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Successful Invasion Into New Environments Without Evidence of Rapid Adaptation by a Predatory Marine Gastropod

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ABSTRACT

Invasive species with native ranges spanning strong environmental gradients are well suited for examining the roles of selection and population history in rapid adaptation to new habitats, providing insight into potential evolutionary responses to climate change. The Atlantic oyster drill (*Urosalpinx cinerea*) is a marine snail whose native range spans the strongest coastal latitudinal temperature gradient in the world, with invasive populations established on the US Pacific coast. Here, we leverage this system using genome-wide SNPs and environmental data to examine invasion history and identify genotype–environment associations indicative of local adaptation across the native range, and then assess evidence for allelic frequency shifts that would signal rapid adaptation within invasive populations. We demonstrate strong genetic structuring among native regions which aligns with life history expectations, identifying southern New England as the source of invasive populations. Then, we identify putatively thermally adaptive loci across the native range but find no evidence of allele frequency shifts in invasive populations that suggest rapid adaptation to new environments. Our results indicate that while these loci may underpin local thermal adaptation in their native range, selection is relaxed in invasive populations, perhaps due to complex polygenic architecture underlying thermal traits and/or standing capacity for phenotypic plasticity. Given the prolific invasion of *Urosalpinx*, our study suggests population success in new environments is influenced by factors other than selection on standing genetic variation that underlies local adaptation in the native range and highlights the importance of considering population history and environmental selection pressures when evaluating adaptive capacity.

1 | Introduction

Biological introductions are increasing at unprecedented rates across the globe (Seebens et al. 2017), and pose significant threats to native taxa by reducing ecosystem function, driving biodiversity loss and altering ecosystem dynamics (Anton et al. 2019; Bax et al. 2003; Grosholz 2002; Molnar et al. 2008). Despite the global rise, only a small fraction of all biological

introductions are able to successfully establish invasive populations (Mack et al. 2000; Williamson and Fitter 1996). We currently lack a complete understanding of the mechanisms that underpin how and why certain species can establish and proliferate after being introduced to new environments (Flanagan et al. 2021; Seebens et al. 2017), including the ways in which genetic, ecological and demographic factors interact to influence invasion success (Jaspers et al. 2021). Understanding the mechanisms that shape organismal capacity to invade and proliferate in new environments also has great importance in the context of climate change, as species will need to adapt to rapidly changing environmental conditions in their current habitats or shift into new geographical areas (McGuire et al. 2023).

Invasions pose a paradox because high genetic diversity in founding populations is a presumed requirement for successful establishment (Bock et al. 2015; Le Cam et al. 2020), yet many invasive populations are established by a small number of individuals possessing low genetic diversity and leading to elevated rates of inbreeding (Blackburn, Lockwood, and Cassey 2009), suggesting that other factors beyond diversity play pivotal roles in success (Allendorf and Lundquist 2003; Gaither, Toonen, and Bowen 2012; Gutekunst et al. 2018; Jaspers et al. 2021). For example, invasion success may be more likely if organisms have the standing capacity for phenotypic plasticity that allows them to tolerate conditions in the new environment (Blakeslee et al. 2020; Riis et al. 2010), or if founders originate from an environment similar to the introduced habitat and thus show some pre-adaption to those conditions (Sax and Brown 2000). Alternatively, following introduction, selection may lead to rapid adaptation to the new environment in the founding population, even when genome-wide diversity is low (Reznick and Ghalambor 2001; Schoener 2011; Willoughby et al. 2018), as long as alleles advantageous in the new environment are present within the founding population for selection to occur (Tepolt et al. 2022). While rapid adaptation has been attributed to the success of many invasive species (e.g., Colautti and Barrett 2013), the conditions under which it is favoured or constrained remain unclear. For example, genetic isolation of invasive populations was found to enhance rapid adaptation because limited gene flow precludes the dilution of adaptive alleles (Sexton, Hangartner, and Hoffmann 2014). In contrast, such isolation may instead result in maladaptive consequences due to genetic drift that causes precipitous population declines (Frankham 2005). Additionally, although there are many examples of rapid phenotypic adaptation following invasion, including morphology (Brandenburger et al. 2019; Garcia Castillo et al. 2023; Lescak et al. 2015), life history traits and behaviour (Ruland and Jeschke 2020), there is still limited genomic evidence for rapid adaptation to new environments (Matheson and McGaughran 2022; Tepolt et al. 2022). Theory predicts that moving to a new environment may reveal trade-offs, for example, due to antagonistic pleiotropy or conditional neutrality, that would result in selection and allele frequency shifts to optimise performance and increase fitness (Kawecki and Ebert 2004); however, the rates and directions of change are likely influenced by multiple factors (e.g., the relative costs and benefits to fitness and the genomic architecture of underlying traits). Similarly, when plasticity is present in the source population and the introduction is into a more 'benign' environment (e.g., fewer extremes or less seasonal variation), plasticity may carry costs and therefore be selected against (Murren et al. 2015). Disentangling the roles of phenotypic plasticity, pre-adaptation, trade-offs and rapid genetic adaptation in the establishment success and evolutionary trajectories of invasive populations requires an understanding of source population environments and demographic histories, which is challenging for many species, especially where historical records are sparse. Combining population genomics with environmental and phenotypic data is emerging as a powerful integrative approach to reconstruct invasion histories, enabling deeper understanding of the influence and interactions of these processes in successful biological invasions (Flanagan et al. 2021; Tepolt and Palumbi 2020).

Beyond invasions, understanding the evolutionary processes that promote or inhibit species' persistence in new environments has profound importance in forecasting biological responses to climatic change. Experimental evolution studies have demonstrated that both phenotypic plasticity and rapid adaptation can play important roles in population resilience to changing environments (e.g., Brennan, deMayo, Dam, Finiguerra, Baumann, Pespeni et al. 2022; Brennan, deMayo, Dam, Finiguerra, Baumann, Buffalo et al. 2022). However, experimental evolution approaches are typically constrained to species with short generation times that can be reared in laboratory settings, creating uncertainty in how representative these studies are to longer-lived species in natural environments. In contrast, studies *in situ* have shown that local adaptation (i.e., the fine-tuning of traits to local conditions) can be instrumental in species' persistence across heterogeneous environments in their biogeographic ranges (Derycke, Backeljau, and Moens 2013; Liu et al. 2016; Miller et al. 2020), and are often critical to include in assessments of species' climate risk (Cacciapaglia and van Woesik 2018). Studies in this realm frequently identify putative loci underlying adaptive divergence across land- or seascapes, and then estimate how allele frequencies at those loci would need to change to keep pace with forecasted future environmental conditions (i.e., genomic vulnerability or offset; Bay et al. 2018; Forester et al. 2023), although the ability of these methods to accurately predict climate risk and resilience remains unclear (Lotterhos 2024). While these approaches can be applied to species with a greater breadth of life history traits to understand how microevolutionary processes contribute to current population success in natural environments, it is still largely unknown if such rapid adaptation will continue to occur in future populations and/or if this correlates with persistence.

Invasive species that are locally adapted to environmental gradients in their native range that are established in environments differing from their origin provide opportunities where predictions of rapid adaptation, akin to genomic vulnerability, can be made and then tested (Kim et al. 2023). In essence, invasive species provide large-scale *in situ* accidental anthropogenic experiments where we can detect rapid adaptation by examining whether allele frequency shifts in invaded locations are in accordance with estimates generated from the native range based on genetic–environmental correlations (Balanyà et al. 2003; Battlay et al. 2023; Sparks et al. 2023). If evidence for adaptation is not supported despite the successful establishment of invasive populations, other factors and processes may drive persistence in new environments. For example, the traits involved in adaptation could

be polygenic and genetically redundant and therefore unique genetic variation could be involved in adaptation in the invaded habitat. Alternatively, selection may be relaxed at the predicted adaptive loci if there is sufficient plasticity to tolerate the new environment, and if the cost of maintaining plasticity under relaxed selection is minimal. Finally, there may be costs of moving towards new optima that are greater than the benefits in the new environment. Combined with genomic approaches to reconstruct invasion history and identify source populations as described above, invasive species provide ideal opportunities to examine the roles of selection, plasticity and population history in establishment and persistence in new environments relevant to biological invasions and potential responses to climate change.

Here, we use Atlantic oyster drills (*Urosalpinx cinerea;* hereafter referred to as *Urosalpinx*) as a system for understanding invasion success and the roles played by rapid adaptation and population history following introductions to thermal environments that differ from their origin. *Urosalpinx* are predatory marine gastropods native to the US Atlantic coast that have successfully established multiple invasive populations globally, including several populations on the US Pacific coast through incidental introductions from the deliberate translocation of Eastern oysters (*Crassostrea virginica*) following the completion of the transcontinental railroad in 1869 (Carlton 1979, 1992; Miller 2000). Native populations of *Urosalpinx* have been well-documented historically along the North American Atlantic coastline from northern Florida to Massachusetts (Carriker 1955), with additional northern contemporary populations also observed in New Hampshire, Maine and Canada that may represent remnants of a wider postglacial warm period distribution (Bousfield and Thomas 1975). Estimates suggest that at least 1.7 million kg of Eastern oysters and associated hitchhiker species were outplanted to the Pacific coast from 1870 to 1900 for human cultivation and consumption, followed by small-scale outplants in multiple estuaries and possible further 'clandestine' translocations throughout the 19th and 20th centuries (Carlton 1979). Historical reports are incomplete but indicate that oysters were harvested from the Chesapeake Bay area and possibly transported to other mid-Atlantic and New England locations to replenish local fisheries before transcontinental transportation (Hoos et al. 2010; Miller 2000). As such, the source populations for the invasive Pacific coast populations remain unclear, yet is an important consideration when examining potential adaptations across the invasive range.

