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RESEARCH

Landscape connectivity and genetic structure of animal populations in urban ponds

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Abstract

Animal populations in urban landscapes tend to have lower genetic diversity and higher genetic differentiation than those in rural landscapes. The extent of these effects can be influenced by the dispersal rates of the organisms and the connectivity of the landscape. Here, we explore associations of landscape connectivity with genetic diversity and genetic differentiation in urban ponds in the City of Stockholm, Sweden. Our focus is on three invertebrate species and one vertebrate species, each with different dispersal rates and life-history traits. We sampled 30 ponds and collected genetic data by using double-digest restriction-site associated DNA sequencing (ddRADseq). Our results showed moderate differences in genetic diversity among populations for all species. Additionally, all populations showed a heterozygote deficiency, suggesting inbreeding. We found significant genetic structure among populations for the three species categorized as low to intermediate dispersers: *Asellus aquaticus* (Isopoda), *Planorbis planorbis* (Gastropoda) and, *Rana temporaria* (Amphibia). In contrast, the species with presumably the highest relative dispersal capacity, *Haliplus ruficollis* (Coleoptera), exhibited no significant population structure. Furthermore, genetic differentiation in *A. aquaticus* and *P. planorbis* was significantly correlated with geographic distance. For *A. aquaticus*, genetic differentiation was also significantly correlated with landscape connectivity across both aquatic (blue) and terrestrial (green) environmental features. Our results highlight the role of landscape connectivity and dispersal ability of species in shaping genetic structure among urban ponds.

Keywords Urban biodiversity · Genetic diversity · Genetic differentiation · ddRADseq · Landscape connectivity · Dispersal

Introduction

Urbanization is a major factor affecting the current decline in biodiversity, primarily due to habitat change, loss, and fragmentation (Goddard et al. 2010; UN 2019). As a consequence, urban areas often exhibit higher environmental heterogeneity at smaller spatial scales compared to rural areas (Savard et al. 2000). The negative impact of urbanization on biodiversity can be mitigated by blue-green spaces, which can serve as biodiversity hotspots by providing habitat and dispersal possibilities (Pautasso et al. 2011; Ives et al. 2016; Keinath et al. 2023).

Urban ponds, whether artificial or natural, along with the surrounding terrestrial environments, are examples of blue-green spaces renowned for supporting high biodiversity (Biggs et al. 2005; Goertzen and Suhling 2013; Hassall 2014; Blicharska and Johansson 2016; Johansson et al. 2019). Ponds are thus valuable for conserving biodiversity in urban environments. The capacity of urban ponds to support biodiversity is influenced by various environmental factors, including pond depth, size, and vegetation (Goertzen and Suhling 2013; Hassall and Anderson 2015; Heino et al. 2017a; Hill et al. 2018). Pond biodiversity is also negatively affected by land use such as built-up areas (i.e., artificial surfaces; Heino et al. 2017a). In addition to these environmental variables, high landscape connectivity positively affects biodiversity by facilitating the dispersal of organisms between suitable habitats (Hyseni et al. 2021). Finally, species diversity tends to be maximized when the constituent

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species have intermediate dispersal rates (Cadotte 2006). While we have knowledge on how environmental variables affect species diversity in urban ponds, our knowledge on the genetic diversity patterns in urban ponds is still limited (but see Babik et al. 2023).

Like species diversity, genetic diversity within species can be considered a measure of biodiversity (Hoban et al. 2022). The processes that influence genetic diversity are often similar to those underlying species diversity. Connectivity and environmental heterogeneity affect dispersal and gene flow, demographic stochasticity, and genetic drift, as well as selection, which simultaneously influence patterns of species and genetic diversity. Knowledge about genetic diversity is important because it is a key factor affecting adaptation and evolution to environmental change in urban landscapes (Fusco et al. 2021a). Habitat loss or fragmentation in urban areas has the potential to impede dispersal and reduce gene flow, leading to genetic isolation of populations. This isolation increases the likelihood of inbreeding, which leads to a loss of genetic diversity due to genetic drift (Montgomery et al. 2000; Frankham et al. 2010), ultimately raising the risk of extinction. Studies on genetic diversity in urban areas have reported a decline in genetic variation and an increase in genetic differentiation among populations in a variety of organisms (Desender et al 2005; Gortat et al. 2012; Rochat et al. 2017; Brav-Cubitt et al. 2022). Small populations are even more vulnerable to fragmentation (Frankham 1996). For instance, species with limited dispersal capability and small population size, occurring in urban areas, such as the red-backed salamander (*Plethodon cinereus*) (Noël et al. 2007) and the Panama City crayfish (*Procambarus econfinae*) (Duncan et al. 2020), exhibit decreased genetic diversity and noticeable genetic divergence when found in fragmented urban environments. In order to contribute to new insights and improve knowledge of patterns of genetic diversity and genetic divergence in urban areas, we studied how these variables were associated with connectivity in an urban pond system.

