

ORIGINAL RESEARCH

Habitat Quality and Water Availability Affect Genetic Connectivity of Platypus Across an Urban Landscape

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ABSTRACT

Freshwater ecosystems are among the most threatened globally. Understanding how environmental variables influence the gene flow of freshwater species can help identify landscape features requiring conservation management. We used landscape genetic resistance modelling to assess the influence of climate, topography and vegetation cover on genetic structure and gene flow in platypus (*Ornithorhynchus anatinus*) in an urban region of south-east Queensland, Australia. Thirty DNA samples were genotyped using the DArTseq platform, and data were filtered to produce a panel of 5478 neutral single nucleotide polymorphism (SNP) markers. We used fastSTRUCTURE, partial redundancy analysis and maximum likelihood analyses to understand platypus genetic structure and landscape influences on gene flow. Between one and three genetic clusters were detected using fastSTRUCTURE. Partial redundancy analysis identified Topographic Wetness Index and antecedent rainfall as driving genetic differentiation between samples. A maximum likelihood population effects model suggested gene flow was spatially structured by Normalised Difference Vegetation Index at a fine scale (100 m) and antecedent rainfall at a landscape scale (1 km). Thus, less vegetated areas appear to restrict the gene flow of platypus in urban systems. Rainfall, vegetation cover and topographic wetness are also important for maintaining platypus gene flow across landscapes. Our research recommends conservation management through restoration of water flow and riverine vegetation to help maintain platypus connectivity and increase gene flow among populations.

1 | Introduction

Freshwater ecosystems are increasingly threatened by habitat degradation, over-exploitation, water pollution, flow modification and invasive species (Dudgeon et al. 2006; Reid et al. 2019, 2020; Cresswell, Janke, and Johnston 2021). Global wetlands are declining at a rate three times faster than forests (Tickner et al. 2020) and freshwater megafauna populations declined by 88% from 1970 to 2012 (He et al. 2019). Both terrestrial and aquatic species contend with natural barriers in the landscape that limit gene flow (Bowler

and Benton 2005; Maclagan et al. 2020; Fusco, Pehek, and Munshi-South 2021). However, dispersal patterns of aquatic species differ fundamentally from terrestrial species because they are restricted to the linear connectivity of waterways (Furlan et al. 2013; Davis et al. 2018; Martinez, Willoughby, and Christie 2018; Tickner et al. 2020; Washburn, Cashner, and Blanton 2020; Hannah 2022). This connectivity can be natural, but many river systems have been highly modified by anthropogenic landscape change which increases fragmentation and limits dispersal for aquatic species (Brauer and Beheregaray 2020). Identifying the genetic impacts of both

natural and human-made features across waterways is necessary to ensure key landscape features are managed in a way that maintains species' potential to adapt to environmental change (Allendorf et al. 2013; Nonić and Šijačić-Nikolić 2019).

Stream networks are naturally influenced by topographic features such as elevation and slope (Keller, Van Strien, and Holderegger 2012; Hromada et al. 2020) which influence gene flow and dispersal in freshwater species (Fusco, Pehek, and Munshi-South 2021; Sunny et al. 2022). For example, aspect and slope influenced patch suitability for the Yosemite toad (*Anaxyrus canorus*) because heat load and radiation created migration corridors that followed gentle slopes (Maier et al. 2022). Similarly, the Neotropical otter (*Lontra longicaudis*) in Veracruz, Mexico, was genetically structured by two basins naturally separated by slope and stream order (Latorre-Cardenas et al. 2020). On the other hand, rapid modification of waterways and removal of riparian vegetation by human activity can intensify these natural dispersal barriers (Baguette et al. 2013; Dudgeon 2019; Latorre-Cardenas et al. 2020). For example, connectivity can be hindered within waterways by the construction of weirs and dams (Dudgeon 2014; Hawke, Bino, and Kingsford 2021; Hawke et al. 2021; Mijangos et al. 2022) and between waterways by the development of buildings and roads that create impervious structures (Martin et al. 2014; Serena and Pettigrove 2005; Kimmig et al. 2020; Mijangos et al. 2022). Such impacts were seen in the Macquarie perch (*Macquaria australasica*) where barriers degraded preferred habitat and reduced dispersal, contributing to genetic differentiation among populations (Faulks, Gilligan, and Beheregaray 2011). Such changes in vegetation cover and structure that result from urbanisation can be detected using remotely sensed environmental data and vegetation indices (McGranahan and Satterthwaite 2014; Martinez and Labib 2023) and could assist conservation managers to understand key landscape features that impact gene flow across landscapes.

The semi-aquatic Australian monotreme, platypus (*Ornithorhynchus anatinus*), has both terrestrial and aquatic modes of dispersal, with a high dependence on freshwater waterways for survival (Serena et al. 2001; Grant and Fanning 2007; Carrick, Grant, and Temple-Smith 2008; Coleman et al. 2022). They have linear home ranges of less than 15 km (Serena 1994; Serena and Williams 2013) and rely on connected waterways to disperse. Although platypus can travel overland between adjacent river basins (Burrell 1974; Akiyama 1998; Kolomyjec et al. 2009; Furlan et al. 2013), terrestrial movement has decreased on mainland Australia because of habitat fragmentation caused by natural and artificial barriers, such as dams and weirs (Kolomyjec et al. 2009; Furlan et al. 2013; Hawke et al. 2021; Mijangos et al. 2022; Musser, Grant, and Turak 2024). Terrestrial dispersal increases predation risk, energy expenditure and their risk of heat stress (Robinson 1954; Grant and Dawson 1978; Fish et al. 2001; Grant and Fanning 2007). Despite documented declines in its population size (45%–58%) and distribution (22.6%) (Hawke, Bino, and Kingsford 2020), the platypus is currently not listed as nationally threatened because of insufficient information regarding rates of decline (Grant and Temple-Smith 1998; Serena et al. 1998; Grant and Fanning 2007; Serena and Williams 2013; Department of Climate Change 2022). Urbanisation around freshwater ecosystems reduces water and habitat quality and availability for platypus (Martin et al. 2014;