The Atlantic coastline of the continental United States is characterised by the strongest cline in coastal latitudinal temperature gradients in the world (Baumann and Doherty 2013). Local adaptation of numerous species distributed across the Atlantic coastline has been directly attributed to this thermal variation (Stanley et al. 2018; Wilder et al. 2020). *Urosalpinx* exhibits a direct development form of reproduction, laying egg capsules on coastal substrate that produce 'crawl away' hatchlings instead of pelagic larval dispersal, a life history strategy that is thought to facilitate rapid colonisation and promote local adaptation across heterogeneous environments like those in its native range (Chang et al. 2011; Johannesson 1988; Miller et al. 2007). Recent work in this species has demonstrated that *Urosalpinx* exhibits local adaptation for key thermal phenotypic traits such as thermal tolerance and growth performance (Villeneuve, Komoroske, and Cheng 2021a, 2021b), making it an especially well-suited system to examine the genomic underpinnings of invasion success and test predictions of rapid adaptation in new habitats. Locations along the US Pacific coast where *Urosalpinx* has established invasive populations exhibit environmental profiles distinct from the native range, differing from potential source populations in key thermal parameters that can exert strong selection pressures (Sanford and Kelly 2011). Here, we combine population genomics and environmental data to reconstruct the invasion history of *Urosalpinx*, quantify genomic signatures of local adaptation in its native range, FI and then leverage this knowledge to examine evidence for rapid adaptation in new environments. Specifically, we use genotype–environment associations (GEAs) to identify putatively thermally adapted loci in native populations and then use these to predict shifts in allelic frequencies (AF) across the invasive range. We then compare our predictions against observed frequencies to determine whether selection is acting to drive evolutionary change in an expected direction at the same loci in invasive populations. For example, if an invasive population is established in an environment with a lower temperature from its origin, we might expect trade-offs to result in selection for alleles most fit at low temperatures. Conversely, if existing plasticity capacity is sufficient to tolerate the new temperature regime, and the costs to move towards the new optimal allele are greater than the benefits, we would expect that no adaptation would be observed. By combining invasion and local adaptation genomics approaches to conduct explicit tests of these hypotheses, our study demonstrates how invasive species can be leveraged to advance our understanding of how populations establish and persist in new environments.

2 | Methods

2.1 | Sample Collections and Processing

We collected adult *Urosalpinx* samples at locations spanning the native and invasive ranges along the Atlantic and Pacific coasts of the continental United States, respectively (see Table S1 for locations and abbreviations; Figure 1A,B), following methods described in Villeneuve, Komoroske, and Cheng (2021b). Across the native range, samples were collected from regions defined as Northern New England (DB and GB; see Figure 1), Southern New England (CT and WH), the mid-Atlantic (OY and DB) and the south-Atlantic (BF, HP, FB and SK). Prior to tissue sampling, we sexed the snails as per (Hargis 1957) and placed individuals in an ice-water slurry to slow metabolism, before measuring shell height and removing soft tissue from the shell using a C-clamp. We subsequently dissected the foot below the mantle tissue, removed the operculum with a razor blade and stored tissue in 96% ethanol before freezing it at −80°C until DNA extraction.

2.2 | DNA Extraction, Library Preparation and Sequencing

We used Qiagen DNeasy blood and tissue kits (Qiagen, Valencia, California) to perform genomic DNA (gDNA)

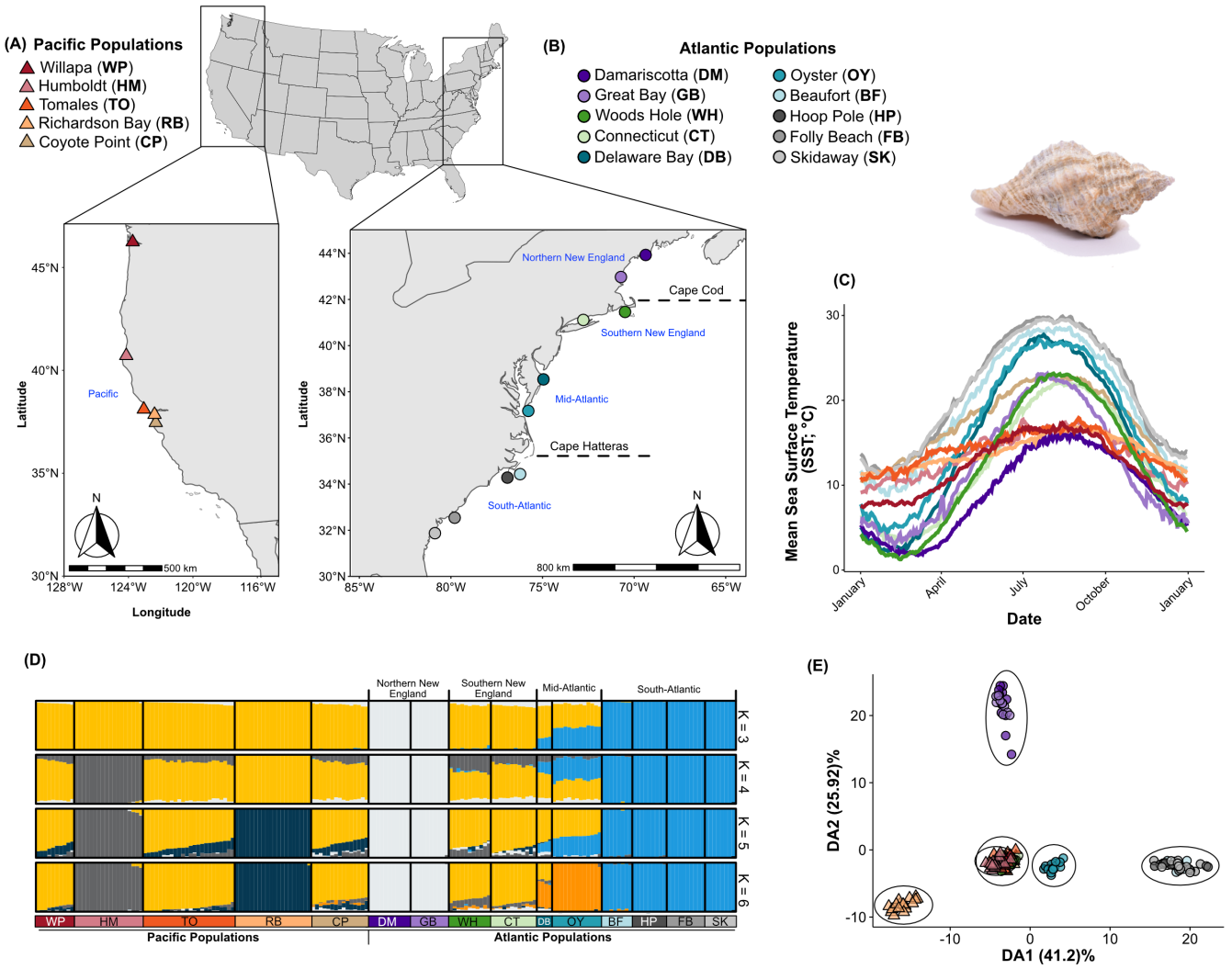

FIGURE 1 | Locations and population structure of the 15 sampling sites across the continental United States for Atlantic oyster drills (*U. cinerea*) used in this study. (A) Invasive Pacific coast collection locations; and (B) native Atlantic coast collection locations [see Table S1 for detailed sampling locations]. (C) Mean sea surface temperatures for each day of the year for each sampling site averaged over 2005–2021 (but see Table S1), with colours corresponding to those in (A) and (B). (D) Outputs from Admixture analysis with $k = 3$, 4, 5 and 6. (E) Output from the discriminant analysis of principal components (DAPC) with $k = 6$, and ellipses represent the identified clusters [see Figure 2 for fine-scale structuring results for the mid-Atlantic and Pacific coast locations]. Photo credit: A. Rugila.

extractions, modified with an additional ethanol clean-up step and double elution to prevent residual reagent contamination in the final product. After extraction, we employed a 1:1 Ampure bead cleanup step to reduce the amount of mucopolysaccharides coprecipitating out with the DNA that commonly occurs in gastropods (Sokolov 2000). We assessed DNA integrity using a Fragment Analyser (Agilent, Santa Clara, California) and quantified it using a Qubit 4.0 Fluorometer (Invitrogen, Waltham, Massachusetts) following the recommended protocols provided by the manufacturers. We normalised DNA concentrations for each sample (150–200 ng total input) and prepared RAD-Seq libraries following the BestRAD protocol described in Ali et al. (2016) using *SbfI*-HF and NEBNext Ultra DNA Library Prep Kits for Illumina (New England Biolabs, Ipswich MA). Finally, we evaluated library sizes and concentrations using a Fragment Analyser followed by Illumina 150 bp paired-end sequencing at Novogene Corp. (Sacramento, California).