Genetic diversity and genetic divergence between populations are affected by geographic distance (Eckert et al 2008; Sexton et al. 2014; Hoban et al. 2022). Traditional methods to estimate geographic distance among patches of fragmented habitats rely on the shortest distance between these patches. However, these geographic distance estimates may miss the main dispersal routes (Cañedo-Argüelles et al. 2015; Kärnä et al. 2015). The reason is that animals and plants may disperse through suitable habitats, referred to as functional connectivity (Baguette and Van Dyck 2007), rather than through the shortest path between two points (Heino et al. 2017b). There are several methods available to estimate functional connectivity. One is electrical circuit theory which has been applied in diverse fields, including conservation, ecology, epidemiology, and evolutionary biology (Dickson et al. 2019). It treats landscapes as resistance surfaces, assigning higher values to areas that hinder movement (McRae et al. 2008; Spear et al. 2010). This method has been used to explore patterns of species diversity and connectivity in urban pond metacommunities (Hyseni et al. 2021). However, no studies have looked at the influence of landscape connectivity on genetic diversity patterns across multiple species in urban pond metacommunities. The present study fills this gap.

The aim of this study was to test how genetic diversity is affected by connectivity. We did this by examining four aquatic species with varying dispersal capability—ranging from weak to strong dispersers—that inhabit urban ponds in the Stockholm region of Sweden. Genetic diversity was estimated using data obtained through double digest restriction-site associated DNA sequencing (ddRADseq). We predicted that weak dispersers would be characterized by strong genetic structure and lower genetic diversity compared to strong dispersers. We also predicted a positive correlation between genetic diversity and functional connectivity.

Material and methods

Sampling

Sampling was conducted in September 2020 at 30 ponds (Fig. 1) in the metropolitan area of Stockholm, Sweden. To estimate genetic diversity and structure in urban pond metacommunities, we collected data for three invertebrate species: *Asellus aquaticus* (Isopoda), *Haliplus ruficollis* (Coleoptera), *Planorbis planorbis* (Gastropoda). We also included a vertebrate species, *Rana temporaria* (Amphibia). All these species are hereafter referred to by the name of their genus. The extent of the study area covered the entire metropolitan area, yet the focus was on densely populated areas. The city center itself has ca. 900000 inhabitants, with another 600000 living in the suburbs of the Stockholm metropolitan area. The ponds studied consisted of a range of natural and human-made water bodies, retaining water for at least four months, with pond areas varying from 2 to 20000 square meters. The invertebrates were collected with an aquatic hand net and *Rana* specimens were sampled by collecting eggs from clutches of frogs at the shoreline of ponds. One egg was collected from each clutch, each representing eggs from a single female. Unfortunately, not all species could be sampled from all ponds (Table S1). *Haliplus* was classified as having a high dispersal capacity due to its fully developed wings, allowing it to fly between aquatic habitats (Boda et al. 2014), whereas *Rana* was classified as a species with low dispersal capacity, given the generally limited dispersal capability of anurans (Sinsch 1990). The remaining two taxa were designated as having intermediate dispersal

