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Limited migration from physiological refugia constrains the rescue of native gastropods facing an invasive predator

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Abstract

Biological invasions have caused the loss of freshwater biodiversity worldwide. The interplay between adaptive responses and demographic characteristics is expected to be important for the resilience of populations to biological invasions, but the interaction between these factors is poorly understood. The native freshwater gastropod Amnicola limosus is distributed along spatial variation in impact from an invasive molluscivorous fish (Neogobius melanostomus), as well as in calcium concentration, which limits the distribution of this invader and thus provides refuges for the gastropods. We investigated if refuge populations could provide migrants to declining invaded gastropod populations through gene flow (i.e., demographic rescue), which could also help maintain genetic diversity (i.e., genetic rescue). We also tested for genetic adaptation of A. limosus to the invasive predator and the low calcium habitats. We conducted pooled whole-genome sequencing of twelve gastropod populations from the Upper St. Lawrence River, complemented with a laboratory reciprocal transplant of wild F0 A. limosus to measure survival and fecundity in treatments of water calcium concentration (low/high) and round goby cue (present/absent). We found that gene flow is restricted from the low-calcium uninvaded refugia towards high-calcium invaded populations, implying that the potential for demographic and genetic rescue is limited. We also detected signatures of divergent selection between habitat types and evidence of low fitness of individuals from refugia populations in both habitat types, which could be either a cause or consequence of the population structure between habitat types and highlights the potential conflict between demographic/genetic rescue and adaptation.

Title: Limited migration from physiological refugia constrains the rescue of native gastropods facing an invasive predator

Running title: Adaptation of gastropods to an invasive predator

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MS, LA, APH, AMD, and RDHB designed the study. LA collected the field samples. LA and MS conducted the laboratory experiment. LA and AP performed the DNA extractions. LA prepared the initial pool-seq processing pipeline with input from FC, finalized by MS. MS ran the bioinformatic and statistical analyses with input from AMD and RDHB. The article was written by MS with input from AMD and RDHB. All authors contributed to the review and editing. AMD and RDHB funded the study.

Abstract (246 words)

Biological invasions have caused the loss of freshwater biodiversity worldwide. The interplay between adaptive responses and demographic characteristics is expected to be important for the resilience of populations to biological invasions, but the interaction between these factors is poorly understood. The native freshwater gastropod*Amnicola limosus* is distributed along spatial variation in impact from an invasive molluscivorous fish (*Neogobius melanostomus*), as well as in calcium concentration, which limits the distribution of this invader and thus provides refuges for the gastropods. We investigated if refuge populations could provide migrants to declining invaded gastropod populations through gene flow (i.e., demographic rescue), which could also help maintain genetic diversity (i.e., genetic rescue). We also tested for genetic adaptation of *A. limosus* to the invasive predator and the low calcium habitats. We conducted pooled whole-genome sequencing of twelve gastropod populations from the Upper St. Lawrence River, complemented with a laboratory reciprocal transplant of wild F_0 *A. limosus* to measure survival and fecundity in treatments of water calcium concentration (low/high) and round goby cue (present/absent). We found that gene flow is restricted from the low-calcium uninvaded refugia towards high-calcium invaded populations, implying that the potential for demographic and genetic rescue is limited. We also detected signatures of divergent selection between habitat types and evidence of low fitness of individuals from refugia populations in both habitat types, which could be either a cause or consequence of the population structure between habitat types and highlights the potential conflict between demographic/genetic rescue and adaptation.

Keywords: genetic rescue, aquatic invasive species (AIS), gastropods, whole-genome sequencing, adaptation

Introduction

Invasive species represent a significant threat to global biodiversity (Early et al., 2016; Duenas et al., 2021). They are an important driver of species extinction (Bellard et al., 2017) and can have a strong negative impact on native species abundance (Emery-Butcher et al., 2020). The strength of the impact of invasive species on native populations and communities depends on their abundance and trophic level, with invasive predators typically having the most substantial impact (Bradley et al., 2019). The extent of a biological invasion in a geographic location will be determined by the limits of habitat suitability in the area (Liu et al.,

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2020). Environmental gradients can be important barriers restricting invasive species' unchecked advance (Mothes et al., 2019). For example, the range of the invasive Asian clam in North America and Europe is currently limited by its inability to tolerate minimum temperatures lower than -10degC and by altitudes higher than 2000m (Crespo et al., 2015). Thus, impacted native species that can tolerate a broader range of environmental parameters than the invaders may have access to refuge habitats free from invaders (Chapman et al., 2002; Reid et al., 2013). However, across large spatial scales or strong environmental gradients, it is unclear whether this type of 'physiological refugia' results from universally broad physiological tolerance in native species or local adaptation of populations experiencing distinct environmental conditions. Moreover, invasive predators might impose additional divergent selection on native species because local populations that overlap with the invader could experience selection for anti-predator traits (Strauss et al., 2006; Brookes & Rochette, 2007). Indeed, among natural and anthropogenic stressors, invasive species are one of the strongest drivers of phenotypic change in native populations (Sanderson et al., 2022).

Local adaptation could interact with demographic processes to facilitate or hinder the co-existence of native species with an invasive predator by providing demographic subsidies, genetic rescue, or by introducing maladaptive alleles to recipient habitats. While some sites can have environmental conditions more conducive to invasion and therefore suffer stronger biotic selection from invasion, other sites can have environmental conditions that exclude or reduce the density of invasive predators, thereby acting as uninvaded refuges with lowered ecological impacts from the invaders (Derry et al., 2013; Reid et al., 2013; Astorg et al., 2021; Morissette et al., 2023). In this scenario, refuge populations could also potentially serve as a demographic subsidy of individuals for invaded populations experiencing population decline (Foppen et al., 2000; With et al., 2006), and prevent their extinction through demographic rescue (Hufbauer et al., 2015). Genetic rescue can additionally occur when migrants prevent the extinction of declining populations through increased genetic diversity that reduces inbreeding depression (Carlson et al., 2014; Whiteley et al., 2015; Fitzpatrick et al., 2016); if this genetic variation includes adaptive alleles, then genetic rescue can also lead to an evolutionary rescue, i.e. the avoidance of extinction via adaptation (G. Bell & Gonzalez, 2011; Sexton et al., 2011; Hufbauer et al., 2015).

Conversely, gene flow could instead bring maladapted alleles into invaded populations if populations have experienced strong divergent selection across the environmental gradient (Bolnick & Nosil, 2007). Migration from refuges could consequently pose some risks if recipient and source populations are divergent due to local adaptation, which can cause genetic incompatibilities in hybrids and lead to temporary reductions in fitness (i.e., outbreeding depression, Fenster & Galloway, 2000; Edmands, 2007; Frankham et al., 2011; D. A. Bell et al., 2019). Thus, there could be a tension between demographic rescue (immigrants from refuge populations providing individuals to bolster the shrinking populations in invaded habitat) versus these immigrants potentially being maladapted, thereby reducing the mean fitness of the invaded populations. It is thus important to understand how these two sources of adaptation (to the refuge vs. to the predator) can interact with demographic processes to either facilitate or hinder the ability of a native species to avoid population decline from an invasive predator.

Genomic methods are increasingly used to understand species and populations' responses to sudden environmental changes induced by anthropogenic activities such as invasive species (Stern & Lee, 2020) and are an important tool for informing conservation (Willi et al., 2022; Bernatchez et al., 2023). They can enable the assessment of population connectivity, investigate demographic and genetic changes, and detect the potential for genetic adaptation (e.g., Marques et al., 2019). Reconstructing demographic changes can help assess potential population declines induced by invasive species. Additionally, inference of gene flow can identify the source and recipient populations in a metapopulation impacted by the invaders. Finally, assessing genetic adaptation can determine if source and recipient populations are divergent because of local adaptation (Cure et al., 2017), thus altering the likelihood of genetic and/or evolutionary rescue from genetically differentiated populations. Hence, knowledge of evolutionary forces, which can be elucidated through genomic tools, is critical for understanding the overall response of native species to the impact of biological invasions.