Brunt et al. 2021) and is likely to restrict dispersal, migration and gene flow. Some evidence exists for this in parts of their range across New South Wales and Victoria (Furlan et al. 2013; Hawke et al. 2021), but how geographically consistent these patterns are is unknown. Furthermore, no studies have incorporated multiple landscape-scale variables into spatially explicit genetic analyses for this iconic species. Increasing evidence about their landscape genetic population structure will strengthen calls to assign them an appropriate conservation status (Hawke et al. 2021). Maintenance of quality freshwater habitat will also improve connectivity and dispersal for other semi-aquatic species that co-occur with platypus.

In this study, we aimed to quantify platypus genetic structure using single nucleotide polymorphisms (SNPs) to make inferences about connectivity and dispersal across a subtropical urban to semi-urban region incorporating diverse land uses. Specifically, we used population and landscape resistance modelling to quantify how genetic distance among individuals was influenced by landscape features including topography, climate and degree of urbanisation which we characterised by the Normalised Difference Vegetation Index (NDVI). Habitat modification for agriculture and dam structures have limited dispersal in other parts of the platypus's range (Kolomyjec et al. 2009; Furlan et al. 2013; Hawke et al. 2021), and previous work showed topographic wetness strongly influenced platypus distribution (Brunt 2023). Thus, we anticipated these variables would also influence gene flow in this landscape. Our work differs from previous studies on this species (Kolomyjec et al. 2009; Furlan et al. 2013; Hawke et al. 2021) by specifically incorporating remotely sensed data into models of landscape resistance to understand landscape-scale connectivity. Quantifying connectivity among platypus populations is important because it can inform conservation management actions that will maintain freshwater habitats and thus gene flow.

2 | Materials and Methods

2.1 | Study Region and Site Selection

The study took place in south-east Queensland, Australia, across seven hydrological basins (Figure 1). South-east Queensland has a sub-tropical climate that is influenced by tropical systems from the north, and fluctuations in the high-pressure ridge to the south (Queensland Government 2020). Average annual rainfall is 1030 mm but is highly variable, with variability driven by local factors, such as topography and vegetation, and broader-scale weather patterns, such as the El Niño–Southern Oscillation (Queensland Government 2020). Most rainfall occurs in summer and autumn, with 388 mm and 295 mm on average per year, respectively. Seasonal average temperatures are 24°C in summer, 20°C in autumn/spring and 14°C in winter (Queensland Government 2020). Temperatures and the frequency of hotter days are predicted to increase for the region, while precipitation is predicted to decrease (Queensland Government 2020). Land use primarily comprises agricultural production from relatively natural environments (55.73%), conservation and natural environments (17.93%) and intensive uses (11.72%) (Figure S1) (DSITIA 2014).

The seven hydrological basins in this study and their main rivers are characterised by seasonal flow that reflects the region's