2.3 | Data Filtering, SNP Identification and Genotyping

All scripts associated with data processing and analyses are provided on GitHub (https://github.com/bpbentley/project_uro_RADSeq). Following sequencing, we demultiplexed libraries using the *process_rad-tags* function in STACKS v2.62 (Catchen et al. 2013), with the first two bases of each read trimmed using ATROPOS (Didion, Martin, and Collins 2017). Following recommendations by Paris and Stevens (2017), we employed parameter optimisation steps to identify the most appropriate parameters governing *de novo* locus formation. Due to the strong divergence between the populations across regions, we optimised parameters M (mismatches allowed between alleles within an individual to collapse into a locus) and n (mismatches allowed between loci across individuals to label as homologues) independently, rather than maintaining them as equal through the parameter optimisation steps (Paris

and Stevens 2017). In all cases, we kept the minimum number of raw reads required to collapse into a putative locus constant ($m = 3$). Outcomes from parameter optimisation resulted in downstream data processing using parameters of $M = 1$ and $n = 6$ (see Figure S1) to retain the optimal number of SNPs, while also ensuring representation from all populations (see associated GitHub repository for full details of parameter optimisation). To ensure representation from each population but reduce computational complexity, we used between 4 and 10 individuals from each population (using individuals with a mean coverage ≥ 20×) to construct the SNP catalogue, and then called SNPs for all individuals using the Bayesian genotype caller implemented in STACKS 2 (Rochette, Rivera-Colón, and Catchen 2019). We then ran the 'populations' module of STACKS 2 with the minor allele count (--mac 3) and maximum heterozygosity (--max-obs-het 0.7) parameters to filter across all populations and retained one SNP per RAD tag, retaining the highest-quality SNP per tag.

Following genotyping with STACKS 2, we used VCFTOOLS (Danecek et al. 2011) to remove loci that were missing in more than 50% of the individuals, as well as loci with a mean depth of less than 10× or more than 1000×. We conducted further SNP filtering following recommendations from O'Leary et al. (2018) with all subsequent analyses conducted in R (R Core Team 2023). We identified paralogues using the proportion of heterozygotes (H) and deviations in read ratios (D) following the methods described by McKinney et al. (2017), with the H and D thresholds set to 0.45 and 4.0, respectively (Figure S2). We then visualised the genotype frequencies and heterozygous miscall rate using the WHOA package (Anderson 2021), and set upper and lower mean read depth thresholds per locus for all individuals to 25× and 175×, respectively. We regarded any locus that had six or fewer reads in any one individual as 'missing' to ensure that only loci with sufficient reads were retained for downstream filtering thresholds. We subsequently ran an iterative loop varying the combinations of individuals and loci to retain in our dataset. To obtain our final dataset, we removed any individual missing 85% (Ipass) or more loci in the catalogue, then removed any individuals missing ≥ 40% of the remaining loci, and any locus that was missing in at least 20% of individuals using an iterative loop that filtered in a stepwise manner. We further filtered loci for linkage disequilibrium (LD) using PLINK (Purcell et al. 2007), with a sliding window of 50 bp, a 5 bp step between windows and an R^2 threshold of 0.2 (--indep-pairwise 50 5 0.2). A summary of all filtering steps and parameters is listed in Table S2. These filtering steps resulted in the retention of 183 individuals and 7671 SNPs for downstream analyses.

2.4 | Population Genetic Structure and Diversity

We examined individual-based population structure using principal component analysis (PCA) and a discriminant analysis of principal components (DAPC). We ran PCAs through the 'glPca' function in ADEGENET (Jombart and Ahmed 2011) following conversion of the final vcf to a genlight object with VCFR (Knaus and Grünwald 2017). For DAPC analyses, we used ADEGENET, with the optimal number of clusters identified using K-mean selection and BIC through the 'find.clusters' function of ADEGENET. We also used ADEGENET for DAPC using the 'dapc' function. Our initial DAPC analysis was run with no prior information before a second DAPC was run using the outputs of the initial run as priors and with the optimal number of PCs identified using a-score optimisation through the 'optim.a.score' function. DAPC and ADMIXTURE analyses were also run on individual clusters to resolve fine-scale structuring that may have been swamped by wide-scale genetic differentiation between clusters. To infer population structure among sampled locations, we applied a Bayesian clustering method implemented through ADMIXTURE (Alexander, Novembre, and Lange 2009). To obtain the number of clusters that best explained the data in ADMIXTURE, we iterated over values of K ranging from two to 14 (i.e., $n_{SITES} - 1$) and selected the most applicable value using the in-built cross-validation method (--cv). We visualised outputs using the POPHELPER package for R (Francis 2017).

We also estimated population-level estimates of genetic distance (F_{ST}) using the 'genet.dist' function of the HIERFSTAT package in R (Goudet 2005) with the 'WC84' parameter to leverage equations described in Weir and Cockerham (1984). To examine evidence for isolation by distance (IBD) in the native range, we subset data to only include sites from the Atlantic coast and correlated the pairwise F_{ST} metrics with geographical distance between sites. We then tested for IBD with the 'gl.ibd' function of the DARTR package for R (Gruber et al. 2018) using 999 permutations, with geographic distance calculated through the conversion of latitude and longitude to Mercator projections.

We calculated observed heterozygosity (H_{OBS}) at the 7671 loci for each population using the 'populations' function of STACKS 2. We compared H_{OBS} between populations in the putative source locations (see below) and the invasive Pacific locations using an analysis of variance (ANOVA) with pairwise comparisons tested with Tukey post hoc tests using R (R Core Team 2023).

2.5 | Identification of Invasion Source

We applied a linear discriminant analysis (LDA) to refine the source of the invasive populations using the MASS package for R (Venables and Ripley 2002), with the LDA trained using the first four principal components of a PCA derived from only populations across the native range. Following model training with all the native populations, we used the model to assign the invasive Pacific coast populations to a source location following methods described in Flanagan et al. (2021). For each Pacific population, we summed probabilities across individuals and visualised the outputs using the CIRCLIZE package for R (Gu et al. 2014). In addition to the LDA for assigning the source of the invasive populations, we ran further PCA, DAPC and admixture analyses on subsets of populations, including those with and without any highly divergent invasive populations to provide further evidence for the source locations.

2.6 | Genotype–Environmental Association (GEA) Analyses

We obtained *in situ* sea surface temperatures (SSTs) through the National Oceanic and Atmospheric Administration's

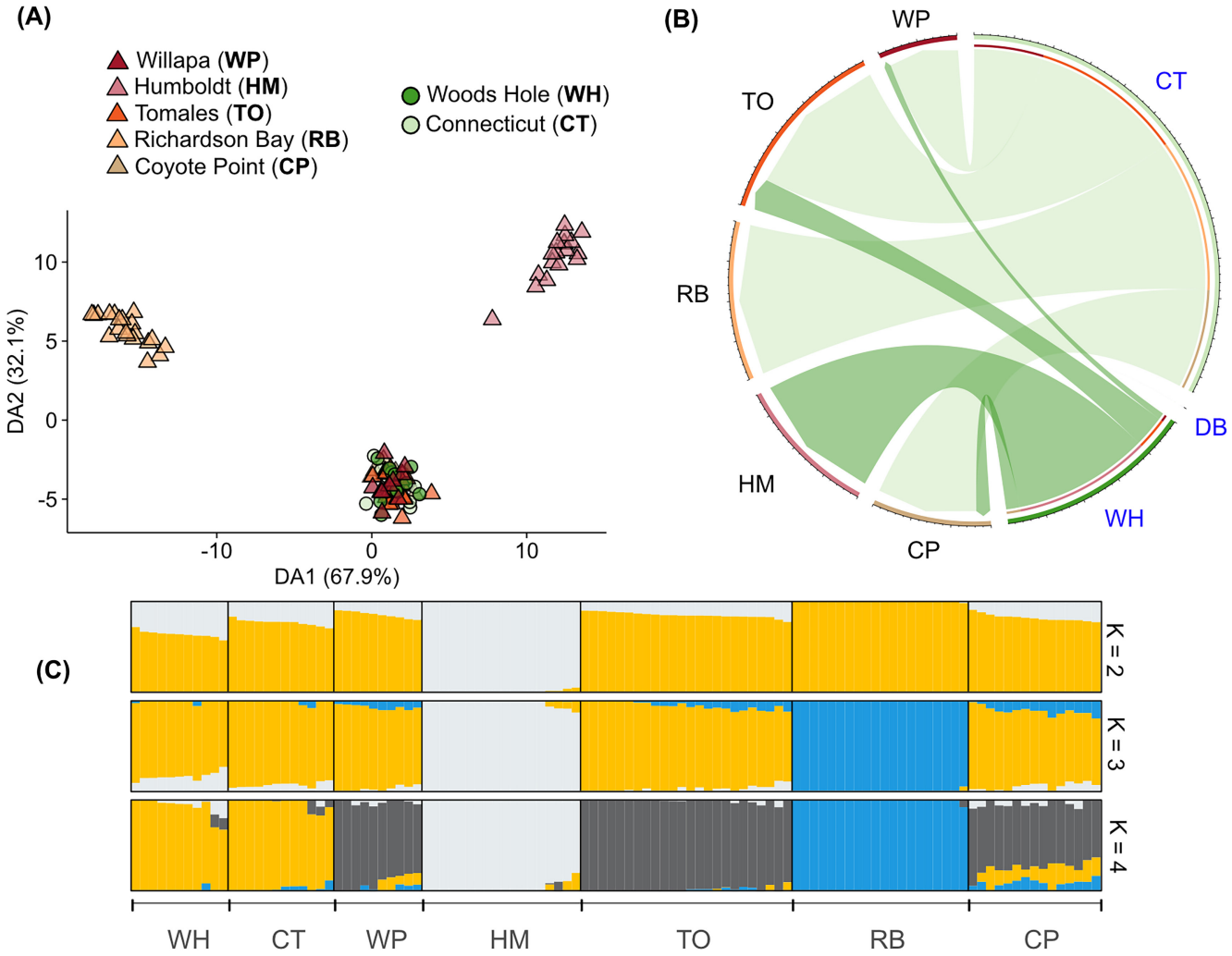

FIGURE 2 | Identification of source location for invasive Pacific populations of *Urosalpinx*. (A) Outputs from a DAPC with $k = 3$; (B) Circle plot showing the likely ancestral source of the invasive Pacific coast populations predicted by an LDA trained with all Atlantic populations where the arrow-head points in the direction of the predicted translocation; and (C) results from an admixture analysis with $k = 2$, 3 and 4.