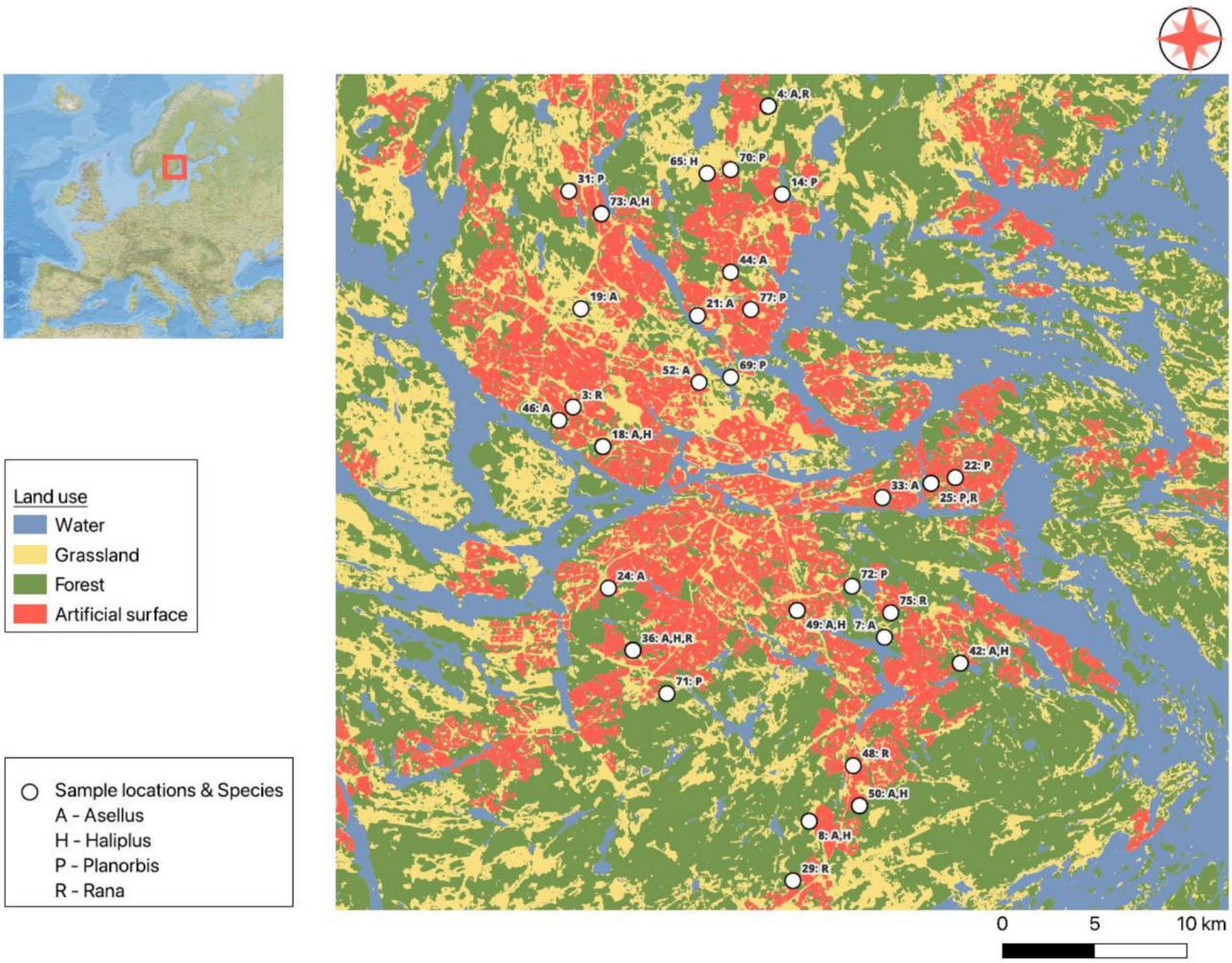

Fig. 1 Sampling locations for all four focal species. The numbers are the codes for each location and the letters represent the species sampled. The sampling pond locations in Stockholm were mapped in Quantum Geographic Information System (QGIS). The Stockholm map is based on Background map: Property map Land data, coordinatesystem: EPSG:3006, Lantmäteriet, and the Europe map was retrieved from Retrieved from: https://server.arcgisonline.com/arcgis/rest/services/NatGeo_World_Map/MapServer/tile/%7Bz%7D/%7By%7D/%7Bx%7D

capabilities. These two taxa were classified as having a higher dispersal than frogs because waterbirds are known to be important dispersal vectors of invertebrates (Green et al. 2023). *Asellus* were collected from 30 ponds (n = 360 individuals), *Haliplus* from 12 ponds (n = 105), *Planorbis* from 13 ponds (n = 126), and *Rana* from 8 ponds (n = 66). However, after ddRADseq sequencing, some samples were excluded due to quality issues. The final number of samples used for each pond in subsequent statistical analyses is shown in Table S1.

Library preparation and ddRAD-sequencing

We made ddRAD sequencing libraries for 657 samples by modifying the protocols from Peterson et al. (2012) and incorporating aspects of the methylation-sensitive ddRAD method (Marconi et al. 2019; Di Marsico et al. 2020). Genomic DNA was extracted using Till et al.'s (2015) salting-out method, optimized for high-throughput in 2 mL 96-well plates, with reduced volumes of DNA binding buffer, lysis buffer, and sodium acetate (400 uL, 500 uL, and 150 uL, respectively). After overnight lysis and centrifugation at 1500 × g for 10 min, each DNA library underwent double digestion with restriction enzyme combinations (AciI + MseI and PstI + MseI).

We then ligated a sample-specific barcoded adapter to the methylation-sensitive restriction end, and a common Y adapter to the MseI sticky end. For either of the AciI and PstI enzymes the protocol was as follows: (1) restriction digestion: Genomic DNA was treated with 5 units of either AciI or PstI, and 5 units of MseI (New England BioLabs), (2) adapter ligation: Digested DNA was ligated to 0.2 µM

of unique barcoded adapters (barcodes by individual; see Supplementary Material) and 0.2 µM of common Y adapter (MseI) using 1 unit of T4 DNA ligase (ThermoFisher), 0.2 mM ATP and 1 × buffer for a final volume of 50 µL.

The libraries were pooled as per the experimental design, purified and size selected by magnetic beads (Sera-Mag SpeedBeads; Cytiva) and E-gel electrophoresis (Invitrogen) for fragments in the range of 250 to 600 bp. Size-selected libraries were quantified using a fluorometer (Qubit; Life Technologies), and a normalized DNA amount (15 ng) was amplified with a primer that introduced an Illumina index (at the Y common adapter site) for demultiplexing by sampling site. Following PCR with uniquely indexed primers, multiple samples were pooled. PCR-enrichment was performed as described by Peterson et al. (2012). Specifically, 15 ng of pooled DNA was amplified using 1 U of Phusion High-Fidelity DNA polymerase (New England BioLabs) in a final volume of 50 µL containing 1X Phusion HF Buffer, 0.2 µM of AciI/PstI PCR primer, 0.2 µM MseI index primer, and 0.2 mM dNTPs (0.05 mM each dNTP). Amplified libraries were purified with magnetic beads and then quantified (Qubit, Invitrogen; and Bioanalyzer, Agilent Technologies). The grouped libraries were pooled in an equimolar fashion, and the final library was Illumina-sequenced using 150-bp paired-end chemistry.

The libraries were sequenced in two lanes on an Illumina Novaseq 6000 machine from both directions (2 × 150 bp) at SciLifeLab, Uppsala, Sweden. Trimming of adapters/primers and demultiplexing of the raw data were performed using CUTADAPT v4.0 (Martin 2011) and fastq_demux, respectively. The demultiplexed data was archived in the NCBI Sequence Read Archive (BioProject ID: PRJNA1026545), link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1026545?reviewer=k77s47nm26he873ahrn69s8jsl).