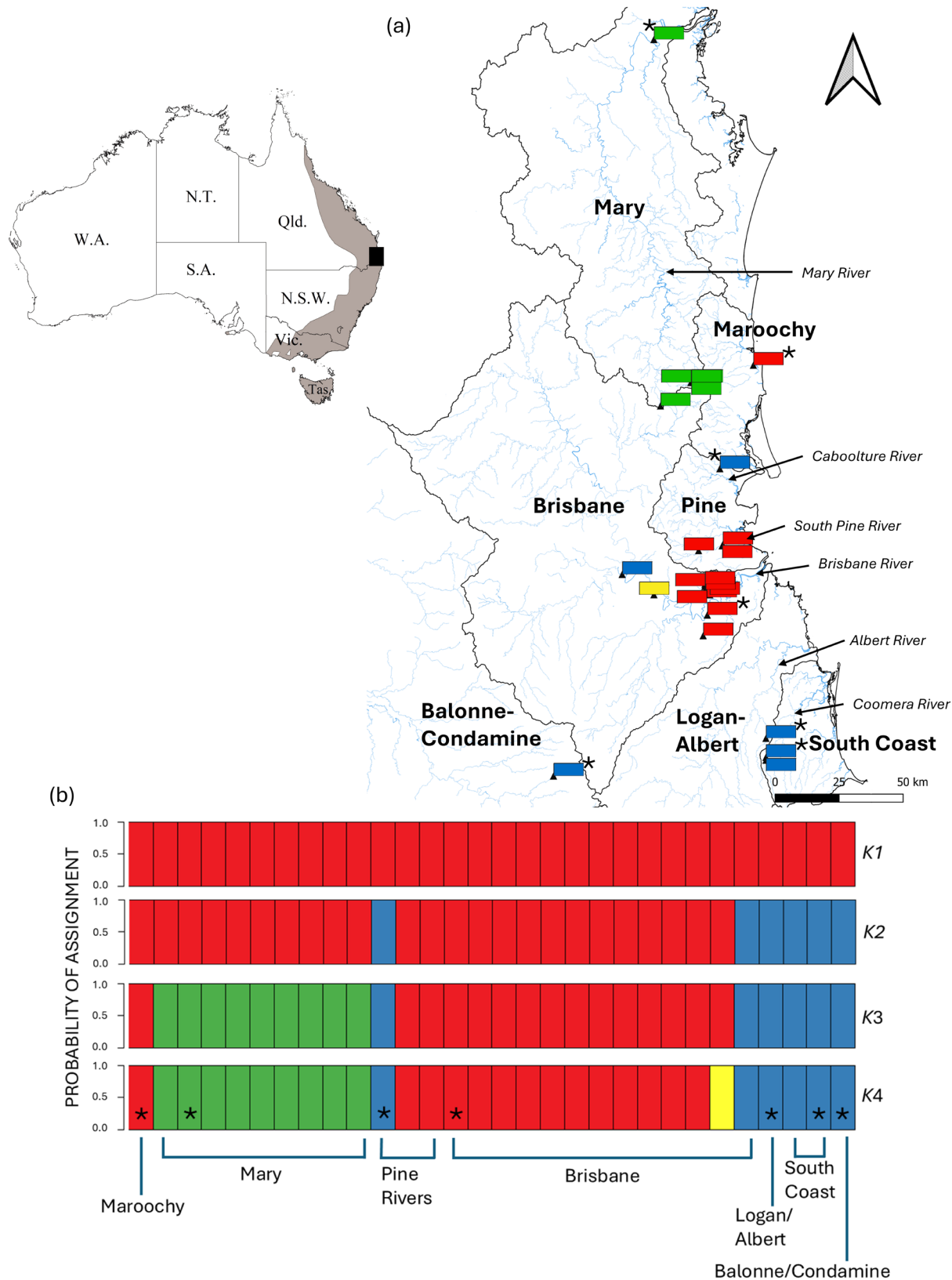


FIGURE 1 | Sampling locations and genetic structure of platypus (*Ornithorhynchus anatinus*) across seven hydrological basins in south-east Queensland, Australia. The number of genetic clusters in the data (K) was estimated to be between $K=1$ and $K=4$. (a) Platypus DNA was sampled from 30 individuals at 21 sites shown on the map with colours representing $K=4$. (b) Assignment probability to each K for the 30 individuals. Inset shows the platypus distribution across Australia in grey and the study location in black. * indicates the museum samples.

annual rainfall pattern, where most rainfall occurs between November and March. The main rivers, Albert, Brisbane, Logan, Coomera and Mary rivers, have perennial flow, whereas the Bremer River may occasionally cease to flow (Australian Bureau of Meteorology 2023). The tributaries of these main river systems are characterised as first-, second- or third-order streams which can be continuously flowing or ephemeral in nature.

In total, 30 platypus DNA samples were collected across the region: 19 from live-captured platypus, seven from museum specimens and four from dead specimens. Live trapping sites were chosen from observational and environmental DNA studies (Brunt et al. 2021) to reduce uncertainty regarding species occurrence and to maximise trapping success. Waterway suitability (width and depth) and accessibility were also used to select appropriate sites because our fyke net method (described below) required a water depth of 20 cm to < 1 m in narrow creeks (Grant 2012; Serena, Griffiths, and Williams 2015). Sampling sites spanned 32,000 km² and were separated by an average of 72 km (range = 0.018–291 km).

Live-captured samples were collected between 2019 and 2021. Trapping was conducted throughout the year, across 55 individual sites each with two fyke net traps. Most sites were sampled more than once, giving a total of 254 trap nights between July 2019 and March 2021. Three to six sites were surveyed on a given trap night. Platypus, as a species are increasingly active in the breeding season (July to October) and when juveniles are emerging from the nest and dispersing (February and March). Most captures (95%) occurred between June and October in 2019 and 2020. Drought in late 2019 and flooding in February 2020 limited survey time because of either too little flow, unsuitable water, or too much water making hard and fast flows dangerous for personnel and animals.

Platypuses were captured using nylon fyke nets (Serena, Griffiths, and Williams 2015)—windsock-shaped nets with hooped sections and wings spanning the width of the stream channel (Grant 2012). The distal end of the net was tied to a stake on the stream bank to keep captured animals afloat (Serena 1994) and the net was anchored to the bank with stakes to block off a section of stream. Two fyke nets were deployed per survey site, one facing upstream and one facing downstream, in an approximately 10-m section of the waterway. Nets were set up in the afternoon, checked before dusk and then checked throughout the night every two to four hours, depending on capture rates and weather. Following capture, each platypus was placed into a calico bag and lightly restrained on a foam pad for processing. A LifeChip microchip (11 × 13 mm) was inserted under the skin between the shoulders to identify recaptures. A small section of hind foot webbing was collected for DNA and immediately preserved in 70% ethanol. Following processing, animals were released at the bank and allowed to swim away.

To increase sample size, seven platypuses were collected from museum specimens, and four from dead specimens (Figure 1). Museum samples consisted of muscle or skin tissue stored in ethanol sourced from the Queensland Museum (five samples collected between 2000 and 2014) and the Australian Museum (two samples collected in 1991 and 1993). Dead specimens were

collected by members of the public and samples and location data were reported to the authors between 2016 and 2021. A muscle or skin tissue sample was taken from each dead specimen and stored in ethanol.