Gastropods have been widely used to study adaptation in response to predation (Brookes & Rochette, 2007;

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Hooks & Padilla, 2021), with abiotic factors such as calcium concentration modulating this response through changes in shell morphology and behavior (Rundle et al., 2004; Bukowski & Auld, 2014). As such, they are a useful biological study model for addressing evolutionary responses to biological invasions. *Amnicola limosus* is a small dominant freshwater gastropod species with a wide geographical distribution in the USA and Canada (www.gbif.org/species/5192461). This gastropod does not have a pelagic larval phase: egg masses are deposited on the substrate, and juveniles move from the substrate to the macro-algal substrate (Pinel-Alloul & Magnin, 1973). Part of the range of *A. limosus* has been invaded by the round goby (*Neogobius melanostomus*), a molluscivorous fish, from the lower Great Lakes and running downstream throughout the Upper St. Lawrence River (Hickey & Fowlie, 2005). *Amnicola limosus* is commonly found in the stomach contents of round gobies, and following the goby invasion of Lake Saint-Louis, *A. limosus*populations experienced a 0.5-1 mm reduction in shell size (Kipp et al., 2012). Because the mean gape size of the round goby is larger than the maximum size of *A. limosus* , round gobies do not have to crush the snail, which suggests that shell size reduction is likely to be due to reduced predation pressure on smaller and less visible individuals (round gobies are visual predators; Kipp et al., 2012). A considerable reduction in small gastropod abundance (down to 2-5% of the original population size, with *A. limosus* being the most abundant species) and species richness in the Upper St. Lawrence River were also reported since the invasion of round gobies in this ecosystem in 2005 (Kipp et al., 2012). However, round gobies cannot tolerate low calcium concentrations (Baldwin et al., 2012; Iacarella & Ricciardi, 2015), and have not invaded the Ottawa River (Ca^{2+} concentrations below 22 mg/L; Sanderson et al., 2021; Morissette et al., 2023) at its junction with the Upper St. Lawrence River. On the contrary, this low calcium concentration is not a physiological limit for *A. limosus*embryonic development ($>$ 1.1 mg/L; Shaw & Mackie, 1990), and Pinel-Alloul & Magnin (1973) showed that *A. limosus* was present in the Ottawa river before the invasion of gobies, indicating that this species can tolerate the calcium concentration found in the Ottawa river. These calcium-poor waters are thus acting as a refuge from goby predation in this system (Astorg et al., 2021; Morissette et al., 2023). Calcium-poor waters could potentially provide demographic subsidies for the native populations at invaded sites (e.g., amphipods; Derry et al., 2013).

This study aims to investigate the potential adaptation of *A. limosus* to the water calcium gradient and the presence of round goby invasion in the Upper St. Lawrence River, as well as the demographic and genetic consequences of the goby invasion on the native gastropod. We tested for evidence of local adaptation via 1) genome scans for SNPs associated with calcium concentration and round goby presence and 2) a laboratory reciprocal transplant of wild *A. limosus* individuals to measure survival and fecundity in factorially-crossed treatments of water calcium concentration and round goby chemical cue. For the local adaptation to the low calcium and high goby predation environmental conditions, we expected to find outlier SNPs associated with one or both of these covariables. In the transplant experiment, we also expected populations to show a home versus transplant advantage in life-history traits relative to the calcium concentration and presence of goby cues. For demography, because of the large decrease in the population size of gastropods observed in the Upper St. Lawrence River following round goby invasion (Kipp et al., 2012), we hypothesized that *A. limosus*populations in invaded habitats could have undergone a similar decrease in abundance, perhaps accompanied by a genetic bottleneck. If so, uninvaded populations could potentially provide demographic, genetic, and evolutionary rescue for invaded populations (Hufbauer et al., 2015; Whiteley et al., 2015). However, this possibility would depend on the level of gene flow between habitats and the extent of adaptive differentiation. Because the life history of *A. limosus* does not involve a pelagic larval phase (Pinel-Alloul & Magnin, 1973), we expected to observe low gene flow in the absence of strong water currents. Low gene flow impedes demographic and genetic rescue, and strong adaptive differentiation will likely hinder evolutionary rescue. As such, we hypothesized that local adaptation to the distinct habitat types might have led to reduced gene flow (isolation by environment; Wang & Bradburd, 2014). Our paper provides a rare empirical study to address how spatial heterogeneity in both abiotic conditions and invasive predator presence can interact with demographic processes to shape the response of a native species to biological invasion. Freshwater environments are deeply impacted by invasive species (Gallardo et al., 2016). It is important to not only consider ecological impacts of invasion but also the evolutionary and demographic responses in native species that can help foster invasive-native species coexistence in invaded freshwater ecosystems.

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Material and Methods

Study sites, sample, and physicochemical data collection

Our twelve study sites are located at the junction of the Ottawa river and the St. Lawrence River near Montreal, QC, Canada (Fig. 1). The Ottawa River water is calcium-poor (10-15 mg/L calcium), and the St. Lawrence River water is comparatively calcium-rich (30-40 mg/L) due to the different geological characteristics of their watersheds. These water masses mix at the junction of two major river systems at Lake Saint Louis, a widening of the St. Lawrence River, but the calcium gradient persists in the north and south shores, and water masses are distinct (Vis et al., 1998). In 2005, round gobies invaded the upper St. Lawrence River and the southern shore of Lake Saint-Louis, but not the calcium-poor Ottawa River nor calcium-poor sites on the north shore of Lake Saint-Louis (Kipp & Ricciardi, 2012).

Twelve *Amnicola* populations were sampled from the study sites in this fluvial ecosystem (Fig. 1), with three populations fully in the Ottawa River, three fully in the St. Lawrence River, and six populations in the Lake St-Louis, including three on the north shore and three on the south shore. We coded populations collected in the Ottawa River water as LCGA (low calcium- water and gobies absent) and populations from the St. Lawrence River water as HCGP (high calcium water and gobies present). Two populations had inverted patterns: RAF is calcium-poor, but gobies are present (LCGP), and PDC is calcium-rich, but gobies are absent (HCGA). It is noteworthy that PDC is located in a refuge habitat (wetland; Astorg et al., 2021) but close to invaded sites, and thus might receive strong gene flow from nearby invaded populations. Field-collected *Amnicola* snails were obtained near the shore via hand picking and brought back to the lab for further processing (DNA extractions and the common garden experiment) in June-October 2017. Goby abundance was measured in the field between July and September 2017 on a single occasion at each site. For this, each site was sampled using three seine net passes, with intermission periods between seining times. The seine net used for sampling nearshore habitats was 30 feet long by 6 feet deep and 1/8 mesh on a 10 m distance. Round gobies were placed into bins and released after the three hauls. The geographic location and environmental characteristics of our sampling sites are detailed in Table S1. We measured dissolved oxygen (DO; mgL^{-1}), pH, water temperature (°C), and conductivity ($\mu S.cm^{-2}$) using a Professional Plus Model YSI multi-parameter probe (model 10102030; Yellow Springs Inc.) at each study site in 2017 at the time of gastropod collection. On the same occasions, we collected water samples and analyzed them for calcium (Ca), total phosphorus (TP), total nitrogen (TN), as well as dissolved organic carbon (DOC) at the GRIL-UQAM analytical lab (Supplementary Methods). Site-specific invasion status by round goby (invaded / uninvaded) is defined by presence/absence (Table S1).

De novo genome assembly and pool-sequencing

For the *de novo* genome assembly, we extracted DNA from the tissue of one individual snail collected in 2017 using a standard Phenol Chloroform extraction method, after removing the shell and excising the mollusk guts to avoid contaminants. Briefly, tissue samples were placed in a digestion buffer containing proteinase K and digested at 55°C. DNA was then isolated using an isoamyl-phenol-chloroform solution, followed by ethanol precipitation. DNA quantity and quality were verified using a combination of different quality control methods: Qubit assay (Thermo Fisher Scientific Inc.), Tapestation (Agilent Inc.), and Femto Pulse (Agilent Inc,). Fragments longer than 1 kb were selected for further processing. Library preparation was performed using 10X Chromium Linked-Read library kit (10X Genomics Inc.) and sequenced on 3 lanes of Illumina HiSeqX PE150 at Genome Quebec. We ran fastp v.0.23.4 on the three 10X paired-end reads to obtain the insert size, using the -Q option to disable quality filtering. Fastp results showed two estimated insert size peaks at 175 and 270 bp. Reads were assembled with Supernova v.2.1.1. The assembled genome is 1,899,346,312 bp in length, with 815,134 scaffolds and a N50 of approximately 5kb. We estimated the genome size with Jellyfish 2.3.0 by reading simultaneously the R1 reads of the three runs of 10X sequencing using the options -F 3, -m 21, and -s 2G. The resulting histogram was then processed with GenomeScope http://qb.cshl.edu/genomescope/ (Vurture et al., 2017), which yielded an estimated haploid genome length of 382,882,063 bp, with 2.25% of

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repeats and 4.72% of heterozygosity. This is much smaller than the assembled genome size (1,899,346,312 bp) due to fragmentation. We also used Benchmarking Universal Single-Copy Orthologs BUSCO v5.2.2 (Manni et al., 2021) to assess gene completeness by searching for core mollusc orthologous genes, using the option -genome and the BUSCO.v4 lineage mollusca_odb10.2019-11-20. Most core genes were missing from our draft genome (74.7%), with only 19% complete core genes recovered (C:19%[S:18.1%, D:0.9%], F: 6.3%, M:74.7%, n=5295, with C: complete single copy BUSCO genes, S: complete and single-copy BUSCOs, D: complete and duplicated BUSCOs, F: fragmented BUSCOs, M: missing BUSCOs, n: total BUSCOs searched).