Data filtering and SNP calling

In the data processing phase, we initiated the removal of adapters and primers, as well as the demultiplexing of raw data, employing CUTADAPT v4.0 (Martin 2011) and fastq_demux, respectively. All reads were uniformly trimmed to a length of 100 bp, and reads with a quality score lower than phred33 were discarded using trimmomatic v 0.39 (Bolger et al. 2014). For SNP calling, we utilized the de novo pipeline within STACKS v.2.62 (Catchen et al. 2011), executing each step of the pipeline (ustacks, cstacks, sstacks, tsv2bam, and gstacks) individually. Across all species, we set the parameter "m" to 3, as it proved suitable for most datasets (Rochette and Catchen 2017). An initial parameter optimization for "M" and "n" was carried out on a randomly selected subset of 16 individuals for each species, following the approach described by Paris et al. (2017). We opted to use "4" for both "M" and "n" parameters across all species except *Haliplus* for which we used 3 (Fig S1 in Supplementary Material). The de novo pipeline was executed separately for each species on all samples using these parameters, along with default settings. Further processing of the catalog was performed utilizing the populations module in STACKS, initially configured with an "R60" setup, where a locus was retained if it appeared in at least 60% of individuals. Additionally, we set a minimum minor allele frequency of 0.05, a maximum observed heterozygosity of 0.7, and selected one random SNP per locus. After removing samples with over 50% missing data for each species, the populations step was iterated with various "R" setups. The goal was to identify an "R" value that would maximize the number of individuals while maintaining an optimal number of loci. We employed "R75" for *Asellus* and *Haliplus*, and "R80" for *Planorbis* and *Rana*. Subsequently, a final dataset for subsequent analyses was derived after excluding samples with one-third missing data. The final dataset for *Asellus* comprised 126 individuals from 16 populations (1336 genetic loci for the entire sample), while *H. ruficollis* consisted of 45 individuals in 9 populations (1179 genetic loci). For *Planorbis* we collected genetic data for a total of 61 individuals across 10 populations (a total of 1922 loci), while for *Rana* we had 40 individuals in 6 populations (1422 loci). On average, the proportion of missing genotypes per SNP was 5.1% for *Asellus*, 9.4% for *Haliplus*, 9.4% for *Planorbis*, and 12.3% for *Rana*, indicating moderate levels of missing data across species. (Please refer to Table S1 for complete sample size information by site and species).

Data analyses

We calculated the following molecular diversity indices: Observed heterozygosity (HO), expected heterozygosity (HE), and the inbreeding coefficient (*F*is) for each population for each species in STACKS' *populations* module. We used the same package to assess genetic diversity using nucleotide diversity (π) and polymorphic nucleotide sites within each population for each species. We chose to focus on π due to a positive correlation between the two estimates for each species, as indicated by Pearson correlation analysis (data not presented). We tested for deviation from Hardy–Weinberg equilibrium for each population using Genepop 4.8.3 (Raymond and Rousset 1995).

A matrix of pairwise genetic distance between individuals was prepared based on Manhattan distances (proportion of alleles shared) for each species to represent genetic differentiation. This matrix was used to test the overall global genetic structure of each species using Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) implemented in the PERMANOVA + add on (Anderson et al. 2008) for add-on in PRIMER-E v.7 (Anderson 2008). PERMDISP (Anderson 2006) in PERMANOVA + was

employed to assess whether the within-group dispersions among populations followed a null hypothesis of homogeneity for the species that PERMANOVA analyses indicated significant population structure. For the species that a global population structure and deviation of within-group dispersion were detected, we performed a pairwise PERMANOVA and PERMDISP analyses between the populations. Additionally, the fixation index F_{ST} (Weir and Cockerham 1984), computed in Arlequin 3.5 (Excoffier et al. 2005), was used to investigate genetic structure. All permutation tests were performed with 9999 permutations. A False Discovery Rate (FDR) correction was applied for multiple testing for pairwise comparisons on the website https://tools.carbocation.com/. We visualized the distance matrix using Principal Component Analysis (PCA) with the package *pcadapt* (Luu et al. 2017).

Correlation between genetic distance and landscape connectivity

To understand whether genetic similarity is influenced by landscape barriers to movement, we used a two-step approach. The first step involves modeling landscape connectivity using the Omniscape algorithm (Landau et al. 2021) This algorithm combines circuit theoretic methods with spatial data to understand how species move across landscapes (analogous to electrical current). In the second step, we determined the relationship between genetic distances and topographic distances. The topographic distance matrices were calculated from Omniscape-computed current flow; then the correlation between genetic and topographic distances were assessed for the different species.