Both male and female platypuses reach maturity around two years of age (Temple-Smith 1973; Grant, Griffiths, and Temple-Smith 2004) with a lifespan in the wild between 6 and 15 years (Grant, Griffiths, and Temple-Smith 2004; Pacifici et al. 2013; Serena et al. 2014; Bino, Grant, and Kingsford 2015) although there are records of wild platypus over 20 years old (Grant 2004; Serena et al. 2024). Our sampling covered multiple generations by including adults and juveniles, but the majority (89%) of live captured platypuses were either adult or sub-adult. Thus, we considered the inclusion of emerging juveniles (11%) to have negligible influence on our estimates of gene flow and retained all age-classes in the data.

2.2 | Genetic Analyses

Total genomic DNA was extracted using a NucleoMag 96 Tissue Kit (Macherey-Nagel) at Diversity Arrays Technology P/L (Canberra, Australia). A double-restriction enzyme complexity reduction and high-throughput sequencing method (DARtseq) was used for genotyping (Kilian et al. 2012). The enzymes PstI and SphI were chosen following tests of different enzyme combinations for platypus. DNA samples were processed in digestion/ligation reactions following Kilian et al. (2012) but substituting the single PstI adaptor for two adaptors corresponding to restriction enzyme-specific overhangs. The PstI adaptor was modified to include Illumina sequencing primers and variable-length barcodes (Elshire et al. 2011). Mixed fragments (PstI–SphI) were amplified in 30 rounds of PCR using the following reaction conditions: 94°C for 1 min and then 30 cycles of 94°C for 20 s, 58°C for 30 s and 72°C for 45 s followed by 72°C for 7 min. After PCR, equimolar amounts of amplification products from each sample were bulked and applied to c-Bot (Illumina) bridge PCR followed by single-read sequencing on an Illumina HiSeq2500 for 77 cycles. Raw sequences were processed using DARtseq analytical pipelines (DARtdb) to split samples by barcode and remove poor-quality sequences. Sequences (69-bp) were aligned to the representative platypus genome (Zhou et al. 2021, GenBank accession PRJNA489114) with a BLAST E-value of 5×10^{-7} and a minimum sequence identity of 90% using proprietary software (DARtsoft), a package developed in-house and used to both identify and score the markers that were polymorphic within such an experiment (C. Cayla, G. Uszynski, D.J., P.W., and A. Kilian, unpublished data).

2.3 | SNP Filtering

Starting with 9800 SNPs that passed DARtseq quality control, we filtered the data (Figure S2) using custom scripts developed by Smith et al. (2020) and modified for the current study (Brunt and Smith 2024). Filter 1 removed SNPs on the same sequence, monomorphic loci and SNPs with > 50% missing data. SNPs on the same 69-bp sequence as another were removed to reduce the chance of disequilibrium from physical linkage, keeping the one with the highest call rate (Reynes et al. 2021). Filter 2 removed SNPs with low minimum minor

allele frequency (<2%), low reproducibility (<98%) and linkage disequilibrium. We used the correlation between genotype frequencies (Chan 2018) to test for linkage disequilibrium between each pair of loci and removed a locus if it was in a correlated pair ($r > 0.75$). The data comprised 5483 SNPs after applying these filters (Figure S2).

2.4 | Detecting Loci Under Selection

Neutrality was an assumption underlying the population structure models that we used, and our aim was to investigate gene flow arising from neutral dispersal processes. Therefore, we investigated whether SNPs were putatively under selection using two individual-level methods: PCAdapt (Luu, Bazin, and Blum 2016) and LFMM (Frichot et al. 2013; Caye et al. 2019). Both methods define background population structure as K principal components derived from individual genotypes (Frichot et al. 2013; Duforet-Frebourg, Bazin, and Blum 2014). In PCAdapt, each SNP is regressed against each principal component. LFMM uses the principal components as latent factors in a Gaussian mixed model, where the genotype matrix is modelled as a function of an environmental matrix (Frichot et al. 2013). Compared with tests designed for site-level sampling, PCAdapt and LFMM are more reliable for species with complex, hierarchical population structure (e.g., multiple divergence events) and are less sensitive to admixed individuals and outliers in the data (de Villemereuil et al. 2014; Luu, Bazin, and Blum 2016). We considered outliers identified in both methods to be putatively under selection and removed them from the downstream analysis (Figure S2).

For both PCAdapt and LFMM, we examined scree plots to determine the number of clusters in the data (K) (Figure S3). For PCAdapt, the most likely K was unclear (Figure S3a, following Cattell's rule, Cattell 1966; Luu, Bazin, and Blum 2016). We therefore tested the sensitivity of choosing K between 1 and 8 and found the choice of K had little influence in the downstream analysis (data not shown). Ultimately, we chose the conservative $K=8$ against which to assess outliers, resulting in 1036 outliers. For LFMM, we explored $K=1$ to 5 and chose $K=2$ based on the scree plot (Figure S3b).

We defined the LFMM environmental matrix using six environmental variables important for platypus distribution (Table 1): Normalised Difference Vegetation Index (NDVI), Topographic Wetness Index (TWI), antecedent rainfall, average temperature of the hottest and coldest months and elevation. To control for the false discovery rate, we calculated q values from p values and classed SNPs as outliers, where $q < 0.05$. When combined with quality control filters, our non-neutral (putatively adaptive) data set comprised 1061 SNPs. Only five outlier loci were common between the two methods, and these were removed from the data set. Our final neutral dataset comprised 5478 SNPs for downstream analysis (Figure S2).