For the pooled sequencing, we extracted DNA from the tissues of 40 individuals per pool/population using the same standard Phenol Chloroform extraction method mentioned above. We quantified all samples using a Picogreen ds DNA assay (Thermo Fisher Scientific Inc.) on an Infinite 200 Nanoquant (Tecan Group Ltd). Samples were normalized to a dsDNA concentration of 15ng/µL, re-quantified, and pooled according to the sampling population. Thus, we created 12 pools of 40 individuals each at 15ng/µL. Libraries were prepared with the NEB Ultra II kit for shotgun sequencing and sequenced on 5 lanes of HiSeq2500 125 bp pair-ended at Genome Quebec. The number of reads sequenced per population was between 187-248 million paired-end reads. We used the following formula to calculate the expected coverage: read length number of reads / estimated haploid genome length. Given an estimated genome size of 382,882,063 bp, a read length of 125bp, and 93.7-124.2 million single-end reads sequenced, we calculated that our expected coverage was between 30-40X. We assessed the quality of our pool-seq illumina libraries with fastqc 0.11.5, from which we obtained a percentage of repeats between 18.4 and 39.7%.

Read processing and SNPs calling

We prepared the assembled reference genome of *Amnicola limosus* by first indexing it with the Burrows-Wheeler Aligner (BWA; Li & Durbin, 2009) v0.7.17 and with Samtools faidx v1.12, and by creating a dictionary with Picard Tools v2.23.3. We then used a custom pipeline for pool-seq quality processing, read alignment, and SNP discovery. We first trimmed reads with the function trim-fastq.pl from popoolation v1.2.2 (Kofler, Orozco-terWengel, et al., 2011) for a base quality of 20 and a minimum length of 50 bp, and assessed the quality of the trimmed reads with fastqc. We aligned trimmed reads to the reference genome with bwa-mem v0.7.17. We filtered out ambiguously aligned reads with samtools v1.13 using a score of 20 and sorted bam files with samtools. We used samtools flagstat to find the percentage of Illumina reads aligned to the reference genome, which was on average 53.5% SD 4.0%. We obtained an mpileup file with samtools mpileup, then filtered SNPs with a minimum global coverage of 5. We converted the mpileup file to a sync file with Popoolation2 v1.10.03 (Kofler, Pandey, et al., 2011), with a quality score of 20. The sync file was then converted to a "pooldata" object with the poolfstat package in R (Hivert et al., 2018), using a haploid pool size of 80 for all populations, a minimum read count per base of two, a minimum coverage of five and a maximum of 300, a minimal minor allele frequency of 0.0125 (to remove singletons) and discarding indels. This pipeline retained 21,312,700 biallelic SNPs.

Detecting genomic signatures of selection

To detect putative loci under selection, we used both outlier and environmental association analyses approaches. We conducted the outlier analysis using the core model from hierarchical Bayesian models implemented in Baypass, using default parameters (Gautier, 2015). Baypass is advantageous in the context of our study (potential bottlenecks in invaded populations) as it enables the detection of outlier SNPs after taking demographic history into account, thus avoiding the confounding effect of demography. The core model estimates the scaled covariance matrix Ω of population allele frequencies, which summarizes population history and is then explicitly accounted for through Ω. The full dataset was divided into 27 pseudo-independent datasets to overcome computing limitations. The "pooldata" object from poolfstat was converted to the 27 sub-dataset Baypass input files with the "thinning" subsampling method and sub-sample size of 750,000

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SNPs. We used the core model to estimate the XtX statistic and associated p-value under a χ^2 distribution with 12 degrees of freedom (bilateral test, Baypass manual). We considered SNPs as outliers when their p-value derived from the XtX estimator was < 0.001. The shape of the histogram p-values derived from the XtX statistics confirmed that they were well-behaved (A peak close to 0 for loci putatively under selection and a uniform distribution between [0,1] for neutral loci; Fig. S1B; François et al., 2016). The Ω matrices from the 27 sub-datasets were compared visually to assess the concordance of the results, and then the statistics obtained for each SNP were combined.

For the environmental association analysis, we opted for the standard model STD under the Importance Sampling approach in Baypass, in which the association between covariables and SNP allele frequencies is assessed independently. This model computes for each SNP its regression coefficient β_{ik} of the association between the SNP allele frequencies and a covariable to compare the model with association (β_{ik} [?] 0) against the null model ($\beta_{ik} = 0$), from which a Bayes factor BF_{is} is derived. We selected two environmental covariables: invasion status (presence/absence of the gobies) and calcium concentration. We also estimated the C_2-statistic with the STD model (Olazcuaga et al., 2021), which is more appropriate for binary variables and was used for the association with goby presence/absence. We checked the Pearson correlation coefficient between covariables with the function pairs.panel() in the package psych in R, which was $r = 0.71$ (slightly above the recommended threshold for the regression method of $|r| < 0.7$, Fig. S2).For the calcium covariable, we ran three independent runs of the STD model with the -seed option to ensure consistency of the MCMC results, then computed the median BF_{IS} across runs. To check for convergence of the independent runs, we calculated the Forstner and Moonen distance (FMD; Förstner & Moonen, 2003) between Ω matrices from each sub-dataset with the fmd.dist function in R (included in Baypass). Results were found to be consistent, with all FMD values < 0.12. Covariables were all standardized to$\hat{} = 0$ and$\hat{} = 1$. For the calcium association, SNPs were considered significantly associated with a covariable when $BF_{is} > 20$ dB . For the association with goby presence/absence, we used the R package qvalue to correct for multiple hypothesis testing on the p-values derived from the C_2-statistic and applied a False Discovery Rate of $\alpha = 0.01$ as a q-values cut-off for outlier detection.

As a complementary analysis to investigate the potential for adaptation to the invasive predator and low calcium concentrations, we identified outlier SNPs showing consistent allele frequency differences between environment types using poolFreqDiff (Wiberg et al., 2017). Note that with this approach, our aim was not to identify independent instances of parallel adaptation but rather detect genotype-environment associations; consistent allele frequency differences could arise due to adaptation occurring in a shared recent ancestor. This method relies on modeling allele frequencies with a generalized linear model (GLM) and a quasibinomial error distribution, which should result in a uniform distribution of p-values between [0,1] under the neutral (null) scenario (Wiberg et al., 2017). It also accounts for bias in allele frequency estimation (e.g., Gautier et al., 2013) by rescaling allele counts to an effective sample size n_{eff} (Feder et al., 2012). We ran the analysis separately for the two covariables as binary comparisons: invasion status (presence/absence) and calcium concentration (low < 24.3 mg/L, high > 34.3 mg/L). For the minimum read count per base, and the minimum and maximum coverage, we used the same values as for the poolfstat filtering, and we also rescaled the allele counts with n_{eff} and added one to zero count cells. To account for demography and genetic structure, we applied the empirical-null hypothesis approach (François et al., 2016) to recalibrate p-values based on a genomic inflation factor of $\lambda = 0.85$. We confirmed that recalibrated p-values were well-behaved based on the observed peak close to 0 and the uniform distribution between [0,1] (Fig. S3; François et al., 2016). We then transformed the recalibrated p-values into q-values with the R package qvalue, and defined outliers if their q-value was below the FDR $\alpha = 0.01$.

Reciprocal transplant experiment

We conducted a laboratory reciprocal transplant experiment at UQAM with field-collected F_0-generation *A. limosus* to investigate the response of gastropods with different source population habitat types (low calcium/uninvaded Ottawa River or high calcium/invaded St. Lawrence River) to home and transplant

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water (calcium-rich water from the St. Lawrence River or calcium-poor water from the Ottawa River), in the presence or absence of goby cues. The goby cue treatment was used to test for predator effects on life history fitness components (survival and fecundity). Amnicola snails that were involved in the experiment were mostly at adult or sub-adult stages as we selected the largest individuals collected in the field and the dates of collection correspond to the presence of adult cohorts in the field (Pinel-Alloul & Magnin, 1973). Two additional water treatments were also tested: the artificial freshwater medium COMBO , with and without the addition of calcium, to test for the specific effect of calcium (Ca) concentration on fitness components. The overall design was therefore a two (origin water: St. Lawrence River SL versus Ottawa River OR)× four (treatment water from St. Lawrence versus Ottawa River, growth media with/without Ca) × two (presence versus absence of round goby cue) factorial experiment, with 12 replicates (corresponding to our sampling populations) per treatment combination.