Using the Omniscape algorithm implemented in the Julia programming language, we modeled omni-directional connectivity (cf. pairwise connectivity, as implemented in Circuitscape). Omniscape combines circuit theoretic methods with continuous spatial data to model connectivity. It allows sources, destinations, and movement intensity to be informed by spatial data. For the latter, we used resistance surfaces (barriers to movement) parameterized for entire metacommunities in this region in a previous study (Hyseni et al. 2021). We used a composite blue-green resistance surface, optimized using the automated solution implemented in the 'resistanceGA' package in R (Peterman 2018). 'ResistanceGA' allows for the optimization of multiple resistance surfaces to create a composite resistance surface. The resistanceGA model automatically assigns resistance values to landscape features based on their potential to impede or facilitate dispersal. Distances from water bodies were used as landscape features representing the blue part of the blue-green composite, while the green portion of the composite was derived from the S2GLC land cover map. We used block and buffer sizes of 2 and 1, respectively. We performed Omniscape modeling with two different radius sizes, 50 and 100 (i.e. 5 and 10 km), and the parameterized resistance surfaces scored in two different ways: Maximum resistance of 50 and 100.

We then calculated topographic paths and distances using the topoDistance R package (Wang 2020), which quantifies movement across a landscape based on topographic features (i.e., elevation). However, instead of using elevation, we used cumulative current flow (i.e., total current flowing through the landscape) obtained from Omniscape. We computed topographic distances for each species using two functions: *topoDist*, which calculates distances along shortest topographic paths and *topoWeightedDist*, which considers weighted topographic paths (exponential weights for current flow across the landscape).

Finally, for each species, we tested the correlation between genetic distance and the eight topographic distance matrices (2 values for maximum resistance X 2 values for radius X unweighted and weighted topographic distances) using the Mantel test (Mantel 1967). The function mantel from the R package vegan (R Development Core team 2022) was used for this task. The genetic distance between each pair of populations was estimated in PERMANOVA, where it was calculated as the distance between the centroids of each population, based on Manhattan distances. To test for correlation between genetic distances and distance matrices capturing blue-green connectivity, we used exponentially weighted shortest paths based on current flow across the landscape. The "Moran spectral randomization" method (Wagner and Dray 2015) was used to compute p-values for blue-green connectivity models by generating spatially-constrained random variables preserving global autocorrelation (Moran's I) and spatial structures at multiple scales.

Results

Genetic diversity

Molecular diversity indices for each population for each species are presented in Table S1. Values of π ranged between 0.175 and 0.221 in *Asellus*, between 0.111 and 0.253 in *Haliplus*, between 0.090 and 0.170 in *Planorbis*, and between 0.128 and 0.367 in *Rana*. *F*is values ranged between 0.16 and 0.26 for *Asellus,* 0.06 and 0.37 in *Haliplus.,* 0.02 and 0.12 in *Planorbis,* and between 0.05 and 0.52 in *Rana. H*O was in general lower than *H*E in all species (Table S1), supporting high *Fis* values and suggesting low genetic variation. All of the populations showed heterozygosity deficiency ($P < 0.001$) for all species suggesting inbreeding.

A closer look at individual ponds and their π and *Fis* values, at the species level suggests a moderate difference in genetic diversity between ponds within species.

Genetic structure

PERMANOVA revealed a significant genetic differentiation among populations for three of the species investigated: *Asellus* ($F_{15, 125} = 3.02$, $P < 0.001$), *Planorbis* ($F_{9, 60} = 5.62$, $P < 0.001$), and *Rana* ($F_{5, 39} = 5.64$, $P < 0.001$). Notably, *Haliplus* did not exhibit a significant population structure ($F_{8, 44} = 1.20$, $P = 0.25$). Complementing these findings, the analysis of population structure using F_{ST} yielded consistent results. For *Asellus*, F_{ST} values ranged from 0.007 to 0.18, with the majority of pairwise comparisons (115 out of 120 cases) demonstrating significant differentiation ($P < 0.05$, Fig. 2A). In contrast, *Haliplus* displayed F_{ST} values ranging from 0.021 to 0.503, but none of the 36 pairwise comparisons were significant after false discovery rate (FDR) correction (Fig. 2B). In the case of *Planorbis*, F_{ST} values ranged from 0.136 to 0.417, and all 45 comparisons indicated significant differentiation ($P < 0.05$, Fig. 2C). Finally, *Rana* exhibited highly variable F_{ST} values, spanning from 0.016 to 0.472, with the majority of comparisons (12 out of 15) showing significance ($P < 0.05$, Fig. 2D). Pairwise PERMANOVA results among the populations were in parallel with F_{ST} comparison. The majority of the comparisons were significant ($P < 0.05$) after FDR correction for *Asellus, Planorbis,* and *Rana* (Table S2, S3 and S4). The visualization of population structure through PCA plots indicated a lack of population structure in *Haliplus*, while the remaining species demonstrated distinct population structures (Fig. 2). Notably, *Planorbis* exhibited a particularly pronounced population structure (Fig. 2C).

The test of homogeneity of multivariate dispersions (deviations from centroid) for the three species that exhibited significant population structure according to PERMDISP tests revealed significant differences in genetic dispersion between populations for all three species (*Asellus*: $F_{15,125} = 3.06$, $P = 0.102$; *Planorbis*: $F_{9,60} = 76.48$, $P = 0.0001$; *Rana*: $F_{5,39} = 20.43$, $P = 0.0003$). After FDR correction, none of the comparisons for *Asellus* remained significant. For *Planorbis*, 34 out of 45 comparisons were significant, and for *Rana*, 9 out of 15 comparisons were significant. It is worth noting that homogeneity violations may lead to significant PERMANOVA results (Warton et al 2012). However, population pairs with significant PERMANOVA results did not always show significant dispersion violations. It is important to acknowledge that the imbalance in population sizes among sampling locations may contribute to observed differences in dispersion.