2.5 | Population Genetic Structure

We used fastSTRUCTURE to assess genetic relationships among individuals (Raj, Stephens, and Pritchard 2014). This model

determines the number of genetic clusters in the data (K) that would maximise Hardy–Weinberg and linkage equilibrium. We investigated $K=1$ to $K=20$ and assigned each individual to a cluster based on the model complexity that maximised marginal likelihood and the model components used to explain structure in data (Raj, Stephens, and Pritchard 2014). If dispersal and gene flow were restricted to hydrological basins, we expected to see genetic differences across catchments. Previous versions of STRUCTURE had methods for determining the most likely K (Pritchard, Stephens, and Donnelly 2000; Evanno, Regnaut, and Goudet 2005), while fastSTRUCTURE provides a range in K (Raj, Stephens, and Pritchard 2014; Enloe et al. 2022). We therefore visualised and assessed population structure between both $K=1$ and $K=4$ to determine if structure corresponded to these individual basins (Figure 1b). We also included a point on the sample map related to $K=4$ as the clusters corresponded broadly to three main hydrological basins, namely, Mary, Brisbane and South Coast (Figure 1a).

2.6 | Defining Environmental Variables

The environment at each sampling site was characterised using biologically significant variables with the potential to influence platypus movement (Table 1). Rainfall and topographic wetness index (TWI) relate to the reliance of platypus on water (Grant and Fanning 2007; Serena et al. 2014). TWI was calculated as $\log(e)$ (specific catchment area/slope) as an estimate of the relative wetness at each site (Gallant and Austin 2012). Temperature relates to platypus thermoregulation (Robinson 1954; Grant and Dawson 1978; Bethge et al. 2004; Klamt, Thompson, and Davis 2011; Bino, Kingsford, and Wintle 2020). Normalised Difference Vegetation Index (NDVI) was quantified from satellite imagery and was used to represent habitat quality. This metric effectively distinguishes between vegetation, impervious surfaces (i.e., that arise with urbanisation) and highly modified vegetation such as agricultural crops (Martinez and Labib 2023; Pettorelli 2013), making it appropriate for our study questions.

Spatial data were clipped to the study region boundary ($-25^{\circ}25'59''$ – $28^{\circ}12'29.98''$ S and $152^{\circ}5'59.96''$ – $153^{\circ}30'00''$ E) and resampled to a spatial resolution of 100 m and 1 km using QGIS version 3.22 (Table 1). This dual-scale approach aimed to sample environmental data at a resolution fine enough to relate to platypus movement and dispersal, while also working within the constraints of data availability. Average temperatures and antecedent precipitation could only be obtained at 1 km resolution from WorldClim (Fick and Hijmans 2017). Topographic variables (TWI, NDVI and elevation) were downloaded at the highest raw resolutions available (Table 1) and resampled to 100 m to standardise among variables while capturing fine-scale landscape variation (hereafter 'fine-scale' resolution). All other data were sampled at the 1 km scale (hereafter 'coarse' resolution) because this was the finest scale available (Table 1).

To quantify NDVI, high-resolution (10 m) images from Sentinel 2 (European Space Agency 2021) were downloaded between November 2021 and February 2022, a period known to be greener after rain. NDVI was calculated in QGIS using the raster calculator as $(\text{NIR} - \text{R}) / (\text{NIR} + \text{R})$, where NIR is the near-infrared band and R is the red band. We used NDVI as a proxy for

TABLE 1 | Spatial variables used to analyse environmental influences on platypus (*Ornithorhynchus anatinus*) gene flow across south-east Queensland, Australia.

Habitat variable	Relevance	Raw resolution	Resampled layer resolution	Temporal resolution	Data layer source
<i>Climatic variable</i>					
Average temperatures of the:	Thermal tolerance (Robinson 1954; Grant and Dawson 1978; Kolomyjec 2010; Klamt, Thompson, and Davis 2011)	30 arcsecond	Unchanged	Average 1970–2000	Fick and Hijmans (2017)
– Hottest month January					
– Coldest month July					
Antecedent precipitation	Dependence on water, 5 months prior to breeding season (Serena et al. 2014; Kolomyjec 2010)	30 arcsecond	Unchanged	Average 1970–2000	Fick and Hijmans (2017)
– Rainfall five months prior to breeding season: Feb–Jun					
<i>Topographic variable</i>					
Topographic Wetness Index (TWI)	Wetness within a catchment and water availability (Ellem, Bryant, and O'Connor 1998; Serena et al. 1998; Grant and Fanning 2007; Brunt, Adams-Hosking, and Murray 2018)	1 arcsecond (27 m pixel size)	100 m and 1 km	—	Gallant and Austin (2012)
Elevation	Natural barrier (Bino, Kingsford, and Wintle 2020)	3 arcsecond (90 m pixel size)	100 m and 1 km	—	Gallant et al. (2009)
<i>Land use</i>					
Normalised Difference Vegetation Index (NDVI)	Vegetation cover and impervious surface barriers reflect urbanisation (Bryant 1993; Woon 1995; Serena et al. 1998; Rohweder and Baverstock 1999; Grant 2014)	1 arcsecond	100 m and 1 km	Nov 2021; Jan 2022	European Space Agency (2021)

Note: Temperature and precipitation were used at a 30 arcsecond (1 km pixel size) scale ('coarse') and the remaining variables were used at a 100 m scale ('fine').