(NOAA) National Data Buoy Center (https://www.ndbc.noaa.gov/), the NOAA National Estuarine Research Reserve System (NERRS) database (https://coast.noaa.gov/nerrs/) and through data made available by the Wiyot Tribe (https://www.wiyot.us/Archive.aspx?AMID=40); (but see Figure 1C and Table S1 for site-specific details). We summarised these data for each site to generate 13 temperature metrics: mean, minimum and maximum SSTs for annual, winter and summer periods, as well as the estimated length of reproductive season (number of days with mean SST ≥ 10°C, as per Carriker 1955; Villeneuve, Komoroske, and Cheng 2021b). Subsequently, we reduced this matrix to four variables (annual mean, annual minimum, annual range and annual maximum) by removing one variable in a pairwise correlation with a value > 0.7 (Figure S3; Dormann et al. 2013).

To determine correlations between genotypes and environmental variables in the native range that may contribute to local thermal adaptation, we first ran redundancy analyses (RDA) between the AF of the Atlantic populations and temperature variables described above using a multivariate ordination approach following methods in Forester (2018) and Capblancq and Forester (2021). As RDA does not allow missing data, we initially imputed missing values as the most common genotype at each locus across all individuals (Forester et al. 2018), which accounted for imputation of approximately 12% of the total dataset. Following this, we filtered out rare alleles by removing any locus with a mean minor allele frequency (MAF) of less than 0.05 within each population. This subsequently resulted in a dataset of 4567 SNPs being retained for RDA analyses from the 10 populations across the native range.

We generated a full RDA model of the AF of the Atlantic coast populations against the four temperature variables following the procedures outlined by Capblancq and Forester (2021), which we then compared to a null model to identify which climatic variables significantly explained the data. To partition the variance of the model, we used partial RDAs (pRDAs), with four pRDAs applied to the data using combinations of climate, geospatial and genetic structure variables. To account for geospatial autocorrelation in the data, we decomposed locations

into multivariate spatial vectors. To do this, we converted latitude and longitude to Cartesian coordinates using the 'geoXY' function of the SoDA package for R (Chambers 2008) and then calculated Euclidean distances between sites which were used as inputs for the 'dbmen' function of the ADESPATIAL package (Dray et al. 2020) to generate distance-based Morans' eigenvector maps (db-MEMs). To determine which db-MEMs were significantly associated with genotypes, we ran an RDA of AF against the 14 identified db-MEMs with the 'ordistep' function of VEGAN used in the forward selection process of model selection (Blanchet, Legendre, and Borcard 2008). To account for genetic structure, we additionally included the first two principal components of the PCA described above, where we only used individuals from the native range as inputs.

After variance partitioning, we used the climate-driven pRDA (*clim*) to scan for potential outlier loci that are linked with climatic variables following the methods described in Forester et al. (2018). This method searched for outliers based on their loadings along each retained RDA axis, with outliers identified as those with loadings 3 standard deviations (SD) from the mean on each axis (i.e., two-tailed $p < 0.0027$). Loci that were identified as outliers through this method were then associated with a specific SST variable by selecting the variable that showed the highest correlation with AF.

As RDA are known to have high Type-I error rates (Booker et al. 2024; Lotterhos 2023), we also ran a latent factor mixed model (LFMM; Frichot et al. (2013)) on the same set of four environmental variables used in the RDA as described above with missing genotypes imputed using the 'pmm' method in MICE (van Buuren and Groothuis-Oudshoorn 2011) and five iterations. LFMM is another GEA approach that accounts for population genetic structure in the data. We, therefore, used the outputs from the population genomic analyses on the native populations to run the LFMM assuming six latent factors through the LEA package in R following methods described in Frichot and Francois (2015). We ran the model five times to increase the likelihood of detecting true associations between the environmental variables and genotypes. From each of these five runs, *z*-scores were extracted, with a median taken for each locus. We then recalibrated these, by initially calculating the genomic inflation factor (λ), and adjusting the *p* values by dividing the median *z*-score by this value (see Frichot and Francois (2015) for full calculation). Finally, *p* values were adjusted for multiple comparisons using the Benjamini–Hochberg algorithm with a *q* value of 0.001 to avoid false-positive detection. Only loci that were associated with the same environmental variable in both the RDA and LFMM analyses were retained for subsequent analyses.

We also tested for associations between environmental variables and genotypes across the invasive range to assess if local adaptation has occurred between the established populations since introduction. To achieve this, we ran the same GEA analyses on the Pacific coast populations using the methods described above, which resulted in 3744 loci being used as input across the five invasive populations. The RDA was run as described above. As the RDA for the invasive populations failed to detect any loci, we compared the loci detected in the LFMM with the loci that intersected in both analyses in the native range.

2.7 | Predicting AF in Invasive Populations

To determine if AF in putatively adaptive loci varied between each of the invasive populations and the source locations, we conducted pairwise *T*-tests for the loci sets associated with the three climate variables where multiple loci were jointly detected in the RDA and LFMM analyses. To evaluate if observed differences were likely due to signals of rapid adaptation or simply due to chance (i.e., genetic drift), we bootstrapped these comparisons by randomly selecting the same number of loci used in each comparison from the full locus dataset and compared the AF in these between source and invasion populations 1000 times. We then compared the results of the initial *T*-tests of the putatively adaptive loci with the distributions of *T*-values from the randomly selected comparisons to assess whether each population had significantly diverged from the source population at the putatively adaptive loci.

We then examined if AF at these temperature-associated loci were shifting within the invasive populations as would be predicted by their new environmental conditions and the genotype—environmental relationships established in the native range. For each locus, we ran a linear regression model of AF against the SST variable observed from all the native populations (e.g., AF~annual mean SST). We then used this regression to predict the AF at the invasive locations based on the observed SST values at these sites. If selection is acting on these loci, then the differences between AF predicted by the regression and the values observed at these locations should approach zero (see Figure 6A,C). For each of the three SST variables, we then calculated the median difference between predicted and observed AF for each of the five populations. Similar to above with the *T*-tests, we assessed the likelihood of obtaining our results due to chance; we randomly selected the same number of loci from the full dataset, bootstrapped this analysis 1000 times and then compared the distribution of median differences between predicted and observed values against the results of the sets of putatively adaptive loci. Taken together, we concluded that any invasive population that had significantly diverged from the source populations, and also had a lower median difference in AF of putatively adaptive loci than 95% of the random comparisons was likely reflective of rapid adaptation due to selection.

3 | Results

3.1 | Sequencing, Catalogue Assembly and Genotyping

Sequencing produced a total of 1.9 billion raw reads across a total of 299 individuals. From the 262 individuals that passed read number thresholds (> 10,000 reads) for ustacks, we retained a mean of 1.32 million filtered reads per individual (median = 1.30 million) with a mean depth coverage of 30.9× (SD: 18.9×). Using a subset of 136 individuals representing all 15 collection sites, we identified a catalogue of 264,989 SNPs (see Table S3). Following SNP filtering pipelines, we retained a final dataset of 183 individuals and 7671 loci for downstream analyses.

3.2 | Population Genetic Structure and Genetic Diversity

Admixture analyses indicated that the genetic variation in the dataset was best explained by five or six clusters (Figure 1D), with individuals from the two northern New England sites (DM and GB) represented in one distinct cluster, and individuals from the south Atlantic region (BF, HP, FB and SK) in another. The southern New England (CT and WH), mid-Atlantic (OY and DB) and Pacific locations (WP, TO, RB, CP and HM) showed evidence of mixed ancestry from three clusters, but with HM and RB assigned to their own unique clusters and the remaining populations exhibiting contributions of varying percentages from each (Figure 1D). Unlike the southern New England populations, individuals from OY and DB also showed substantial contributions from the south Atlantic cluster. The DAPC analysis also suggested six clusters best explained the data ($k=6$; Figure 1E), and results were largely concordant with the Admixture findings, with the largest genetic separations between clusters containing the northern New England, southern New England and Pacific, mid-Atlantic and south Atlantic regions. All Pacific populations except RB overlapped with southern New England populations in the DAPC of the full dataset; however, HM formed its own distinct group within this larger cluster (Figure 1E). Further exclusive analysis of the southern New England and Pacific invasive populations to clarify fine-scale structure that could be obscured given the strong divergence in the full dataset confirmed the presence of three clusters ($k=3$; Figure 2A,C) when all locations in these regions were included (invasive populations at WP, CP and TO grouped with southern New England, while HM and RB represented exclusive clusters), although analyses of locations only within the multisite cluster also revealed evidence of finer-scale differentiation (Figure S4).