Correlation between genetic distance and landscape connectivity

We found a significant correlation between genetic differentiation and geographic distance in *Asellus* and *Planorbis* (Table 1), suggesting that geographic distance was associated with spatially structured genetic differentiation in these two species (cf. *Haliplus* and *Rana*, where genetic distance is independent of geography or landscape connectivity). Furthermore, the genetic differentiation of *Asellus* shows a significant ($P < 0.05$) association with blue-green connectivity (Table 1). Such a correlation was not observed for the other three species. The fact that blue-green connectivity contributes to genetic differentiation when geographic distance is taken into account, is also reflected in the shortest paths maximizing current flow among any two sampling sites (Fig. 3). These blue-green shortest paths are different from the shortest geographic distance between points, suggesting that not only geographic distance was a factor influencing genetic differentiation.

Discussion

Genetic diversity and population structure

Few studies have investigated the genetic diversity of organisms inhabiting urban ponds. Such information is important because it provides information about potential adaptations to the urban environment (Mable 2019). We found variation in molecular diversity indices such as expected heterozygosity (HE), population levels of inbreeding (*Fis*), and nucleotide diversity (π) in the four species studied. Notably, all four species exhibited a heterozygosity deficiency across their populations, indicating some level of inbreeding. This observation is consistent with the effects of urbanization and habitat fragmentation, which often lead to reduced gene flow and genetic isolation in fragmented populations (Frankham et al. 2010).

Asellus, *Planorbis* and *Rana* displayed significant population structure as indicated by both PERMANOVA and F_{ST} values, suggesting restricted gene flow and genetic differentiation among their populations. In contrast, *Haliplus* exhibited no significant population structure, implying a more panmictic population. This difference in population structure between species could be attributed to the varying dispersal capability of the species (Richardson 2021). We classified *Haliplus* as a high dispersal species compared to the other three study species due to its ability to fly between aquatic habitats (Boda et al. 2014). In contrast, *Asellus, Planorbis*, and *Rana*, which probably have more limited dispersal capability, exhibited a more pronounced population structure. This result aligns with our initial hypothesis that weak dispersers would have a stronger genetic structure compared to strong dispersers. Other studies in urban landscapes have also found an association between dispersal and genetic structure. For example, difference in F_{ST} values in four butterfly species was suggested to some degree to be a