We raised wild F_0 individuals from the 12 populations in the laboratory for up to 73 days. Between 15 and 22 individuals (average: 19.6±1.3) were initially placed in 250 ml plastic cups with river water and reared in growth chambers (Thermo Scientific Precision Model 818) at 18°C with a light:dark cycle of 12:12 hours. We fed *Amnicola* snails ad libitum with defrosted spinach every 2-3 days if needed or at each water change. Water in the water treatments was changed, and old spinach was removed every 3-4 days. For the goby cues treatment, gobies were kept in a 50-liter aquarium for two weeks prior to the experiment, set in a growth chamber at 18°C with a 12:12h light. Gobies were fed 3-4 times a week with flake fish food (TetraFin). The goby cue treatment was added as 5 mL of water from the goby aquarium per Amnicola culture at each water change (every 2-3 days), which represents 2% of the volume of the culture. The addition of water was done manually with a 30 mL syringe. We recorded survival and fecundity (total number of eggs produced per individual) as response variables every 19 ± 13 days throughout the experiment, using high-resolution stereomicroscopes (Olympus). However, due to the very low survival for all populations for the treatment testing the effects of calcium in growth media, we removed this comparison from further analyses (see Fig. S4).

We analyzed fecundity (total number of eggs produced) and survival rates with a generalized linear model (GLM) and a generalized linear mixed effect model (GLMM) using the lme4 package in R respectively. We modeled fecundity with a negative binomial distribution while survival was modeled with a binomial distribution and a logit link function. We checked the models for overdispersion using the overdisp_fun function from https://bbolker.github.io. We tested both models with and without the random effect of populations, using an AIC approach corrected for small sample size (AICc) and the ΔAIC criterion to evaluate the random effects (kept when $\Delta AIC > 2$) with the R package bbmle. Likelihood ratio tests were used to evaluate the fixed effects for both the GLM and GLMM models. For the GLM model of fecundity, we checked for the influence of outliers on the model, by using both visual and quantitative diagnostics of the leverage and Cook's distance. We did not find a consistent effect of outliers on this model and thus did not remove outliers. Fixed effect coefficients and their confidence intervals were converted to incident rate ratios (fecundity) and odd ratios (survival) using an exponential function.

Population structure, genetic diversity, and demography

We first estimated population structure with the core model from Baypass, with the scaled covariance matrix Ω of population allele frequencies summarizing some aspects of population history. We also obtained a genome-wide pairwise F_{ST} matrix from the poolfstat package, using the same parameters as described above. We used the pairwise F_{ST} matrix to assess the potential for isolation by distance, using the relationship between the genetic distance (F_{ST}/(1- F_{ST}); Rousset, 1997) and the log of the geographical distance (2D distribution of populations) with a Mantel test (9999 permutations) using the vegan package in R. Distances between sites were obtained by measuring paths between populations along the rivers (in m) with Google Earth v.10.38.0.0. We also tested for isolation by environment, by first calculating the environmental distance between population pairs using the squared Mahalanobis distance, calculated from the calcium concentration and goby presence/absence with the R package ecodist. We verified that there was no correlation between

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the environmental distance and geographic distance (non-significant Mantel test with 9999 permutations: $r^2 = 0.03$, p-value = 0.20). Then we tested for a relationship between environmental distance and genetic distance as $F_{ST}/(1\text{-}F_{ST})$ with a Mantel test (9999 permutations).

We obtained the observed heterozygosity from the poolfstat package and compared heterozygosity levels between habitat types with a t-test after checking for the assumptions of normality (qqplot) and homoscedasticity (Bartlett test). We calculated genome-wide diversity indices (Tajima's pi, Watterson theta, and Tajima's D) using popoolation (Kofler, Orozco-terWengel, et al., 2011). First, we generated mpileup files for each population separately with samtools (Li et al., 2009) from the sorted.bam files output by the custom pipeline. Then we computed the genome-wide diversity indices using non-overlapping windows of 100kb, a minimum coverage of 20 (as recommended in Kofler, Orozco-terWengel, et al., 2011 except for Tajima's D with minimum coverage = 13, as the corrected estimator requires the pool size < 3 minimum coverage), a minimum quality of 20, a minimum fraction covered of 0.05 and a pool size of 40. It should be noted that popoolation calculates the diversity indices along chromosomes; thus, due to the fragmentation of our draft genome, the diversity indices were calculated mostly among separate contigs and in windows $<$ 100kb. The minimum number of SNPs per window across populations using this filter was 25. We used Hedge's G to detect a potential difference in the three diversity indices between the St. Lawrence and Ottawa rivers.

We also investigated the demographic history of three population pairs using the diffusion approximation method implemented in δaδi (Gutenkunst et al., 2009). We aimed to detect a potential bottleneck in the invaded populations and to quantify the magnitude and direction of gene flow between the two habitat types (invaded and refuge). We selected the populations PB-LCGA, IPE-LCGA, and PDC-HCGA as refuges, paired with PG-HCGP, BEA-HCGP, and GOY-HCGP as invaded populations respectively. PDC-HCGA was of particular interest as a high calcium population located in an uninvaded wetland (refuge). Note that the limited number of population pairs investigated is due to the large computation time required to analyze the various models considered. Our most complex model (Fig. S8) has defined effective population sizes after the split (nu1 and nu2), followed by a bottleneck in both populations (modeling a scenario in which the goby invasion impacted population abundance at the whole ecosystem scale) followed by exponential recovery in both populations. T_S is the scaled time between the split and the bottleneck and T_B is the scaled time between the bottleneck and present. Migration rates are asymmetric but constant through time after the split, with m_{IR} the migration from refuge to invaded populations and m_{RI} in the opposite direction. As we knew the time of the potential bottleneck (12 years before sampling with one generation per year), we set T_B as a fixed parameter. As we set T_B as a fixed parameter, the parameter $\theta = 4\mu L$ was an explicit parameter in the models that included a bottleneck. We defined $\theta$with μ the mutation rate of 7.6×10^{-9}substitutions per site per year from the Caenograstropoda species*Nucella lamellose* , and L the effective sequenced length, calculated as$L \approx$ *total length of sequence analyzed* $\times$ *SNPs retained for use in dadi/total SNPs in analyzed sequence.*

We investigated six additional non-nested models: a) bottleneck and growth only in the invaded population (constant N_e for the refuge population) with uneven migration, b) only bottlenecks in both populations without recovery and uneven migration, c) only bottleneck in the invaded population with uneven migration (constant N_e for the refuge population), d) a simple population split at T_S with uneven migration, e) a population split with symmetric migration and f) a population split without migration. The default local optimizer was used on the log of parameters with random perturbation of the parameters to obtain a set of parameter values resulting in the highest composite likelihood. Optimization was conducted repeatedly until convergence was reached (i.e., three optimization runs with log-likelihood within 1% of the best likelihood). Only one model in one population pair did not reach convergence after 30 optimization runs (bottleneck without recovery for the PDC-HCGA and GOY-HCGP population pair). Finally, we compared our seven models based on the differences in the likelihoods and plots of residuals of the models. As we obtained unlikely results during the conversion of parameters in our best models, possibly due to imprecise mutation rates, we did not conduct parameter conversion. To obtain uncertainties on our parameters while accounting for the effect of linkage, we used bootstrapping and the Godambe Information Matrix approach (Coffman et al., 2016). For this, we generated 100 bootstrapped datasets with a chunk size of

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To address the potential effect of using a pool-seq approach on the variance in allele frequency estimates stemming from differences in coverage between pools (Gautier et al., 2013), we used a filter to obtain relatively homogenous coverage between our two selected populations/pools. From the initial SNPs dataset output by poolfstat (21,312,700 SNPs), we retained SNPs that fell within the 1st and 3rd quartiles of coverage in both populations (11-19X for PB-LCGA and 10-18X for PG-HCGP; 11-18X for IPE-LCGA and 9-15X for BEA-HCGP; 10-16X for PDC-HCGA and 10-17X for GOY-HCGP). We also filtered out SNPs that were detected as outliers (putatively under selection) in the Baypass (core and aux or STD models) and poolFreqDiff analyses and removed uninformative SNPs (fixed or lost in both populations). To accommodate for large computation time during the optimization, the datasets were thinned at random to retain a final dataset of [?] 1 million SNPs per population pair. We used a custom script and the dadi_input_pools function from the genomalicious R package (Thia & Riginos, 2019) with the "probs" parameter in the methodSFS option to transform allele frequency data into the SNP data format from δaδi. We used δaδi to infer the folded SFS as we did not have information on the ancestral allele state. Due to low confidence in the low-frequency estimates, we also masked entries from 0 to 5 reads

Results

Genomic signatures of local adaptation to round goby invasion and water calcium.