The average F_{ST} across all 7761 SNPs and 15 populations was 0.23, with pairwise F_{ST} between populations ranging from 0.02 (BF-HP) to 0.49 (SK-DM). Generally, pairwise F_{ST} between regions were relatively high (Figure 3A and Table S4), indicating high genetic differentiation between regions. This was particularly true for pairwise comparisons between the northern New England region (DM and GB) and all other regions. Genetic differentiation was generally low within regions, including the south Atlantic cluster (mean pairwise $F_{ST}=\sim0.06$) and the southern New England/Pacific cluster ($F_{ST}=\sim0.12$), but not within the northern New England cluster (DM and GB; pairwise $F_{ST}=0.30$) which showed pairwise values that were more similar to the between region values (Figure 2A,B). Concordant with admixture and DAPC analyses, pairwise F_{ST} values were slightly higher between HM and the other southern New England and Pacific sites (mean pairwise $F_{ST}=0.18$; see Figure 2A) than other within region comparisons. Pairwise F_{ST} values were strongly correlated with pairwise geographic distance between sites across the native Atlantic range (mantel $R=0.67$, $p<0.001$; Figure 3B), concordant with a pattern of IBD.

Genetic diversity varied between populations; observed heterozygosity (H_{OBS}) ranged from 0.063 to 0.181 (Table S5), with the highest genetic diversity in the south Atlantic populations (BF, HP, FB and SK) and lowest in the two northern New England locations (DM and GB; Figure 4A). However, we note that the strong genetic divergence between regions in our dataset

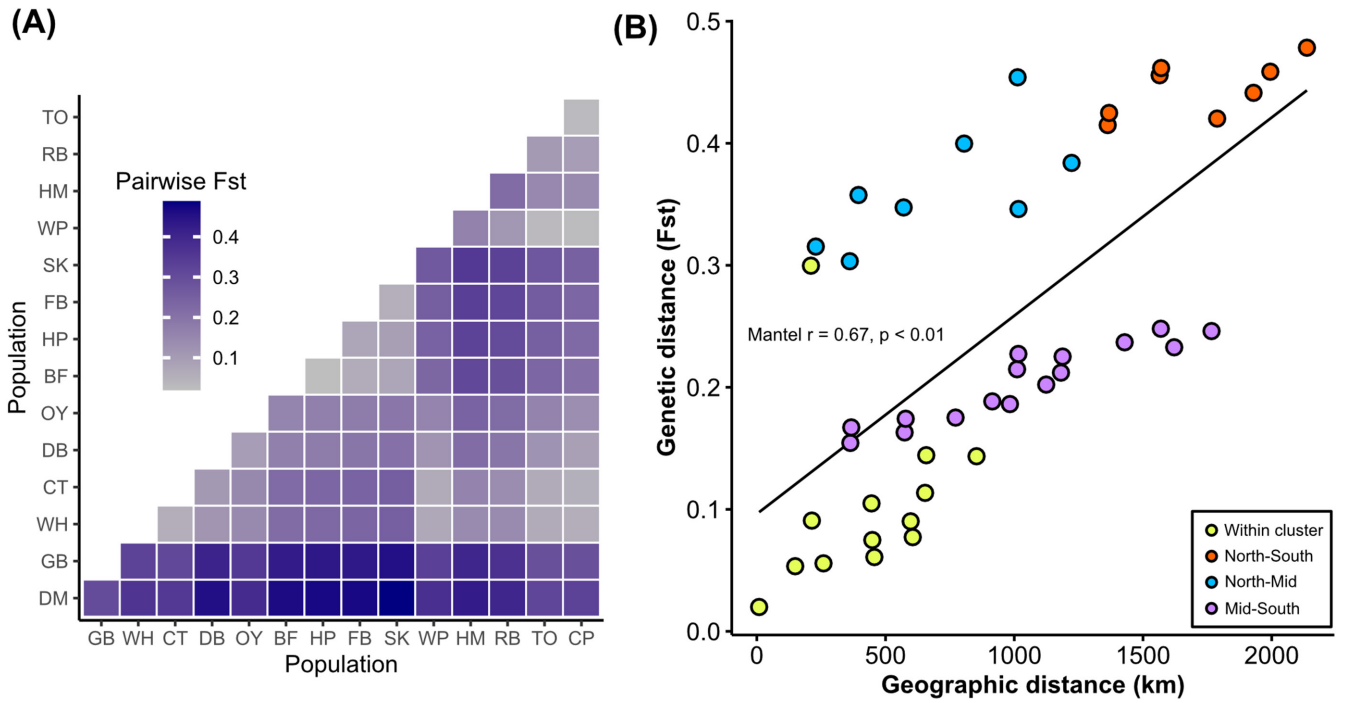

FIGURE 3 | Genetic differentiation between populations. (A) Pairwise F_{ST} values between all sampling locations (also see Table S4); (B) correlations between geographic distance and genetic distance in sites from the native Atlantic coast showing evidence of isolation by distance (IBD), with colours representing comparisons between and within genetic clusters. North: DM and GB; Mid: WH, CT, OY and DB; South: BF, HP, FB and SK.

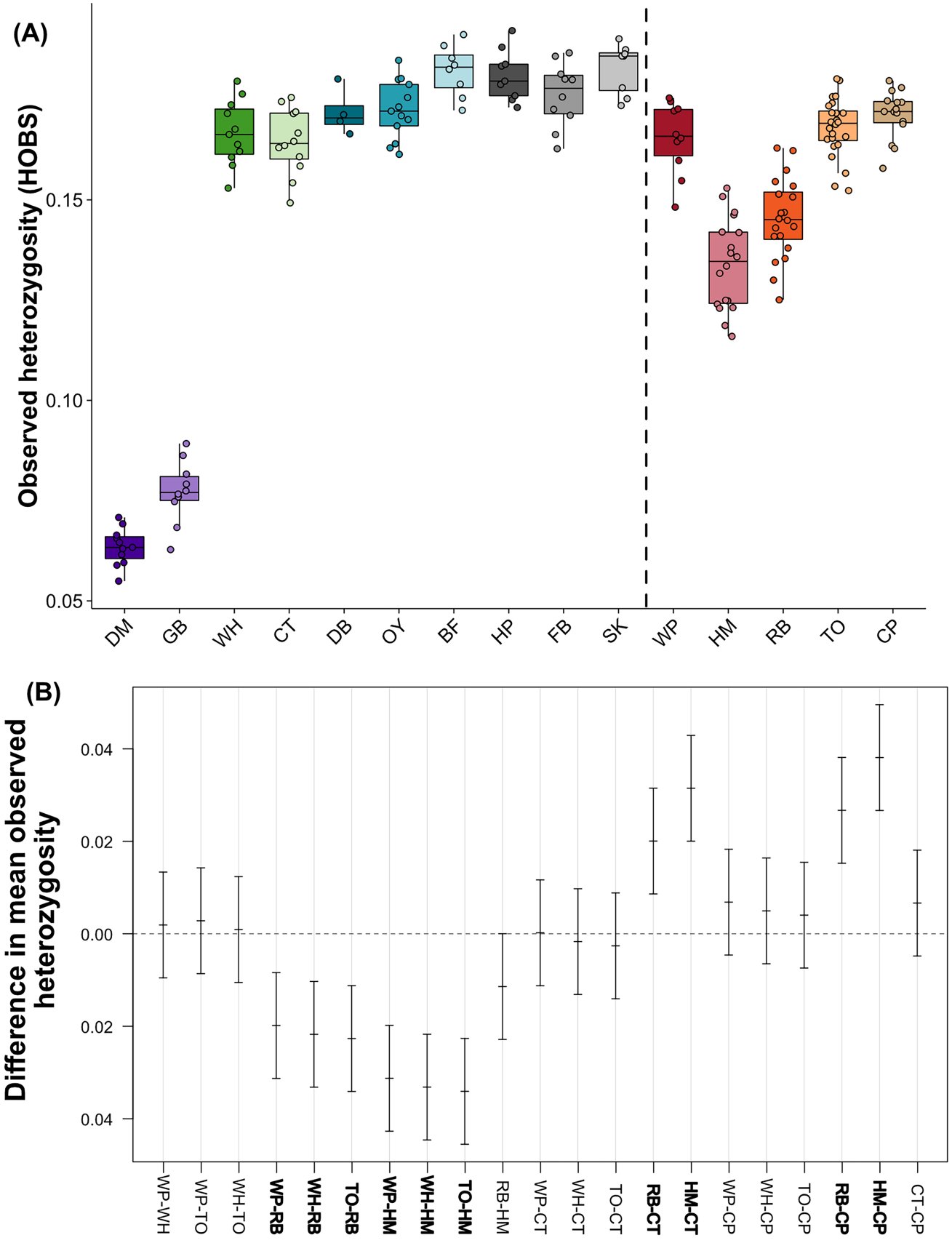

FIGURE 4 | (A) Boxplot of observed heterozygosity for each population of *Urosalpinx*. Populations to the left of the dashed line represent the native range, and to the right represent the invasive populations (see also Figure S5). (B) Differences between observed heterozygosity in invasive Pacific populations, and the two putative source locations in the southern New England region (WH and CT). Bolded values indicate significant pairwise differences ($p < 0.05$) detected by a Tukey post hoc test following a one-way ANOVA between observed heterozygosity and population.