urbanisation and habitat quality because higher values relate to vegetation cover (>0.4) and reflect pervious, connected habitat (Figure S1). Low NDVI values represent low vegetation density, such as open areas (0.2–0.4) and land cover changes associated with urbanisation (Zaitunah, Samsuri, and Sahara, 2021; Martinez and Labib 2023) and impervious surfaces. We expected that impervious structures surrounding waterways and open, sparsely vegetated areas would pose a risk for platypus because impervious structures are a physical barrier and open areas lacking vegetation have been associated with increased predation risk (Stillfried et al. 2017; Potash, Conner, and McCleery 2019). NDVI ranges from –1 to 1, where negative values correspond to water. However, given that water facilitates rather than inhibits movement of the platypus (Grant and Fanning 2007), we reclassified waterways with an NDVI value of 1.

Temperature and precipitation data were obtained from WorldClim (1970 to 2000, Fick and Hijmans 2017) and analysed at a 30 arcsecond resolution. We used the average of the five months total rainfall prior to breeding (hereafter ‘antecedent rainfall’) because this was previously found to influence platypus occurrence (Serena et al. 2014). We also used the average temperatures of the coldest month (July) and the hottest month (January) because they relate to thermoregulation (Table 1). To create local habitat site measures, we used a 1 km radius around each individual sample and calculated the mean of each landscape variable within the buffer (Latorre-Cardenas et al. 2020).

2.7 | Landscape Resistance Modelling Overview

We explored the influence of environmental features on gene flow using two landscape resistance methods: (1) spatial principal component analysis (sPCA) combined with a partial redundancy analysis (RDA) and (2) landscape resistance (or isolation by resistance) using maximum likelihood population effects models (MLPE). Both methods were conducted at the individual level, using the genotype of each platypus as an independent observation (as opposed to site- or population-level sampling). Each of these methods is spatially explicit and incorporates landscape features directly into the analysis. The key difference between them is that the sPCA/RDA analysis is based on the landscape immediately surrounding each sample, while the landscape resistance/MLPE analysis was based on a continuous landscape surface, incorporating environmental variation across the entire study region.

2.8 | Partial Redundancy Analysis

We explored relationships between genetic variation and geographic distances between study sites using sPCA (Jombart 2008; Jombart et al. 2008) in *adeigenet* (Jombart 2008) in R 1.2.1 (R Core Team 2022). This approach detects spatial genetic patterns based on genetic variance among sampling locations and its correlation to distances between locations (Jombart et al. 2008). Moran’s I is used to measure spatial autocorrelation and the analysis retains positive eigenvalues that correspond to global structures (cline, patch, or intermediates) and negative eigenvalues indicate local patterns (strong differentiation between neighbours) (Jombart 2008; Jombart et al. 2008).

We then used partial redundancy analysis (pRDA) to identify landscape features associated with genetic structure of platypus at local sites, while controlling for spatial location. Partial RDA is a constrained ordination technique that models multivariate response data (in our case multilocus genotypes) to separate the relative contribution of landscape and spatial components on genetic structure (Legendre and Legendre 1998). We analysed the spatially lagged scores from genotypic data and used the first two principal components retained by the sPCA as response variables (Kierepka and Latch 2016; Latorre-Cardenas et al. 2020). We also conducted a permutation procedure with 999 randomisations to test for significant global (‘global.rtest’) patterns in the data. Our six landscape features (Table 1) were used in the model as predictor variables.

Spatial autocorrelation can violate assumptions of independence among data points, inflating Type I errors (Kierepka and Latch 2016). This can be tested for in pRDA using Moran’s I, which identifies spatial autocorrelation in the data. Geographic coordinates for each sampling location were used as a conditional variable to control for geographic distance (and therefore, spatial autocorrelation).

The pRDA analysis was conducted in the *vegan* package (Oksanen et al. 2022) following Kierepka and Latch (2016). We first determined the variance inflation factor (VIF) of each explanatory variable and excluded variables with a VIF > 10. We ran a null model (with no environmental variation) using the *ordistep* function (Oksanen et al. 2022) and conducted forward stepwise model selection to find the predictor variables that best explained genetic differentiation among individuals (Oksanen et al. 2022). The *anova.cca* function was used to test the significance of the models. The final model was selected as the model with the lowest Akaike’s Information Criterion (AIC) (Nakagawa and Schielzeth 2013) and that which improved the fit over the null model by $\Delta AIC > 12$.

2.9 | Isolation by Resistance

For analysis of isolation by landscape resistance, we calculated a genetic distance matrix as the response variable, derived from the Euclidean distance between all pairs of individual platypuses (435 distance observations from 30 samples), using the *dist* function in base R. This is widely used as an individual based genetic distance metric in landscape genetics (Beninde, Wittische, and Frantz 2024) and regularly correlates highly (over 90%) with other measures of genetic distance (Shirk, Landguth, and Cushman 2017). To generate continuous environmental resistance surfaces as predictor variables, we used CIRCUITSCAPE to calculate pairwise resistances between all 435 pairs of samples (McRae and Beier 2007; Shirk, Landguth, and Cushman 2018).

