (i.e., the middle 50% of the records), the horizontal line is the median, and the whiskers are the highest and lowest values which are no greater than 1.5 times the IQ range. Outliers (i.e., black circles) are values between 1.5 and 3 times the IQ range, beyond the whiskers. Different letters above each box plot indicate significant differences ($p < 0.05$). (B) Linear relationships and the strength of those relationships (R^2 value) between the shell lengths (mm) of brooding female *H. japonica* and the number of live, internal embryos counted during dissection.

found with higher numbers of live young during dissections (Figure 4B). Therefore, the relationship between shell length of a brooding female and the number of live embryos that was counted during dissection differed between sites (N = 286, LM, $F_{(5,280)} = 54.05$, $p < 0.001$, Adjusted $R^2 = 0.482$). Brooding females from the Rappahannock were never observed with more than 42 live young, while there were clear positive associations between shell length and numbers of live young at all other sites (Figure 4B).

Discussion

Here, we genetically confirmed that mystery snails present in Virginia and Washington, D.C., USA were the Japanese mystery snail, *H. japonica,* and that these non-indigenous populations were genetically depauperate. In demographic analyses, we determined that mystery snails were the most abundant snails present and that their populations were skewed towards large brooding females, suggesting stable and successfully reproducing populations.

Genetic confirmation and population genetics of H. japonica

While Van Bocxlaer and Strong (2016) previously identified *H. japonica* in the Potomac River in Alexandria, Lake Barcroft, and Gunston Cove, Virginia via morphological distinctions, here we confirm identification of *H. japonica* via genetic methods. Moreover, we detected little to no genetic structure in the populations of *H. japonica* from the United States. All sequences, regardless of collection site, were comprised of just three haplotypes, and diversity indices (h and π) were 7 and 48 times lower in the non-indigenous regions, suggesting very limited genetic diversity for the invasive snail in its non-indigenous range.

Many non-indigenous populations exhibit significantly lower genetic diversity, which may occur as a result of founder effects (Dlugosch and Parker 2008), where just a subset of a species' source genetic diversity is transported (naturally or anthropogenically) to a new region, resulting in substantially lower genetic diversity compared to the source (Barton and Charlesworth 1984; Grosberg and Cunningham 2001). Frequent additions to the founding population could introduce new genotypes and increase standing genetic variation in the non-indigenous range that could help alleviate deleterious effects of low genetic diversity (Roman and Darling 2007; Dlugosch and Parker 2008; Lejeusne et al. 2014). However, even though we observed lower haplotype and nucleotide diversity in its non-indigenous populations, *H. japonica* was the most abundant snail at the majority of our sample sites and also demonstrated a very high reproductive capacity (discussed further below). This species is another example of a demographically successful invasive species demonstrating limited genetic diversity—and thus represents a "genetic paradox".

The genetic paradox refers to species that exhibit significantly reduced genetic diversity in founding populations, yet are demographically successful by adapting to novel environments (Estoup et al. 2016). While seemingly paradoxical, these species may have biological traits that allow them to circumvent possible deleterious effects predicted in founding populations (e.g., accumulation of deleterious alleles due to low founding sizes and subsequent genetic drift) (Schrieber and Lachmuth 2017). However, neutral markers commonly used in single-gene approaches may not reflect genetic diversity across the genome or genes associated with ecologically relevent traits (Estoup et al. 2016). Thus, further examination of genetic diversity across multiple markers could reveal genetic mechanisms for the demographic success of mystery snails under a scenario of depauperate genetic diversity, as in the invaded North American range.

In addition, it was not possible for us to pinpoint source locations from the indigenous range for the species' introduction using genetic data presently available. This is because the genetic diversity in that region is vast in comparison to what we sampled in the non-indigenous range (e.g., Blakeslee et al. 2008). Greater spatiotemporal sampling of indigenous and non-indigenous ranges could provide better understanding of the impact of genetic diversity on the species' distribution and its spread in the non-indigenous range, as well as likely sources of introduction.

Population demography of H. japonica

While the invasive Japanese mystery snail *H. japonica* was the most frequently encountered snail across all sites, two indigenous snail species had higher catch-per-unit-efforts at particular sites (i.e., *Campeloma decisum* at Anacostia and *Elimia virginica* at Leesylvania and Rappahannock). One of these, the pointed campeloma – *C. decisum*, was the largest indigenous

snail we encountered and can reach sizes of up to 40 mm shell length (Brown et al. 1989). Brown et al. (1989) observed that individuals can aggregate at high densisties of up to 800/m^2, but we did not observe large aggregations at our sites. Similar to *H. japonica*, *C. decisum* is also a deposit and filter feeder and has ovoviviparous (broods live young) reproduction (Brown et al. 1989). The Piedmont elimia, *E. virginica*, can reach sizes of up to 33 mm shell length, is dioecious, and has been observed at densities over 400 individuals/m^2 (Smith 1980). This species grazes on epilithic periphyton (Harman 2000), in contrast to *H. japonica* and *C. decisum*.