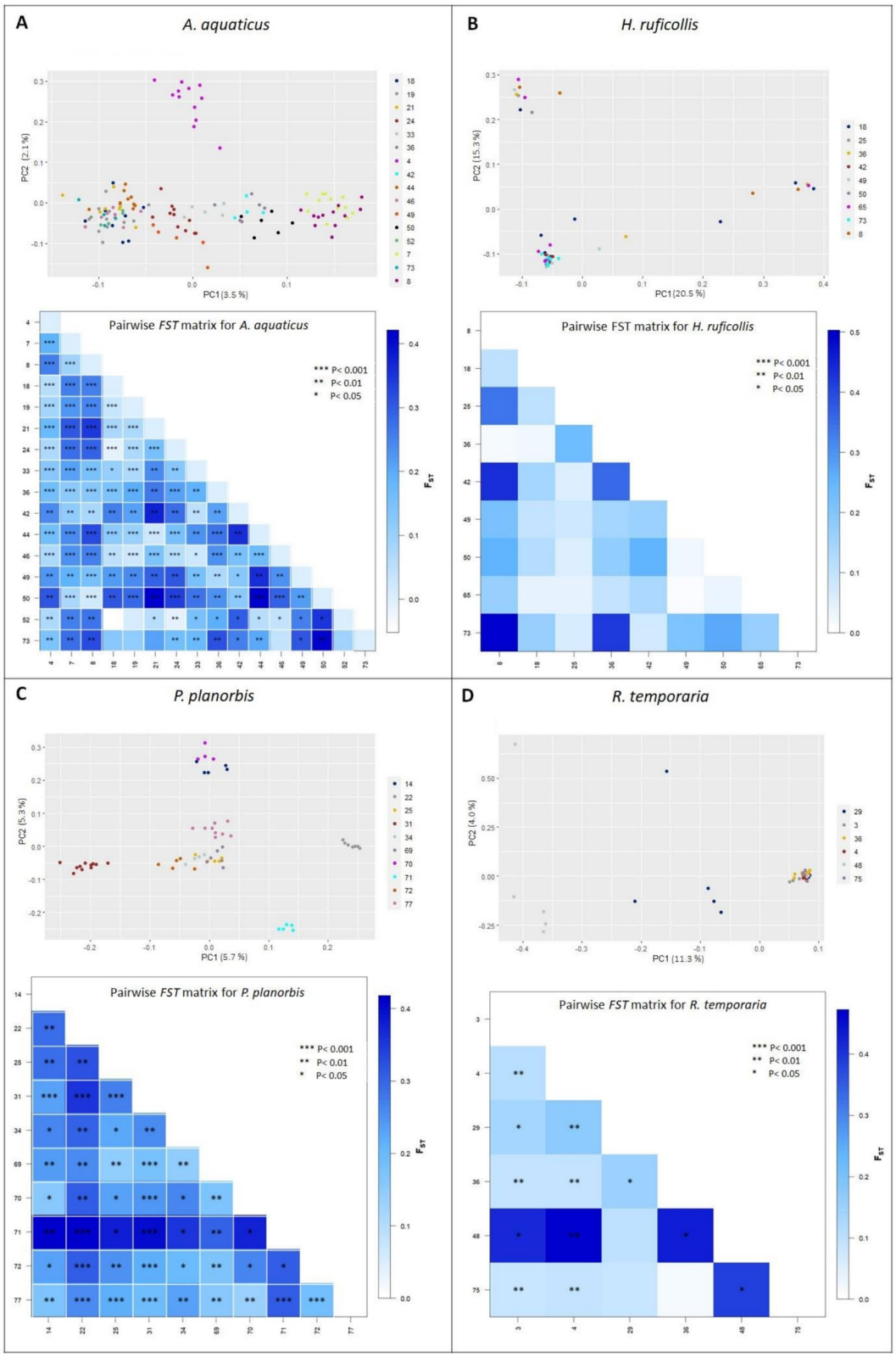

Fig. 2 Pairwise FST matrices and PCA plots illustrating the population structure of the study species. **A** *A. aquaticus*, **B** *H. ruficollis*, **C** *P. planorbis*, and **D** *Rana temporaria*

Table 1 Correlation between genetic and geographic/blue-green distances

	Geo	BG_r100_10k	BG_r100_5k	BG_r50_10k	BG_r50_5k
Asellus	**0.630**	**0.679**	**0.676**	**0.678**	**0.678**
Haliplus	− 0.030	− 0.116	− 0.118	− 0.115	− 0.119
Planorbis	**0.460**	0.418	0.425	0.418	0.421
Rana	− 0.031	− 0.072	− 0.071	− 0.070	− 0.068

The first column (Geo) represents the correlation between genetic and geographic distances by species, and shows that genetic differentiation and geographic distances are correlated in *Asellus* and *Planorbis* but not in *Haliplus* and *Rana*. The other columns represent the correlations between genetic distances and distance matrices capturing blue-green connectivity, calculated as shortest paths (BG), with blue-green distances being weighted exponentially. Habitat patches with resistance below 50 (r50) and 100 (r100) represent sources/sinks. Bold values correspond to $P < 0.05$

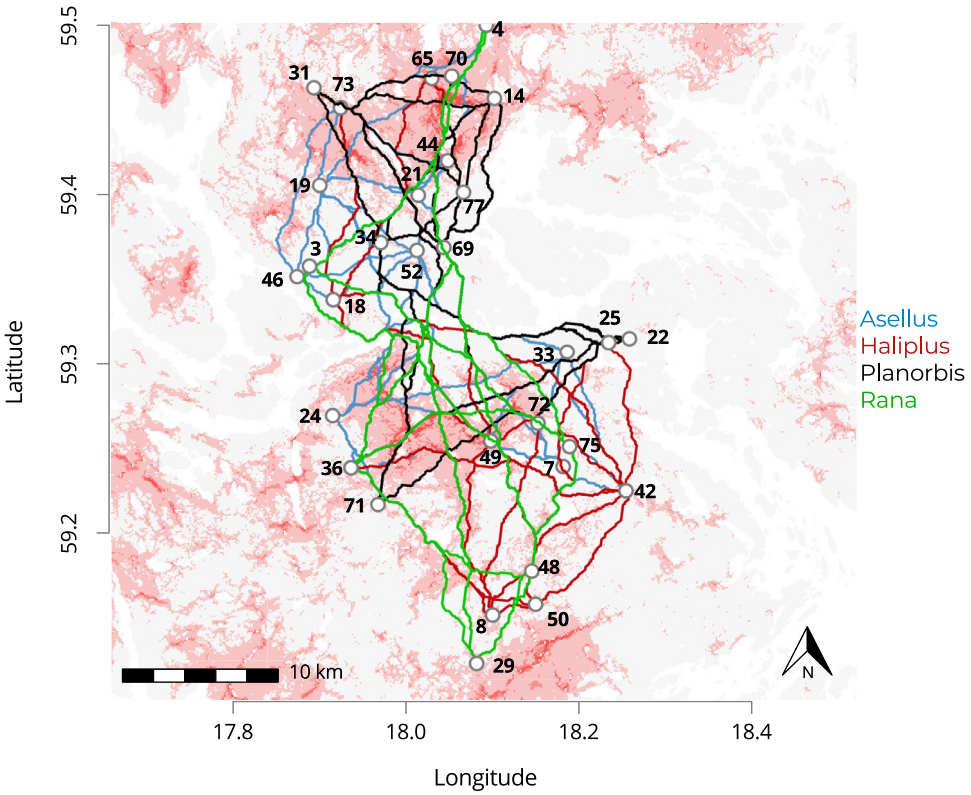

Fig. 3 A map of cumulative current flow (i.e., blue-green connectivity) across the landscape based on the Omniscape algorithm. The red surface color represents resistance, with darker red indicating higher resistance. For each of the four species (*Asellus* = blue; *Haliplus* = red; *Planorbis* = black; *Rana* = green) we calculated shortest paths based on the inverse of current flow across the landscape. These shortest paths represent movement from any given sampling site to another along routes of higher current flow (i.e. lower values for the inverse; similar to lower elevation in a purely topographic model). The numbers corresponds to the codes for each location, see Fig. 1

consequence of dispersal capability differences between species (Wood and Pullin 2002). Whether the genetic structure in *Asellus, Planorbis and Rana* is a result of genetic adaptation or genetic drift requires further data and analysis which is beyond the purpose of our study.