Using the core model in Baypass as our outlier analysis, we found 226,794 outliers SNPs with a p-value < 0.001 and either high or low XtX values, which represented [?]1.1% of the dataset (Fig. S1A). Outlier SNPs with high XtX values can be interpreted as putatively under positive selection, while low XtX values indicate balancing selection (Gautier, 2015). We also investigated the association of SNP allele frequencies with the selected environmental variables (invasion status and calcium concentration) using the STD model in Baypass. We found 778 outlier SNPs significantly associated with calcium concentration across the three replicate runs (BFis > 20, [?] 0.004% of the dataset) and 88,277 SNPs associated with the goby presence/absence (q-value < 0.01, [?] 0.4% of the dataset). Using the poolFreqDiff analysis with a FDR of 1%, we identified 54,285 outliers displaying consistent differences in allele frequency in the same direction between the low and high calcium habitats (0.3% of the dataset) and 23,651 outlier SNPs between the populations from the invaded and uninvaded habitats (0.1% of the dataset; Fig. S5). Of those calcium concentration outliers, 18 were in common between the Baypass STD model and the poolFreqDiff analysis, whereas 3,324 of the predator status outliers were in common between the Baypass STD model (using the C_2-statistic) and the poolFreqDiff analysis (Fig. 2, Fig. S5). Most of the outliers were uniquely associated with a single covariable, with 2,668 SNPs associated with both calcium and invasion status across methods (Fig. S5). Overall, we found 1,050 SNPs in common between the Baypass core and STD models, as well as 7,009 SNPs in common between the Baypass STD model and the poolFreqDiff analyses including both the invasion status and calcium concentration (Fig. S6).

(Mal)adaptive responses to round goby invasion and water calcium levels

We found life history differences between the gastropod populations from the two environments, using fecundity and survival as fitness components (Fig. 3). For fecundity, the model with the random effect of population origin was not better than the model without (ΔAICc = 1.2), and only the origin water (Ottawa River: OR, LCGA vs St. Lawrence River: SL, HCGP) effect was significant (p = 0.015). Even though the interaction between origin water and treatment water was not significant (p = 0.076), fecundity was higher for SL and OR populations in home water (13.30 SD 19.70 and 2.42 SD 6.27 respectively) than in transplant water (3.00 SD 4.33 and 0.92 SD 1.83 respectively). SL populations produced [?] 5 times more eggs than OR populations (4.90, 95% CI [1.54-15.58]). For survival, the model with a random effect of the population was better than without (ΔAIC = 250.7). The fixed effects of origin and treatment water were significant

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(p = 0.020 and p = 1.496×10^{-9}, respectively), but their interaction and the goby cue effect were not (p = 0.203 and p = 0.794, respectively). SL populations were 10 times more likely to survive compared to OR populations (9.55, 95% CI [1.70-53.73]). However, exposure to treatment water from the St. Lawrence River significantly lowered the odds of survival, with survival rates less than one-third that of populations exposed to Ottawa River water (0.31, 95% CI [0.21-0.46]). There was considerable variation in survival rates between populations, as demonstrated by the significant effect of population on survival. Variation in survival among populations (random effect of the population of origin) did not depend on geographical location or habitat of origin (Fig. S7): OKA-LCGA, PG-HCGP, and PON-HCGP had significantly higher survival rates, while BEA-HCGP, PDC-HCGA, and PST-HCGP had significantly lower survival rates.

Demographic and genetic effects of the invasion

Genome-wide nucleotide diversity π was relatively high overall with 0.011 (SD 0.006) on average. Diversity was similar between the populations from the invaded St. Lawrence River habitats (0.011, SD 0.007) and the populations of the uninvaded Ottawa River (0.011, SD 0.006), with a negligible effect size of habitat type (Fig. 4A; Hedges'g = 0.09, 95% CI [0.16, 0.02]). Estimates of$\theta_{\text{Watterson}}$ were identical between the two habitat types (Fig. 4B; SL populations: 0.015 SD 0.008; OR populations: 0.015 SD 0.008), and the effect size of habitat was therefore negligible (Hedges'g = 0.06, 95% CI [0.13, 0.01]). This resulted in a slightly negative overall Tajima's D (-0.38 SD 0.43), which was lower for SL populations (-0.41 SD 0.44) than for OR populations (-0.36 SD 0.43), but the effect size of the difference was negligible (Hedges' g =0.11, 95% CI [0.10, 0.13], Fig. 4C). Observed heterozygosity was not significantly different (p-value = 0.289, t = -1.153, df = 6.5; Fig. 4D) between the populations from uninvaded (0.168 SD 0.003) and invaded sites (0.171 SD 0.003).

Consistent with the known population declines in invaded habitats, we found a significant association between invasion status and the effective population size of our study populations based on our demographic modeling (Table 1, Fig. S9). For the pair PB-LCGA (refuge) and PG-HCGP (invaded), the best model included bottlenecks in both populations, but the inferred parameters indicated that only the invaded population showed N_e recovery (Table S2), suggesting an alternative model that we did not test (bottlenecks in both population but N_e recovery only in the invaded population). In the case of IPE-LCGA and BEA-HCGP, we did not detect a significant bottleneck, but the invaded population had an effective population size 40 times lower compared to the refuge (Table S2). Finally, the best model for PDC-HCGA and GOY-HCGP included a bottleneck without recovery only for the invaded population (Table 1).

Isolation by environment and variable gene flow between habitat types

We found that populations clustered by environment type (Fig. 5), particularly by the presence/absence of the round goby, with RAF-LCGP and PDC-HCGA both clustering with the invaded populations and showing lower pairwise F_{ST} values within those clusters. Our results from the scaled covariance Ω matrix of population allele frequencies inferred with Baypass indicate that there is also positive covariance in allele frequencies within clusters and negative covariances between clusters. These population structure results were concordant between the Ω matrix (Fig. 5A) and the pairwise F_{ST} matrix (Fig. 5B). Population structure was also explained by isolation by distance and by the environment (Fig. S10), as the positive correlation between the genetic distance $F_{ST}/(1\text{-}F_{ST})$ and the log of the geographic distance or the Mahalanobis were significant (Mantel test, 9999 permutations: p = 0.007, r^2 = 0.209 and p = 0.044, r^2 = 0.078 respectively). We found low but significant asymmetric gene flow in most cases with the scaled migration rates $0.05 < 2N_em < 5.5$ (Table S2). However, gene flow was non-significant ($2N_em < 0.05$) from the low calcium refuge toward high calcium invaded populations (IPE-LCGA toward BEA-HCGP and PB-LCGA-HCGP toward PG). For these two population pairs, the lower migration rates were consistent with higher values of pairwise F_{ST} and time since the population split (Fig. 5 and Table S2). Finally, the population pair GOY-HCGP and PDC-HCGA (wetland refuge) had much higher migration rates in both directions compared to the other

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population pairs, consistent with lower pairwise F_{ST} and these two populations belonging to the same cluster in Baypass (Fig. 5 and Table S2).

Discussion

Knowledge of evolutionary and demographic processes is crucial for our understanding of how native species will respond to biological invasion and the mechanisms that facilitate or inhibit their co-existence with invasive species. We investigated the interaction between adaptation and demography to gain insight into the persistence of a native gastropod (*Amnicola limosus*) following approximately 12 years of exposure to an invasive predator, the round goby, in the Upper St. Lawrence River. Our genomic results indicate that *A. limosus* has locally adapted to the invasion in the span of ≤12 generations. We also find evidence for adaptation to differences in water calcium over the longer geological history of the ecosystem. Despite evidence of local adaptation in invaded populations, they are experiencing demographic decline, whereas refuge populations show relative stability. While these results could imply that the low-calcium refuge populations have the potential to provide migrants and generate demographic and genetic rescue of invaded populations, this hypothesis was not supported. We detected restricted gene flow and strong population structure between the physiological refuge and invaded populations. Moreover, we found evidence that individuals in uninvaded refuges appear to be maladapted for life history traits, showing low fitness overall. Therefore, despite the current persistence of native *A. limosus* gastropods in the Upper St. Lawrence River system following the invasion by round gobies, this native gastropod could become vulnerable due to reductions in effective population sizes and limited potential for genetic rescue of impacted populations by the populations in physiological refugia.