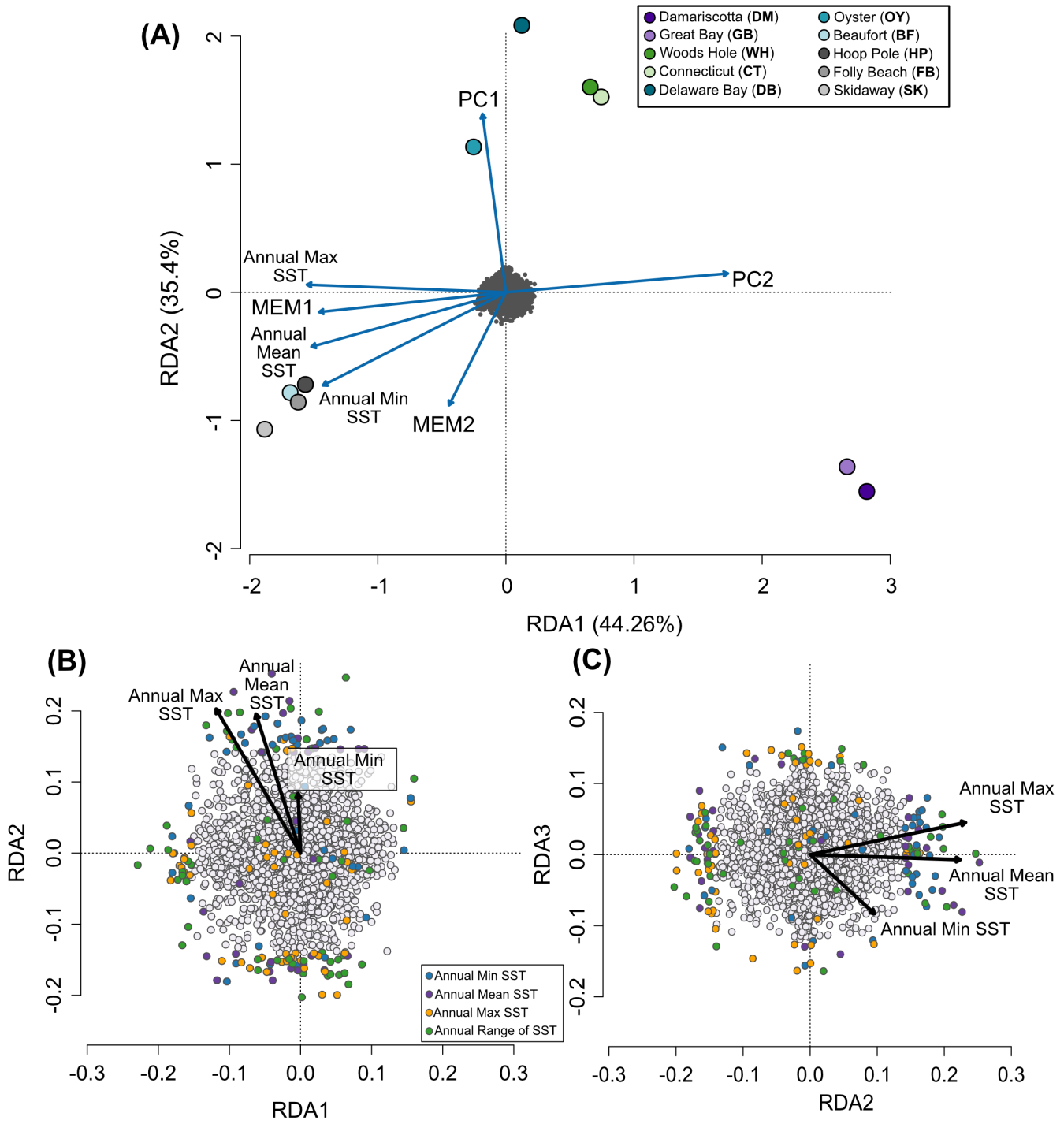

FIGURE 5 | (A) Output from partial RDA full model (*pRDA_full*), with populations represented by coloured points, and loci included as grey points. Bi-plots of loci identified as outliers (±3 SD) on the first three axes of the climate partitioned RDA (*pRDA_clim*) and the associated predictor variables for (B) RDA Axes 1 and 2; and (C) Axes 2 and 3.

can affect estimates of heterozygosity and allele fixing using the STACKS 2 workflow (Figure S5B–E), so the lower diversity in northern New England may be partially due to technical bias. Diversity in two of the invasive locations (HM and RB; H_{OBS} = 0.13 and 0.14, respectively) was lower when compared to the other invasive populations and the native sites (Figure 4). The HM and RB populations also contained higher levels of allele fixation than the other invasive populations (Table S6).

3.3 | Identification of Invasion Source(s) and Post-invasion Diversity

Congruent with population structure analyses, LDA strongly supported that all invasive populations on the Pacific coast originated from the southern New England region (Figure 2B) and indicated that most originated from the CT area, with the exception of HM which had a higher likely origin from WH. Heterozygosity was significantly different overall between

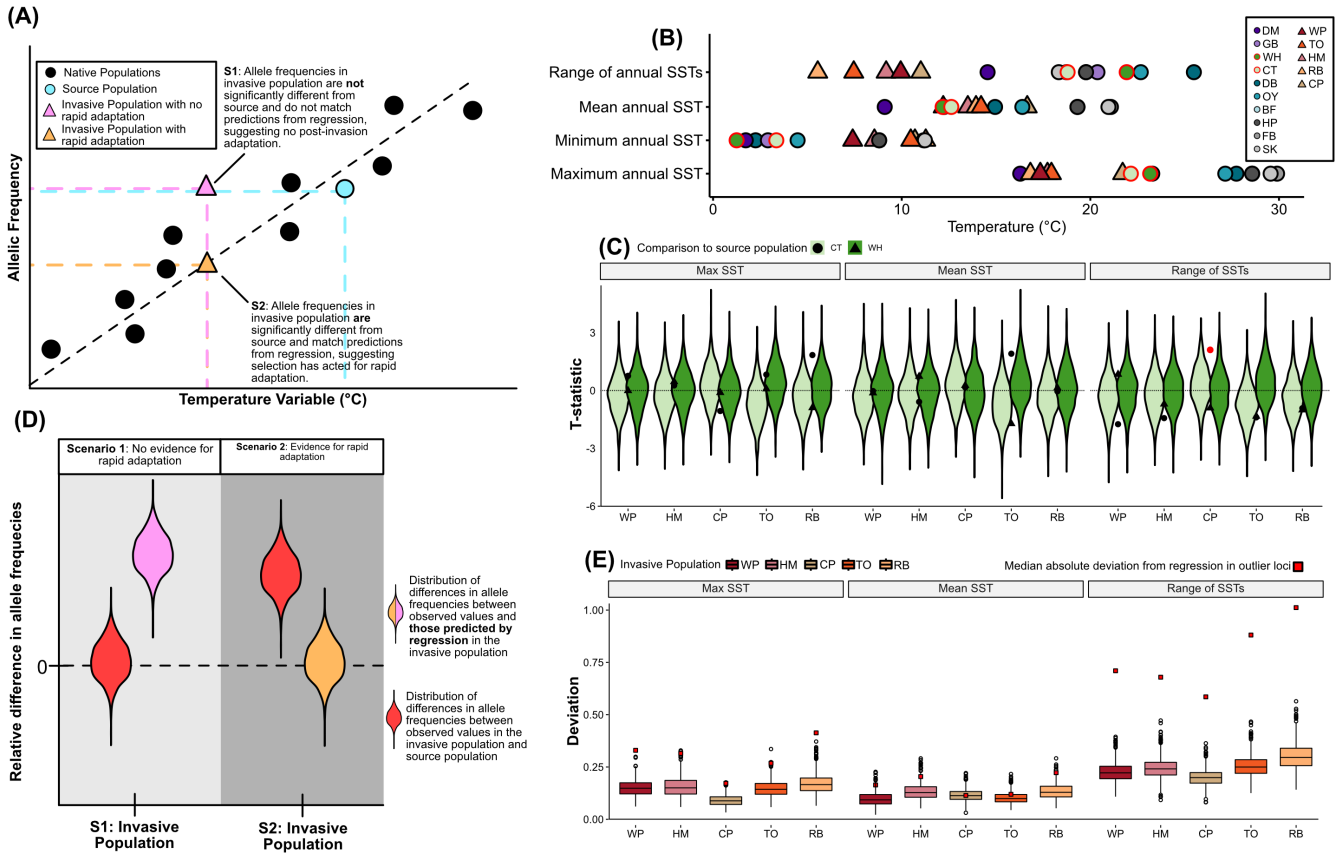

FIGURE 6 | Conceptual and observed shifts in allele frequencies: (A) conceptual figure demonstrating the expectations of allele shifts in invasive populations that have not experienced rapid adaptation in putatively thermal-adaptive loci (S1), and those whose allele frequencies have shifted in a predictable direction due to rapid adaptation (S2). (B) Distribution of range, mean, maximum and minimum SSTs for all native (circle) and invasive (triangle) locations of *Urosalpinx*. The two putative source locations (CT and WH) and denoted with a red border. (C) Deviations of invasive populations from source location outlier loci detected with two GEA analyses (RDA and LFMM) for three environmental variables. Points represent the significance value of the *T*-tests between AF at source and invasive populations, and violin plots show the distribution of T-statistics from 1000 bootstraps of *T*-tests applied to a random sample of the same number of loci. Dotted lines denote *T*-values = 0, and solid red points show significant *T*-test comparisons ($p < 0.05$). (D) Conceptual figure showing the expected distributions of relative differences in allele frequencies under the two scenarios shown in (A). (E) Comparisons of observed median residual values from linear regression models between temperature metrics and identified outlier loci (red squares) and the distributions of median residuals from regressions of the same temperature variables from 1000 randomly selected sets of loci (boxplots).

locations when considering the invasive and source populations ($F = 36.68$, df = 6, $p < 0.001$). Tukey post hoc tests showed that the significant differences were due to comparisons between HM and RB and all other locations, with lower heterozygosity in these two populations (Figure 4B).