The PCA plots visually reinforced the observed population structure, particularly for *Planorbis*, which displayed a pronounced genetic differentiation among its populations. Three of its populations – 31, 69 and 77 (see Fig. 1), showed a distinct genetic isolation. Interestingly these three ponds were not geographically isolated from the rest of the ponds suggesting that urban landscape features rather than straight-line Euclidean distances, might limit gene flow in this species. Such effects have also been suggested for *Apodemus* mice inhabiting rural areas, where it was inferred that artificial barriers within the city were more important than simple geographic distances in shaping the genetic differentiation found in the city (Gortat et al. 2012). Similarly, it was found that green toad populations show higher genetic differentiation and lower gene flow in urban compared to rural areas, although geographic distances between sites were significantly shorter in the rural area (Vargová et al. 2023). Our PCA plot also showed that four of the six *Rana* populations clustered together, suggesting a low genetic differentiation among these four populations. This pattern was somewhat surprising given that the distance between them was more than 1 km, and that anurans in general have low dispersal ranges (Sinsch 1990; Berven and Grudzien 1990). Similarly,

Rana did not show much lower diversity (π) compared to the other study species. One possible explanation for the low genetic divergence between some of the *Rana* populations could be that humans have translocated frogs to these ponds. However, some studies suggest that weak dispersers may not be necessarily more vulnerable to urbanization and increased land use compared to strong dispersers (Martin et al. 2023). In addition, Fusco et al. (2021b) found no difference in overall levels of genetic diversity across urban, suburban and rural habitats in stream salamanders around the New York City metropolitan area.

Correlation between genetic distance and landscape connectivity

We also examined the relationship between genetic distance and connectivity, considering both geographic distance and a blue-green connectivity approach. We found that the genetic differentiation of *Asellus* and *Planorbis* exhibited a significant association with geographic and the former with also blue-green connectivity. However, such correlations were not observed for the other two species. This suggests that the impact of landscape connectivity on genetic structure varies among species with different dispersal capacities. We categorized *Asellus* and *Planorbis* as intermediate dispersers, and we suggest that at the scale of our study intermediate dispersers are more dependent on geographic distance and connectivity. Strong dispersers such as *Haliplus* can disperse to the ponds despite the distance between them, and therefore no association between genetic distance and connectivity was found. In contrast, weak dispersers, such as *Rana*, probably need a shorter geographic distance or more blue-green connectivity between ponds to show a correlation between genetic distance and connectivity.

The lack of significant association with and blue-green connectivity and genetic differentiation for *Rana* can be explained by the fact that amphibians need some heterogenous landscape to maintain the genetic diversity and connectedness. Johansson et al. (2005) showed in their study with *R. temporaria* that in northern latitudes, areas with some level of agricultural activities exhibited higher genetic diversity than more natural boreal forests, suggesting that moderate levels of human impact may create heterogeneity in habitats which results in genetic diversity, as seen in our study species.

These differences between species emphasize the role of species-specific dispersal abilities and life history traits in shaping their genetic diversity and population structure in fragmented urban environments (Wood and Pullin 2002; Schleicher et al. 2011). They also highlight the importance of considering such factors when studying the effects of urbanization on genetic diversity.

Implications for biodiversity conservation in urban environments

Our findings have important implications for biodiversity conservation in urban environments. As urbanization continues to fragment natural habitats, understanding the genetic diversity and population structure of species in urban settings is crucial for their conservation. Our study highlights the need for tailored conservation strategies that consider the specific dispersal abilities and life history traits of different species. However, dispersal abilities should be considered in the context of landscape features that either hinder or facilitate the movements of organisms between sites (Heino et al. 2017a, b). Hence, incorporating blue-green connectivity models, as demonstrated in our study, can be a valuable tool for assessing and predicting the impact of urban development on genetic connectivity. By identifying landscape features that influence genetic structure, conservation efforts can be more effectively targeted to protect critical corridors for gene flow and maintain genetic diversity in urban populations.

In conclusion, our research provides novel insights into the genetic diversity and population structure of species in urban ponds and highlights the role of landscape connectivity and dispersal characteristics of species in shaping these patterns. As urbanization continues to transform the environment, understanding the genetic dynamics of urban populations is essential for the conservation of biodiversity in urban landscapes.

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Author contributions FJ conceived and designed the study. FJ and CH collected the samples. CH performed the laboratory work and analyzed the data. YY did the data cleaning and analysed the data. FJ, YY and LMB wrote the manuscript; CH, JN and JH helped with the writing. All authors approved the final manuscript.

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Data availability The demultiplexed data was archived in the NCBI Sequence Read Archive (BioProject ID: PRJNA1026545), link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1026545?reviewer=k77s47nm26he873ahrn69s8jsl).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose

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