Genomic signatures of local adaptation to round goby invasion and low water calcium.

Our genomic data (population structure, Fig. 5 and environmental association analyses, Fig. 2) provide general evidence for local adaptation to the two distinct environment types in *A. limosus* , i.e., low calcium/goby absent and high calcium/goby present. The exceptions were for two populations (RAF-LCGP and PDC-HCGA) that experienced inverse conditions for selection than the other sampled populations, both clustering with the invaded populations. For RAF-LCGP, the results support strong selection from goby predation even under lower calcium conditions, which are less optimal environmental conditions for round goby feeding and performance (Iacarella & Ricciardi, 2015). For PDC-HCGA, the results suggest strong migration from adjacent invaded sites, which was confirmed by our demographic analyses (see below). PDC-HCGA itself remained uninvaded despite higher water calcium concentrations, likely because the site was located within a wetland, which provides less optimal conditions for round-goby establishment due to the substrate properties (Astorg et al., 2021).

Our EA analyses with Baypass and poolFreqDiff potentially allowed us to disentangle the signals of the two putative selective pressures (i.e., the effect of selection from goby predation at invaded sites and low calcium levels at uninvaded sites), even though invasion status and calcium concentration were strongly correlated. We found SNPs uniquely associated with invasion status and calcium concentration, which can be interpreted as signatures of local adaptation to predation by the round goby fish, and to the more limiting calcium concentrations at uninvaded sites. However, a more comprehensive understanding of the relative roles of these distinct selection mechanisms on genomic variation will require functional validation and ideally a different sampling design that includes additional sites with less correlation between these two environmental factors. Due to the unavailability of an annotated reference genome for *A. limosus* or a closely related species, we were unable to investigate putative physiological functions of the SNPs showing significant differentiation between environment types. Adaptation to calcium likely involves different functions from

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adaptation to predation. Differences in calcium concentrations between the water masses from the two rivers are related to the geological characteristics of the river watersheds and therefore represent environmental differences over the long evolutionary history of this species in the St. Lawrence River. On the other hand, predation from the invasive round goby on mollusks is a recent and novel stressor in the St. Lawrence River. Putative physiological functions that would be worth investigating in future studies include transmembrane calcium transport and biomineralization pathways that might be involved in adaptation to low calcium concentration (Clark et al., 2020), as well as shell development regulatory genes that could play a role in the evolution of smaller-sized shells at maturity, which has been observed in populations subject to goby predation (Kipp et al., 2012; Johnson et al., 2019).

(Mal)adaptive responses to round goby invasion and water calcium levels

Our results from the reciprocal transplant experiment give insight into potential adaptive and maladaptive responses in life history traits between SL (HCGP) and OR (LCGA) populations to divergent calcium concentrations and round goby predation regimes (Fig. 3). Indeed, we found important differences in life-history traits (fecundity and survival as fitness components) between the populations from the two habitat types. Both fecundity and survival were higher in the HCGP populations than in the uninvaded LCGA populations, regardless of water treatment. The potential for local adaptation to the calcium gradient is suggested by a slight home advantage in fecundity for populations from both habitats in their origin water versus transplant water (water treatment LCGA vs HCGP), although the interaction between origin and treatment water was not significant. While our laboratory reciprocal transplant experiment could indicate that *A. limosus* responded to round goby invasion through shifts in life-history traits, populations from the uninvaded habitats are also possibly maladapted, as shown by low fitness across treatment water and goby cue treatments. This suggests the LCGA populations might be generally maladapted (Brady et al., 2019), which could occur through a trade-off of adaptation to low calcium water, as intracellular transport of calcium is energetically costly (Clark et al., 2020). In addition, individuals from the Ottawa River might allocate more resources toward calcium transport and be less able to invest in life-history traits such as reproduction.

Survival rates between populations varied widely, especially for the HCGP populations. This could reflect potential local (mal)adaptation to other biotic or abiotic parameters that we did not consider in the present study (e.g., temperature, substrate, nutrient availability, and food quality). As it was conducted within a single generation, we acknowledge that our reciprocal transplant experiment did not allow us to differentiate plastic vs genetic vs maternal effects on the measured traits. However, our genomic results support the idea that the life-history differences observed between the two population types could be at least partially explained by adaptive genetic differences between the environments.

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Demographic and genetic effects of the invasion.

Given prior findings showing a decline in gastropod population abundance following invasion by round gobies, we hypothesized that invaded populations might suffer from population bottlenecks and reduced genetic diversity. However, we did not find a negative effect of the invasion on genetic diversity (Fig. 4), with high

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levels of nucleotide diversity found in all populations. The nucleotide diversity reported here is relatively high compared to other species observed across phyla (Leffler et al., 2012), although lower than what has been observed in other gastropod species (Redak et al., 2021; Oswald et al., 2022). The slightly negative Tajima's D found in all populations indicates that there was an excess of rare alleles compared to the neutral model, which could reflect a recent population expansion or positive selection. However, our demographic analysis revealed the occurrence of genetic bottlenecks or reductions in N_e in invaded populations, and even in one refuge population (Table 1, Table S2). This reduction in N_e was only followed by recovery in one of the cases, hinting at a possible case of genetic rescue (i.e., due to an increase in genetic diversity), although the results must be interpreted with caution as there was considerable uncertainty around the parameter estimates. The source of migrants that could potentially be generating a rescue is also presently unknown and is unlikely to be due to the migration from the low-calcium physiological refugia populations as the gene flow in this direction was non-significant. Further validation of the potential for genetic rescue would require obtaining census data and collecting new genomic samples targeted at identifying the potential source populations and quantifying the level of hybridization in recipient-invaded populations (Fitzpatrick et al., 2020).

Surprisingly, the effective population size reductions did not trigger major declines in genetic diversity levels (Fig. 4). This could be due to low but significant gene flow ($0.1 < 4N_em < 11$; Hämälä et al., 2018) within habitats, as suggested by the gene flow estimates detected between PDC-HCGA and GOY-HCGP populations and the lower F_{ST} within clusters (Table S2, Fig. 5). Similar rates as observed here have been shown to be sufficient to maintain genetic diversity despite low effective population size (Gompert et al., 2021). Invaded populations thus do not appear to be currently in need of genetic rescue from refuge populations to recover genetic diversity, or perhaps genetic rescue has already occurred or is ongoing and is the reason for the high genetic diversity observed at invaded sites.

Interaction between local adaptation and demographic/genetic rescue

A core goal of this study was to determine if local adaptation could interact with demographic and genetic rescue. We found relatively low or non-significant levels of gene flow between the populations from the two habitat types (from LCGA refugia to HCGP populations and inversely), but high gene flow within habitat types (Table S2). Pairwise-F_{ST} values were within the range of what has been observed in other egg-laying marine gastropod species . Thus, our analyses of gene flow indicate that refuge populations in low calcium habitats do not provide migrants to invaded populations, and this is further supported by the significant pattern of isolation by environment (Wang & Bradburd, 2014). This pattern could be generated by selection against migrants (Nosil et al., 2008; Orsini et al., 2013; Tigano & Friesen, 2016) related to calcium limitation and round goby predation. Individuals from the LCGA populations had lower fitness overall compared to HCGP populations and thus might have low reproductive output and survival in invaded habitats. Given that goby predation has been shown to cause selection for smaller shell sizes at maturity (Kipp et al., 2012), LCGA individuals might be also more vulnerable to predation than HCGP individuals if they are more conspicuous due to larger shell sizes. Similarly, hybrids might be selected against if intermediate phenotypes have lower fitness in their local environment (Thompson et al., 2022). In addition, because individuals from uninvaded populations have lower reproductive output and survival, they provide a more limited demographic subsidy to invaded populations. This will depend on the magnitude of the relative fitness difference between source and recipient populations (i.e., how detrimental migrant alleles will be in the recipient populations; Bolnick & Nosil, 2007). The recent adaptation to goby predation could have thus reinforced the existing effect of isolation by environment from the adaptation to low calcium, thereby limiting the potential of low calcium physiological refugia to provide migrants necessary for both the demographic and genetic rescue of invaded populations.