3.4 | Genotype–Environment Associations

Annual mean and maximum SSTs were significantly associated ($p < 0.01$) with AF in Atlantic populations in the complete RDA when using stepwise ordination. For all downstream pRDA, all four of the SST variables (annual mean SST, annual minimum SST, annual maximum SST and annual range of SSTs) were retained. The full pRDA (*pRDA_full*) containing the four SST variables, population structure (as principal components) and geographic distance (as db-MEMs) explained approximately 90% of the genetic variation across the Atlantic populations (Figure 5 and Table S7). Despite the overall model explaining such a high proportion of the variation in genotypes, 64% of this total variation was unable to be partitioned into the explanatory variables, suggesting that the variables were confounded. Outputs from the *pRDA_full* showed that from the variation that could be partitioned, the southern New England and mid-Atlantic populations separated out based on genetic structure (PC2), while the south Atlantic populations were defined by their association with minimum and mean SSTs, as well as geographic distance (MEM1 and MEM2; Figure 5). Genetic structure (PC1 and PC2) was also the major driver of difference in the northern New England populations.

When we accounted for genetic structure and geographic distance in a pRDA (*pRDA_clim*), the four climate variables explained approximately 17% of the remaining variation, but this association was not significant ($p = 0.299$). Neither of the models that examined the genetic structure (*pRDA_struc*) or geographic distance (*pRDA_geo*) showed significant associations with the variance in genetic variation either. These results suggest that while the RDA was effective at explaining most of the variance in data, it is unable to disentangle SST correlations from

genetic structure and geographic distance, as expected given that both variables correlate with temperature across the latitudinal cline of the Atlantic coast. However, when considering the SST-driven pRDA (*pRDA_clim*), we identified 189 loci as outliers when using individual RDA axis loadings (greater than 3 SD from the mean). Of these outlier loci, 51 were associated with annual maximum SST, 37 with annual mean SST, 42 with annual minimum SST and 53 with the annual range of SSTs (Figure 5B,C). Additionally, the LFMM analyses identified a total of 1187 loci associated with the four climate variables (FDR adjusted $p < 0.001$), with 56, 267, 515 and 349 identified for minimum, mean, maximum and range of annual SSTs, respectively. A total of 82 loci overlapped between the RDA and LFMM analyses across the native range (Figure S6), and these were used for downstream analyses for predicting allelic shifts in the invasive populations.

Ordination analyses determined that there was no significant association between AF and geographic distance within the invasive range, with no db-MEMs incorporated into the RDA. The full RDA for the invasive range was overall not significant, and the adjusted R^2 was estimated at 0.2. In contrast to the RDA in the native range, the RDA analysis for only the invasive populations yielded no significant associations between the loci and any of the SST metrics (Figure S8). In comparison, the LFMM analysis identified 3008 loci associated with at least one of the four SST metrics, of which 13 overlapped with the 82 loci identified in the native range (Table S10 and Figure S7).

3.5 | Detecting Evidence of Rapid Adaptation in New Environmental Conditions

Thermal environments for the parameters included in the RDA and LFMM analyses were substantially different in the invasive populations to those at the source locations (Figure 6B). However, we did not observe significant differences in AF between source and invasive populations for the 82 loci identified by both RDA and LFMM analyses as putatively adaptive across the native range, with the exception of loci associated with the annual range of SSTs between CP and CT (Table S9).

Furthermore, when considering differences between observed AF in the invasive populations and those predicted through regressions for loci associated with mean SSTs, median residuals in the outlier loci were generally within the distributions generated through bootstrapping random loci (Figure 6C). For loci associated with maximum SSTs, observed median residuals were typically at the upper end of the distributions, with values higher than those generated by the random loci for WP, TO and RB. This observation was further exaggerated in the range of annual SSTs, likely due to the SST range in the invasive populations falling outside of the range of values used to inform the regression (Figure 6A). As only one locus was identified to be associated with the minimum SST through the LFMM and RDA analyses, we were unable to conduct these tests on this variable. These results suggest that AF for these loci in the introduced populations have not shifted predictably towards values expected based on regressions generated across the thermal conditions in the native range.

4 | Discussion

Understanding the mechanisms that shape organismal capacity to proliferate in new environments is key to predicting biological invasions and species' responses to climate change. While rapid adaptation is one possible avenue for resilience to changing environments, whether it occurs in newly established populations may depend on factors such as selection pressures, the amount of standing genetic variation, existing phenotypic plasticity and the demographic history of the founding individuals. Coupling population genomics with environmental data informed by prior knowledge of phenotypic adaptive divergence (Villeneuve, Komoroske, and Cheng 2021a, 2021b), we demonstrate that invasive populations of *Urosalpinx* on the US Pacific coast were founded by individuals from southern New England, and evidence that this species exhibits local thermal adaptation across their native range. Although some invasive populations have undergone evolutionary divergence from their source since establishment, we did not detect evidence that selection on temperature-associated loci drove rapid adaptation to their new thermal environments. Alternatively, the widespread invasion success of *Urosalpinx*, despite originating from a strikingly divergent native environment, may be driven by other processes, including relaxed selection on putatively adaptive loci in invasive habitats and/or capacity for phenotypic plasticity (Villeneuve, Komoroske, and Cheng 2021a). Thus, while rapid genetic adaptation may contribute to species' resilience to new and changing environments in some contexts, our work indicates that other factors likely play key roles in population establishment and persistence in real-world environments.

4.1 | Strong Biogeographic Genetic Breaks Across Native Range

The patterns of genetic divergence we observed across the native range of *Urosalpinx* are concordant with expectations based on their life history (i.e., direct development limiting dispersal ranges) and oceanographic drivers, with a high correlation between genetic and geographic distances and strong breaks between regions around Cape Cod and Cape Hatteras (Figure 1B). Such strong IBD and genetic breaks across these two biogeographical features align with studies of other marine taxa, including those with high dispersal capabilities (Bert et al. 2011; Orth et al. 2020; Ropp et al. 2023; Wilbur et al. 2005; Wilder et al. 2020). This reinforces the premise that local adaptation can occur in marine environments, in contrast with historic assumptions that these are open, homogenised systems (Palumbi 1992). Our results of strong genetic structuring in a species with crawl-away larvae contrast with prior studies in the region which found low genetic differentiation between populations, including marine snails (*Nucella lapillus:* Colson and Hughes 2007; *Littorina obtusata:* Schmidt et al. 2007), although it is unclear if coarse marker resolution influenced these results. A more recent study with genome-wide SNPs in *N. lapillus* found evidence for clade splits around the major biogeographic breaks and differentiation in putatively functional loci, but little neutral structure within regions (Chu et al. 2014) as we found for *Urosalpinx*. Unlike *N.*

lapillus, which inhabit open coastal environments and may be successful at dispersing through rafting within geographical regions (Colson and Hughes 2004, 2007), *Urosalpinx* inhabits patchy estuarine habitats that may constrain rafting or other dispersal mechanisms. This is also supported by the evidence for fine-scale genetic differentiation between locations within regions (Figure S4F–I), including some sites with close geographic proximity to one another (e.g., Connecticut and Massachusetts), suggesting very limited genetic connectivity between populations even at small geographic scales.

We also observed very strong genetic divergence between the northern New England sites (DM and GB) and the other regions, including those in southern New England that are close geographically. Two potential hypotheses have been previously proposed for the origin of northern New England populations: (1) they represent remnant populations of a continuous distribution following Pleistocene glaciation events, or (2) they were recently founded via artificial introduction with oyster farming activities that have since diverged from their source. Our data strongly support the first hypothesis because the strikingly strong genetic divergence of these populations between other regions and each other (Figures 1 and S4A) is aligned with genetic isolation for a much greater extended period due to biogeographical constraints and glaciation. Additionally, the divergence between northern New England locations and candidate source populations to the south is much greater than those between Pacific invasive populations and their source populations in southern New England, which would have occurred on roughly the same timescale as those theoretically established under the second hypothesis. However, it is also possible that the pronounced genetic signature in the northern New England populations is a consequence of an older nonhuman–mediated expansion of *Urosalpinx* back into the area following glaciation events.

4.2 | Reconstructing the Invasion History of Pacific Coast Populations

Our analyses clearly identified southern New England (i.e., CT and WH) as the region of origin of the invasive *Urosalpinx* populations across the US Pacific coast. This finding broadly aligns with historical records indicating that following the completion of the transcontinental railway in 1869, large volumes of eastern oysters and associated 'hitchhiker' species (Carlton 1979) were transported to the New England region from the Chesapeake Bay area for augmentation, before being further translocated to the Pacific coast (Hoos et al. 2010; Miller 2000). The augmentation of New England populations from Chesapeake Bay and subsequent Pacific translocations were reported to continue over the 19th and 20th centuries. Interestingly, although *Urosalpinx* from the Chesapeake Bay outer coastal lagoons (OY) and Delaware Bay (DB) were more similar to southern New England populations than other Atlantic coast populations, they still formed a distinctive mid-Atlantic genetic cluster and were not identified as the origin for any of the invasive populations examined in our assignment analyses (with the exception of one sample from RB). If historical records are accurate and southern New England oyster populations were repeatedly augmented from the mid-Atlantic region, the observed contemporary genetic divergence between these two regions may be explained by recent divergences following the cessation of augmentations, or by the maintenance of unique historical haplotypes (Hoos et al. 2010). Furthermore, the observed genetic divergence between the southern New England populations and the mid-Atlantic region despite historic augmentations may also be due to population perturbations and reductions in this area. For example, a 1969 oil spill in Wild Harbour, Massachusetts, decimated populations around the Cape Cod region, followed by recolonisation of these sites (Cole 1978), although our analyses did not detect any reductions in heterozygosity that may indicate genetic bottlenecks in these populations. While we cannot discount the possibility that invasive Pacific populations originated from some other specific location that we failed to sample in this study, our results provide strong evidence that southern New England represents their most likely source region.