We conducted an analysis of isolation by resistance to determine how landscape resistance surfaces affected genetic differentiation (McRae and Beier 2007) using maximum likelihood population effects model (MLPE) in the ResistanceGA package (*MLPE.lmm* function; Clarke, Rothery, and Raybould 2002). This mixed-effects model includes a random effect term to account for the potential correlation among genetic distances for all $n-1$ pairwise distances involving each individual platypus

(Clarke, Rothery, and Raybould 2002; Shirk, Landguth, and Cushman 2018; Kozakiewicz et al. 2019). We fitted six separate models, with genetic distance modelled as a function of each landscape distance matrix separately. We also included a null model with no predictor variation as a baseline against which to compare the parametrized models.

Models fit with restricted maximum likelihood (REML) cannot be compared with AIC when the fixed effects differ among models (Clarke, Rothery, and Raybould 2002). Thus, we used the Maximum Likelihood estimator (REML=FALSE in MLPE.lmm()) to enable model comparison with AIC (Shirk, Landguth, and Cushman 2018; Thatte et al. 2020). The top model was selected using AIC as described for the pRDA. As an estimate of model fit with and without random effects, we calculated conditional ($R^2_{(c)}$) and marginal ($R^2_{(m)}$) R^2 values, respectively (Nakagawa and Schielzeth 2013), using MuMIn (Barton 2022) in R.

3 | Results

3.1 | Loci Under Selection

We detected 1061 outlier SNPs, 1036 with PCAdapt and 25 with LFMM (Figure S4). The three Principal Components (PC) used in LFMM represented correlations among elevation, July average temperature and January average temperature (PC1), NDVI and antecedent rainfall (PC2) and TWI (PC3). In examining environmental associations with outlier loci, LFMM detected one SNP related to PC1, 22 SNPs related to PC2 and three related to PC3 (Figure S4). Five loci common to both methods were removed from the final dataset.

3.2 | Genetic Structure

The number of genetic clusters (K) among neutral platypus genotypes identified by fastSTRUCTURE was between $K=1$ (model complexity maximising marginal likelihood) and $K=4$ (model components used to explain structure in the data) (Figure 1b). At the south of the Pine basin, three samples clustered with samples from the Brisbane basin, possibly because of the proximity of waterways, with adjacent waterways separated by approximately 1 km.

3.3 | sPCA and RDA Analysis

The sPCA analysis identified global (observed: 0.065, $p=0.001$) but not local (observed: 0.05, $p=0.66$) genetic structure in the overall platypus sample (Figure S5). The first two sPCA axes explained the most variance and had the highest eigenvalues (eigenvalues (variance)=63.27 (255.02) and 31.01 (152.76) for Axis 1 and 2, respectively) (Figure S6a,b). Both axes exhibited high levels of spatial autocorrelation (Moran's $I=0.99$ and 0.81 for Axis 1 and 2, respectively) indicating isolation by distance.

The first sPCA axis revealed genetic differentiation between the north and south of our study region, indicating that individuals

from the Mary hydrological basin are genetically different from those in the Brisbane basin (Figure 2a). The second sPCA axis suggested that platypus, as a species in the Pine and South Coast basins were genetically differentiated, while individuals from the north Mary and Brisbane basins were genetically similar (Figure 2b). Taken together, these analyses suggest that different parts of the platypus genome have a variable influence on spatial genetic structure. In the pRDA analysis, TWI ($F=6.38$, $p<0.025$) and antecedent rainfall ($F=8.27$, $p<0.015$) were retained after model selection, indicating that these environmental features influence genetic structure (Table 2, Figure S7).

3.4 | Landscape Effects of Genetic Variation

The MLPE analysis (where the response variable represented genetic distance in all models) revealed that NDVI explained gene flow between individual platypus samples at a fine scale (100 m) (Table 3a) while antecedent rainfall explained gene flow at a coarse scale (1 km) (Table 3b). In all cases, $R^2_{(c)}$ values were substantially higher than $R^2_{(m)}$ values (Table 3), indicating the random effects improved model fit.

4 | Discussion

Using a range of spatially explicit models for different spatial scales and sampling configurations, our analyses provided insights into the environmental factors influencing platypus gene flow across an urbanised landscape. We found a signal of genetic differentiation between north and south platypus samples that tended to correspond with the Mary and Brisbane hydrological basins. Genetic structure increased with increasing geographic distance (isolation by distance) and was also influenced by TWI, antecedent rainfall, temperature and vegetation cover (characterised by NDVI). Our analyses suggest that vegetation cover was more important driving spatial genetic structure at finer habitat scales, while rainfall and TWI operated at larger scales. This suggests that topographic, climatic and vegetation variables have a distinct influence on platypus gene flow in south-east Queensland, Australia. Our study indicates that increasing environmental water flow to maximise topographic water availability could help to maintain platypus genetic connectivity across the region.

The number of genetic clusters identified in our data ranged between $K=1$ and 4. There was some evidence that when K was more than one genetic structure, it corresponded to four hydrological basins across south-east Queensland, indicating that platypus, as a species are genetically structured by regional topography and river systems. There was no evidence that older samples (i.e., those obtained from museum specimens) were genetically differentiated from contemporary samples, so we considered genetic structure in our data to be spatial rather than temporal (Figure 1). Isolation by distance identified by the sPCA analysis confirmed genetic differences based on geographic positions between the north and south samples, suggesting restricted gene flow between catchments. Other studies of platypus have found that river basins tend to form discrete population units (Akiyama 1998; Kolomyjec et al. 2009; Gongora