In contrast to the low gene flow found between LCGA and HCGP populations, gene flow was relatively high between the wetland refuge PDC-HCGA and the invaded population GOY-HCGP, although it did not result in N_e recovery from the bottleneck in the latter (Table S2). Wetland habitats provide refuge from goby predation by reducing their abundance at a local scale and are known to enhance fish and macroinvertebrate

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diversity (Astorg et al., 2021; Morissette et al., 2023). Their role as a refuge has also been previously recognized in other invaded systems (Reid et al., 2013). Given the prevalence of wetlands in the Upper St. Laurence River (Morissette et al., 2023), wetland refugia with high calcium concentration thus have the potential to provide migrants to invaded sites, particularly due to the lower adaptive divergence between these populations. This suggests that migration of individuals from larger wetland refuge populations might be providing not only a demographic subsidy (demographic rescue; Hufbauer et al., 2015) but could also be replenishing the genetic diversity if indeed it was lost due to population declines in invaded populations (genetic rescue; Whiteley et al., 2015). The role of wetlands as a refuge and their potential implication in the demographic and genetic rescue of invaded gastropod populations therefore warrants further investigation.

Based on our demographic modeling and population structure results (Table S2, Fig. 5), populations from high calcium habitats are more likely to be exchanging migrants, which appears to be sufficient for preserving genetic diversity. However, this beneficial effect of gene flow might have limitations as shown by the absence of effective population size recovery in two out of three populations for which we detected a bottleneck (Table 1). This is particularly important because strong selection such as that detected in the invaded populations can also lead to reduced population sizes and drift, with negative effects on population fitness (Falk et al., 2012). The net outcome of this conflict between adaptation to the invasive predator and low calcium concentrations, and genetic rescue, will thus depend on the severity of population decline in recipient populations (Hufbauer et al., 2015), the extent of adaptive differentiation between populations in each habitat type, and the rate of immigration from high calcium wetland populations.

Potential limitations of genetic rescue during population management.

This study documents the impact of an aquatic invasive predator on evolutionary and demographic processes in a native prey species. Evaluating the potential evolutionary impacts of invasive species on native species is important because they can lead to surprising, unforeseen negative effects, such as the disruption of existing local adaptation . Although genetic rescue has been proposed as a valuable tool for the conservation of small, isolated populations (Whiteley et al., 2015; Ralls et al., 2018), it has also been recognized that genetic rescue carries the risk of outbreeding depression if there is an adaptive genetic divergence between source and recipient populations (Frankham et al., 2011). The present study highlights a case from natural, unmanaged populations where the potential for genetic rescue from physiological refugia is potentially limited by adaptive divergence, and only appears to be possible in the presence of migration from refuge populations of similar habitat types. This implies that the presence of physiological refugia will not necessarily translate into the demographic or genetic rescue of imperiled populations if strong genetic differentiation exists between refuge and recipient populations, for example stemming from isolation by environment. It thus reiterates the importance of considering the local (mal) adaptation of donor and recipient populations during managed introductions that aim to produce genetic rescue (Hoffmann et al., 2021).

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Data Accessibility

The raw sequencing reads are deposited in the National Center for Biotechnology Information Sequence Read Archive SRA repository (BioProject PRJNA1035459) and the metadata are also stored in the SRA (BioProject PRJNA1035459), using the Eukaryotic water MIxS package. The draft genome of *Amnicola limosus* will be deposited in NCBI GenBank and is provided as a downloadable file during the review process. Scripts and input data files used for the analyses are available for download at the following link: Files_upload_Amnicola_limosus_Molecular_Ecology and will be uploaded to a public repository (Dryad Digital Repository).

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Tables legends:

Table 1: Results of the demographic models investigated with dadi for the three population pairs. Three population pairs were selected PG-HCGP (invaded) with PB-LCGA (refuge), BEA-HCGP (invaded) with IPE-LCGA (refuge), and GOY-HCGP (invaded) with PDC-HCGA (refuge). Models are presented according to their log likelihood (log L), with the number of parameters in each model (k). For each population pair, the best model based on composite log-likelihood is underlined.

Figures legends:

Figure 1: Locations of study sites of collection of*Amnicola limosus* populations in the St. Lawrence River system and experimental design of reciprocal transplant experiment. A: The study sites are located near the island of Montreal, QC, Canada (right of the map). Sites are colored based on water calcium concentration (mg/L). Sites with gobies absent are HA-LCGA, OKA-LCGA, IB-LCGA, PB-LCGA, IPE-LCGA, and sites with gobies present are PG-HCGP, PST-HCGP, PON-HCGP, BEA-HCGP, GOY -HCGP. The two exceptions are RAF-LCGP (low calcium and gobies present) and PDC-HCGA (wetland, high calcium, and gobies absent). B: Experimental design showing the two origin waters (Ottawa River in grey or St. Lawrence River in blue) with six populations from each river (replicates), water treatment (OR-LC: Low calcium - Ottawa River; SL-HC: St. Lawrence River - high calcium) and goby cue treatment (+/−: with or without).

Figure 2: Results of the environmental association analyses. (A) Biplot of the q-values obtained from the poolFreqDiff analysis testing for consistent differences in allele frequencies between populations from invaded and uninvaded environments, as a function of the q-values derived from the C_2-statistic in the STD model in Baypass, assessing the association of SNP allele frequencies with goby presence/absence. The dashed vertical and horizontal grey lines indicate the FDR of α 0.01. (B) Biplot of the q-values for the poolFreqDiff analysis comparing populations of low and high calcium habitats as a function of the Bayes Factor (BFis) from the Baypass STD model (median of three independent runs) testing the association with calcium concentration. The dashed vertical line (BFis $>$ 20 dB) indicates outlier SNPs significantly associated with the calcium covariable and the horizontal line gives the q-value FDR of α 0.01.

Figure 3: Fecundity (total number of eggs produced) and survival as a function of water treatment, origin water, and goby cue treatment in the reciprocal transplant experiment. Each dot represents a measurement for one population (blue: St. Lawrence populations HCGP, grey: Ottawa river populations LCGA), summarized by the corresponding boxplots (showing the median, minimum and maximum, values, 1st and 3rd quartiles, and outliers as full dots). The black dots are the mean for each treatment, with squares and triangles for treatments with or without goby cues. Fecundity was analyzed with

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a GLM (negative binomial distribution), with only the origin water being significant (p = 0.015). Survival was analyzed with a GLMM (binomial distribution), with the origin and treatment water being significant (p = 0.020 and p = , respectively).

Figure 4: Genome-wide diversity indices. (A) violin plots of nucleotide diversity pi, (B) Watterson's Theta, (C) and Tajima's D according to habitats (Ottawa River LCGA; St. Lawrence River HCGP), with median and interquartile ranges shown in the box plot insert. (D) Observed heterozygosity per population, comparing habitat types (black dots: average per habitat, error bars show one standard error interval), except for PDC (HCGA, wetland refuge) and RAF (LCGP) which have inverted calcium concentration and goby presence characteristics.

Figure 5: Population genetic structure. (A) Heatmap of the scaled covariance matrix Ω (with ρ_{ij} the correlation coefficient between pairs of populations) with hierarchical clustering tree (using the average agglomeration method), obtained from the core model of Baypass. (B) Pairwise F_{ST} matrix between the twelve study populations. The gray bars correspond to the Ottawa River populations (LCGA) and the blue bars to the St. Lawrence populations (HCGP), except for PDC (HCGA, wetland refuge) and RAF (LCGP).

Table 1:

		Composite Log-likelihood per population pair	**Composite L**
Model	**k**	**PG-HCGP/ PB-LCGA**	**BEA-HCGP**
Bottleneck + growth two populations	10	*-10,380*	-12,816
Bottleneck + growth invaded population	8	-10,736	-12,485
Bottleneck two populations	8	-11,017	-12,884
Bottleneck invaded pop.	7	-10,771	-12,334
Split + uneven migration	5	-10,615	*-11,669*
Split + even migration	4	-10,777	-12,257
Split no migration	3	-24,606	-29,842

Figure 1:

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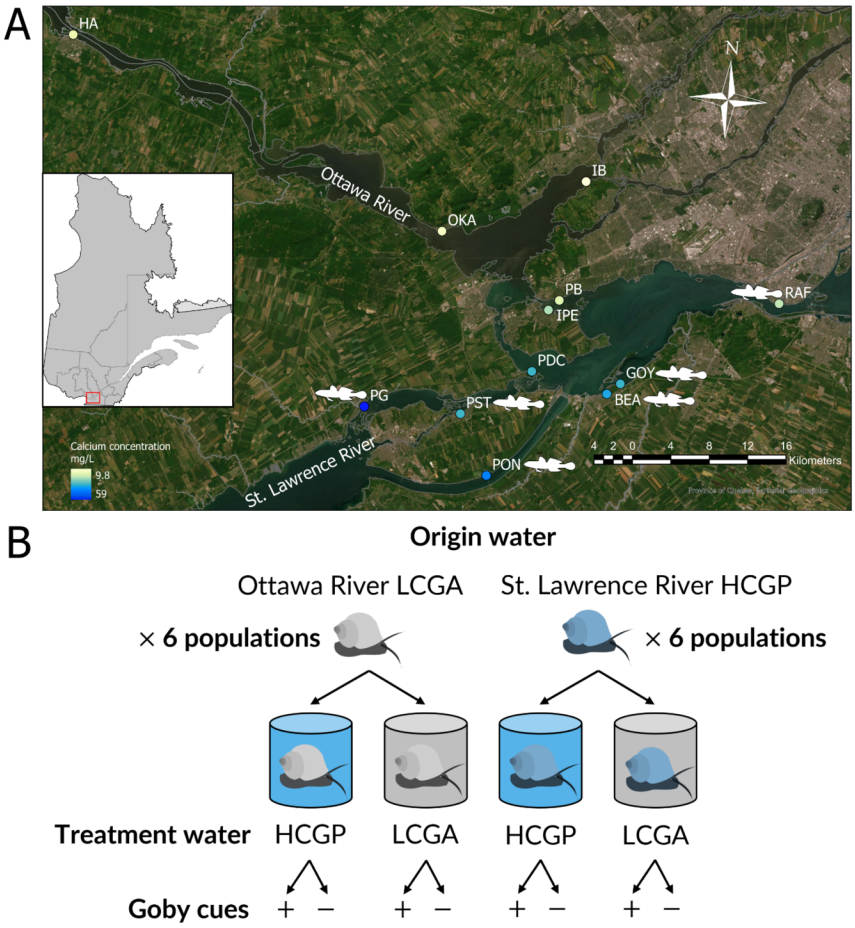

Figure 2

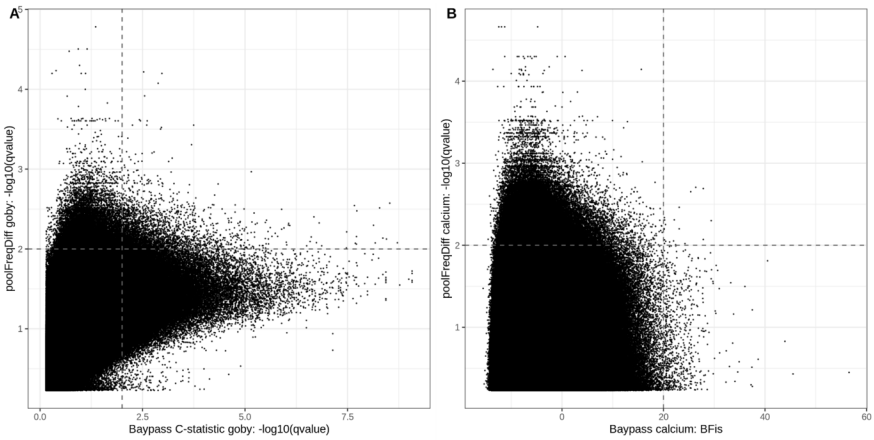

Figure 3

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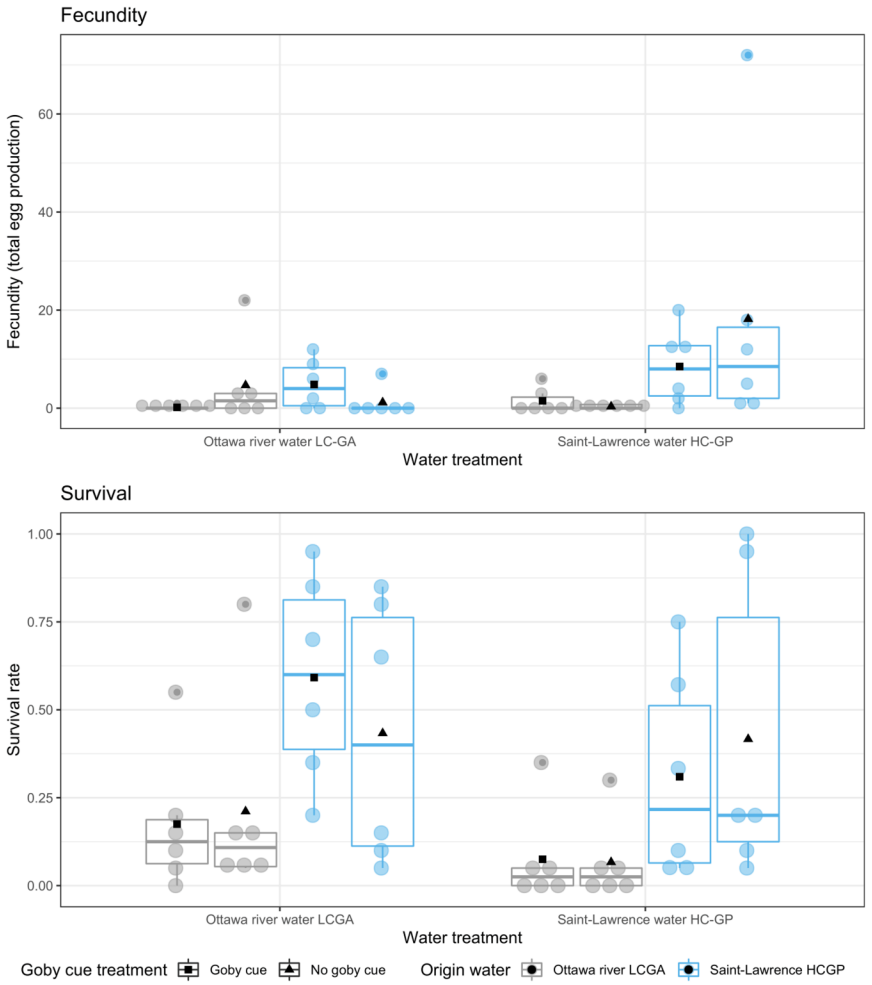

Figure 4

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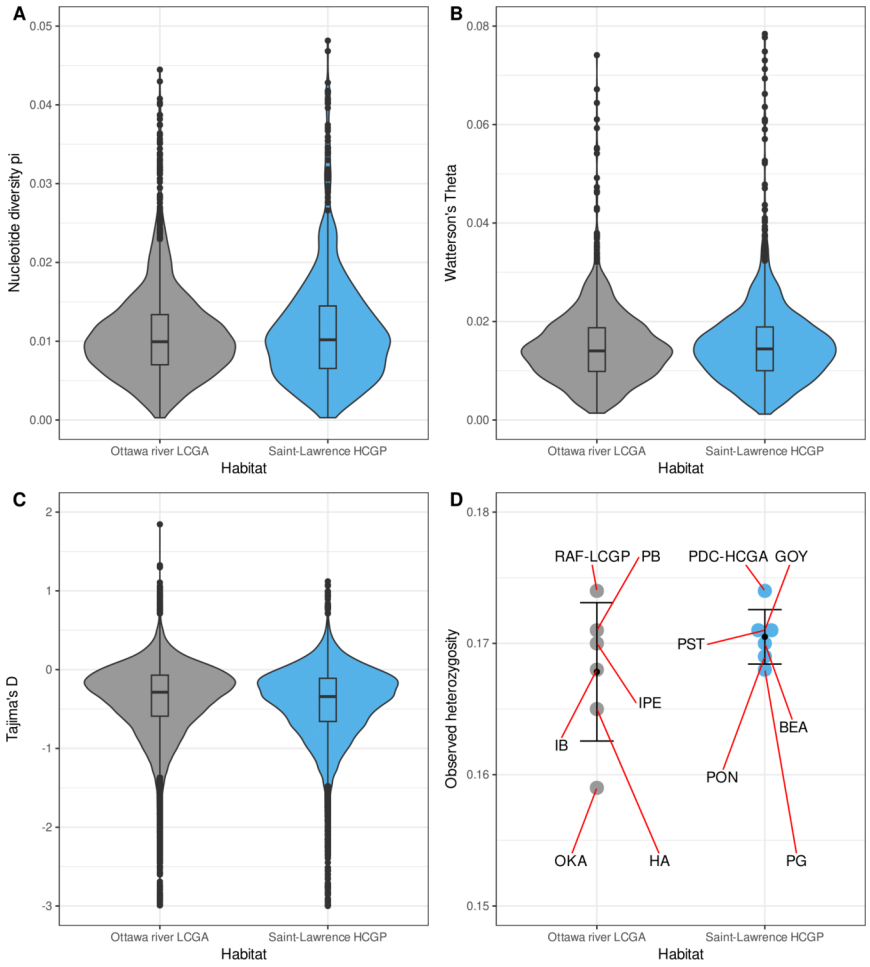

Figure 5

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