Despite our results suggesting that the invasive populations of *Urosalpinx* on the US Pacific coast originated from the same source region, they also suggest that key differences in introduction histories have likely influenced evolutionary and demographic trajectories. Functional sustained gene flow between the US Atlantic and Pacific coasts purportedly ceased almost 100 years ago, yet we only detected evidence for strong genetic differentiation from source populations in the Humboldt and Richardson Bay (RB) populations. In contrast, although we did observe genetic differentiation between all other invasive and source populations (Figure S4C–E), pairwise F_{ST} were much lower and similar to each other, suggesting larger population sizes and/or more recent isolation between these locations. Humboldt and Richardson Bay were also the only invasive populations that displayed signals of genetic drift and reduced heterozygosity which are characteristic of genetic bottlenecks and founding effects (Dlugosch and Parker 2008). Although biological invasions are often presumed to be founded by small numbers of individuals that drive increased rates of genetic drift and reduced genetic diversity (Estoup et al. 2016), scenarios of high levels of persistent propagule pressure over time can also occur, reducing these effects, as can incidents of high levels of sperm storage, which may result in increased genetic diversity within founding populations (Rafajlović et al. 2013). Although we cannot demonstrate conclusively, our data support historic information that continued introductions over many decades may have resulted in overall large founding populations at locations that were the primary recipients of translocations (Willapa, Tomales Bay and Coyote Point), while others were the consequence of more isolated introduction events and small founding populations (Humboldt and Richardson Bays). Interestingly, anecdotal observations indicated that the Humboldt Bay population 'no longer persisted' in the early 2000s, yet individuals were subsequently collected in 2002 (Fofonoff et al. 2023) and again for this study in 2019. While we lack sufficient data to draw clear conclusions, it is possible that the size of this population has remained low enough to evade consistent detection of individuals over time, potentially influenced by the lower genetic diversity and inbreeding effects on fitness that can contribute to population declines (Keller and Waller 2002).

4.3 | Adaptive Divergence and Invasion Success

Combined with prior knowledge of local adaptation in phenotypic traits in *Urosalpinx* (Villeneuve, Komoroske, and Cheng 2021b), coupling invasion and seascape genomics provided an ideal opportunity to examine the genomic underpinnings of adaptive divergence across their native range and detect rapid adaptation to new environments within the invasive populations. We found a total of 82 loci associated with key temperature variables in the native range, suggesting that thermal adaptation is likely playing a critical role in shaping the genetic composition of native populations of *Urosalpinx*. Across this range, over 60% of the putatively adaptive loci were associated with annual maximum SSTs and the range between the minimum and maximum annual SSTs, indicating that upper thermal limits and the ability to tolerate temperature fluctuations are likely under the strongest selection pressures. These results align with observations that maximum habitat temperature best explains variation in thermal tolerance in *Urosalpinx* (Villeneuve, Komoroske, and Cheng 2021a), however, the trade-offs for evolving and maintaining such capacity are not well understood. Season length has also been found to be a key environmental driver of countergradient variation in growth performance in *Urosalpinx* (Villeneuve, Komoroske, and Cheng 2021b), but was not included in our final models because of high correlations with other environmental variables (Figure S3). Thus, it is possible that some of our putatively adaptive loci actually reflect selection pressures from season length or other correlated environmental parameters, but further studies are needed to determine if the loci identified here are involved in local adaptation in growth performance. Similar patterns of countergradient variation in growth have been observed in a variety of other taxa (Conover and Schultz 1995; Conover, Duffy, and Hice 2009), and recent work has identified heritable genomic variation underlying evolutionary responses in growth rates in other species (Therkildsen et al. 2019). Given these findings, and that climate change is forecasted to strongly alter seasonality and related selection pressures for many organisms, future research examining the underlying genomics of countergradient variation in growth performance in *Urosalpinx* and other species would be a valuable next step to advance our understanding of parallel evolution and biological responses to climate change.

The role of rapid adaptation in population persistence in new environments has long been a topic of interest in evolutionary biology, with more recent relevance for understanding and mitigating the impacts of anthropogenic global change. Given the presence of putative environmentally adaptive loci across the native range of *Urosalpinx* and no loss of genetic diversity in most of the established invasive populations, we hypothesised that we would observe allele frequency shifts at these loci as would be predicted by the thermal conditions in their new environments. If observed, this would align with expectations of genomic vulnerability studies (i.e., where adaptive divergence identified across landscapes is used to estimate the evolutionary rates required to keep pace with projected environmental change and the likelihood of future population persistence; Bay et al. 2018). Instead, we found no evidence of rapid adaptation at the putatively thermally adapted loci in the Pacific coast populations of *Urosalpinx*, yet this species has clearly persisted and thrived as invasive species in this region. Importantly, our results do not imply that rapid adaptation cannot facilitate success in new environments or contradict predictions of population persistence based on genomic vulnerability, but rather emphasise that other mechanisms and cofactors may be important to consider (Lotterhos 2024).

We hypothesise that we did not detect evidence of rapid adaptation in the invasive populations due to: (1) relaxed selection following introduction with no costs to maintain plasticity and/or high costs for adaptation, and/or (2) unique responses to environmental selection due to genetic redundancy. First, the apparent lack of adaptive response may be due to a relaxation of selection on thermally linked loci in the new environment with no or limited costs to maintain plasticity. Most studies on genomic vulnerability focus on predictions in a warmer climate that would involve directional selection to a higher thermal optimum. In contrast, while Pacific coast locations have higher mean SST and substantially different overall thermal environments relative to source locations (Figure 6B), other thermal conditions are less extreme (i.e., SST maximum, minimum and range) and most of the putatively adaptive loci we identified were associated with these environmental parameters. For example, in southern New England, SSTs fall to just above freezing during the winter months (~1°C–3°C) and rise to greater than 22°C during the summer. Given the seasonal reproduction of *Urosalpinx*, individuals must cope with these fluctuating conditions to successfully reach maturity and reproduce, likely favouring the evolution of standing capacity for high thermal phenotypic plasticity in source populations. Our predictions that allele frequencies would shift towards those that are optimal in the new environments were grounded in the assumptions that there are physiological or ecological costs to maintaining plasticity to tolerate more extreme conditions than are required and that the benefits of adapting to the new optimum outweigh the costs of doing so. However, if these trade-offs do not occur and/or do not translate to fitness differences, then selection would not result in adaptation in the invaded range. Furthermore, if there are little fitness costs associated with maintaining capacity for high phenotypic plasticity even when it is not needed, it may prime them for establishing in new environments, facilitating invasion without the need for genetic adaptation (Hendry 2016). Finally, given that thermal traits can be polygenic and loci may have complex interactions (Lotterhos 2023), it is possible that population persistence was not associated with predicted allele frequency shifts in putatively adaptive loci due to genetic redundancies or other processes (e.g., antagonistic pleiotropy or conditional neutrality that may impose different selection pressures in new environments). The interactions of phenotypic plasticity and genetic redundancies with genetic adaptation in both biological invasions and population persistence under climate change are active areas of study, for which future work delving further into these relationships in *Urosalpinx* could be highly informative.

Finally, we acknowledge limitations of our approach. First, while we did not detect evidence of adaptation in invasive populations at thermal-associated loci identified in the native range, genetic adaptation may have occurred in invasive populations at other loci (e.g., those associated with environmental variables other than temperature). Additionally, it is possible

that our results were influenced by the reduced representation methods we employed, which can miss important functional regions under selection. Given that we identified a number of loci significantly associated with temperature, future research utilising an exome capture or whole-genome approach (Wilder et al. 2020) that explicitly includes functional genomic regions would provide additional insight into the presence and mechanisms of rapid adaptation in invasive populations.

5 | Conclusion

The mechanisms driving population establishment and persistence in new environments are complex, and the relative contributions of key processes like phenotypic plasticity, preadaptation and rapid evolution are dependent on important contextual factors such as life history, demographic history and environmental selection pressures. Combining invasion and seascape genomics approaches to reconstruct the invasion history and test predictions of rapid adaptation in new environments, we demonstrate that *Urosalpinx* has likely successfully proliferated on the Pacific coast without evidence of rapid thermal adaptation. We suggest that relaxed selection and/or the maintenance of standing genetic variation for phenotypic plasticity in thermal traits may play important roles in their success and identify several key areas for future study. Our work highlights the utility of invasive species for understanding evolutionary responses to changing environments, and the importance of considering population history and environmental selection pressures when evaluating adaptive capacity.

Author Contributions

B.P.B., L.M.K., A.R.V., R.S.B. and B.S.C. conceived the study; B.S.C., L.M.K. and A.R.V. collected the samples; J.D.S. and J.L.A. performed laboratory work; B.P.B., L.M.K., J.D.S., and R.S.B. performed data analysis; B.P.B. and L.M.K. wrote the manuscript; all authors edited and reviewed the article. All authors approved the current version of the article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data and Code Availability

Raw demultiplexed RAD-Seq reads and associated metadata are available on NCBI's short read archive (BioProject: PRJNA1168071) and related scripts are available at GitHub (https://github.com/bpbentley/project_uro_RADSeq).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.