Previous work has also noted that invasive populations of *H. japonica* can attain high densities and likely has significant negative effects on the local ecosystem (Wolfert and Hiltunen 1968). Wolfert and Hiltunen (1968) reported that seine fishermen in Lake Erie, Pennsylvania, USA could catch two tons of the species in a single haul. Similar to our study, Wolfert and Hiltunen (1968) found that the second most frequently encountered snails were either *C. decisum* or a pleurocerid snail similar to *E. virginica*. It is likely that these species overlap in diet and may even compete for food in areas where there are high densities; yet studies thus far examining competition among indigenous freshwater snails and non-indigenous mystery snails have concentrated on *C. chinesis* (e.g., Jokinen 1982; Bury et al. 2007; Clark 2009; Johnson et al. 2009; Olden et al. 2013). While it is likely that the two mystery snails have similar ecological impacts because they share many of the same demographic and morphological traits, more experimental work describing the impacts of *H. japonica* on indigenous species and systems is needed to compare their ecosystem influences.

The broad size range of *H. japonica* collected for both sexes and the high proportion of brooding females in the populations suggests that these multigenerational populations successfully reproduce in these locations. The reproductive mode (i.e., ovoviviparous; eggs hatch within the mother before being expelled as juveniles) and reproductive capacity of this species both contribute to its success as an invader (Van Bocxlaer and Strong 2016). Organisms that brood live young are often less constrained by possible Allee effects (i.e., reduced or negative population growth at low population densities) that could emerge in founding populations (Johannesson 1988). Because ovoviviparous species can release numerous, crawl-away young that do not disperse far from the parent settlement, this reproductive strategy can enhance their colonization success and population growth in newly founded regions; indeed, some of the most globally widespread and invasive aquatic gastropods include brooders (e.g., *Potamopyrgus antipodarum*; Alonso and Castro-Diez 2008). Ovoviviparity has likely enhanced both *H. japonica*'s and *C. chinensis*' colonization success, spread, and population growth in freshwater systems throughout North America. For example, the estimated annual production of an invasive population of *C. chinensis* from a Nebraska reservoir was ~ 2.2–3.7 million

young (Stephen et al. 2013). This reproductive strategy may have also been a contributing factor in its demographic success and geographic spread in North America despite low genetic diversity in the region as detected in our study.

Site-level differences in H. japonica

In general, brooding female *H. japonica* were significantly larger than males, due mainly to the presence of the large brood pouch located in the same location as the much smaller testes in males (Van Bocxlaer and Strong 2016). However, at the Rappahannock site, we found a smaller size range of brooding females in comparison to other locations. Female *H. japonica* in this survey contained developing embryos of several sizes and stages of development, a trait found in other species of Viviparidae snails (Jakubik 2007). However, this characteristic also varied across sites. In general, female shell length and embryo number were positively related to one another except for two locations – Gunston Cove and Rappahannock. Female *H. japonica* in their indigenous range in Japan release live young between April and October, and the number of live brooding young was between 10 and 120 (Taki 1981). Stephen et al. (2013) found that *C. chinensis* females (N = 29) (e.g., species identification was not genetically confirmed) collected in southeast Nebraska contained an average of 25 embryos per female, while the average across all sites in this survey was 2.5x higher (N = 132, average 62, range 16–109). An earlier survey of only seven female *H. japonica* from northern Virginia collected in September 2012 found an average of 108 live young (range 46–220) (Van Bocxlaer and Strong 2016).

It is possible that there were various abiotic and biotic factors that could explain these site-level differences detected between our study and others; for example, the Stephen et al. (2013) survey occurred in September, possibly at the end of the breeding season, while the survey presented here occurred earlier during the summer months. The warmer water temperatures in Virginia/D.C. may have also contributed to the higher abundances of embryos in females as compared to other northern populations. Moreover, snail density, water quality, nutrients, day length, parasites and food availability all likely vary by location and could influence snail growth and reproductive output in these different locations (Ter Maat 2007; Sandland and Josephson 2017; Song et al. 2017; Dickens et al. 2018; Muñoz et al. 2018). Conversely, a genetic explanation for these demographic differences across sites in Virginia/D.C. is unlikely given the little genetic differentiation we observed among populations and low genetic diversity in all non-indigenous populations. Even so, the reproductive mode and the number of developing embryos documented in *H. japonica* in Virginia/D.C. populations suggest that this species has the potential to undergo additional population growth, especially if large females spread to new locations (Keller et al. 2007).

Although the populations studied here are genetically depauperate, the snails are successfully reproducing, and thus appear to represent another invasive species demonstrating a genetic paradox. The species' reproductive strategy as well as the presence of ecologically relevant genes may have helped it avoid deleterious effects often associated with low genetic diversity. Finally, while much of the literature from laboratory and field studies have identified North American mystery snails as *C. chinensis*, our study suggests that Japanese mystery snails are the dominant mystery snail in Virginia. Given the morphological similarities between the two species, genetic confirmation is needed going forward to ensure any new reports and research correctly identifies these two invasive species.

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Author's contribution

AEF conceptualized the research, 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. GAL collected the data and reviewed and edited all other drafts. AMHB 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.

Ethics and permits

With submission of this article, the authors have complied with the institutional and/or national policies governing the humane and ethical treatment of the experimental subjects. Maryland Department of Natural Resources Scientific Collection Permit Number SCP2018861. Virginia Department of Game and Inland Fisheries Scientific Collection Permit Number 062276.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Site names, country, and description of genetic sequences obtained for indigenous and non-indigenous populations of *Cipangopaludina chinensis* and *Heterogen japonica* used to construct phylogenetic trees.

This material is available as part of online article from:
http://www.reabic.net/aquaticinvasions/2022/Supplements/AI_2022_Fowler_etal_SupplementaryMaterial.xlsx