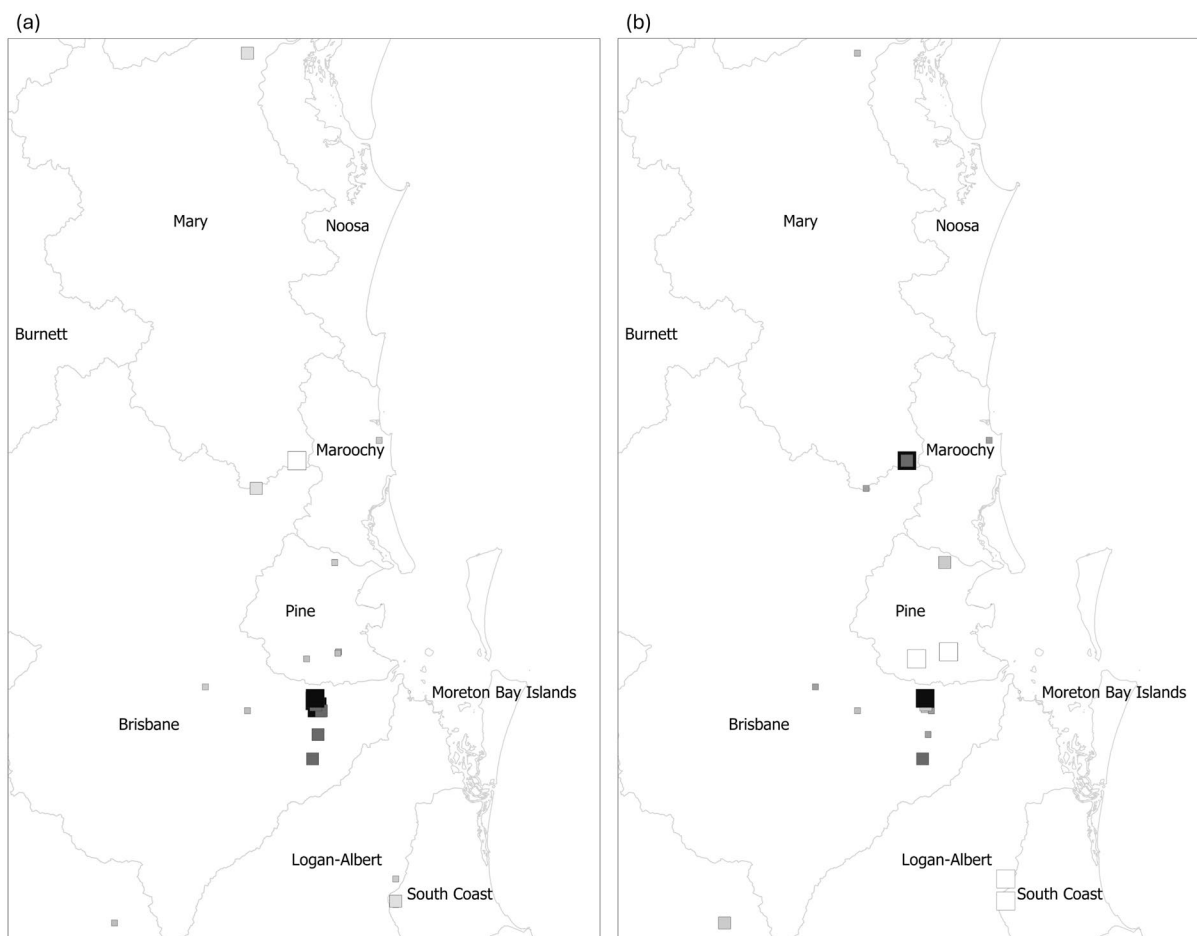


FIGURE 2 | Spatially lagged scores from the first two sPCA axes: (a) axis 1 and (b) axis 2, depicting patterns of genetic structure among north and south platypus (*Ornithorhynchus anatinus*) samples from south-east Queensland, Australia. Squares represent the spatial location of individual genotypes. The shading of the squares represents a gradient from positive sPCA values in white (genetic similarity) to negative sPCA values in black (genetic differentiation). The size of the squares corresponds to the magnitude of the score. Partial RDA suggested these axes of genetic structure were best explained by Topographic Wetness Index and antecedent rainfall.

TABLE 2 | Results of partial RDA analysis indicating environmental variables associated with platypus (*Ornithorhynchus anatinus*) genetic structure across south-east Queensland, Australia.

pRDA model	Parameters	<i>p</i>	AIC	Δ AIC	Variance	VIF
Final model	TWI + rainfall	0.003	129.94	0.00	35.93	—
FULL biological + conditional	—	0.011	130.60	0.66	36.81	—
	NDVI					1.34
	TWI					1.23
	Rainfall					3.41
	Latitude					1.49
	Longitude					2.61
Null	—	—	140.73	10.79	—	—

Note: In the final model, genetic structure was explained by Topographic Wetness Index and antecedent rainfall. The null and full models are shown for comparison, with Δ AIC relative to the final model. The null model included no variation on the predictor variable as a baseline for comparison. The models better than the null are indicated in bold.

Abbreviations: AIC = Akaike Information Criterion, NDVI = Normalised Difference Vegetation Index, TWI = Topographic Wetness Index, rainfall = 5-month total rainfall average, VIF = Variance Inflation Factor.

et al. 2012). Between adjacent river systems further south in their range (greater Sydney region, New South Wales), isolation by distance was also detected, indicating that major catchment

systems influence gene flow at a regional scale (Kolomyjec et al. 2009). Other semi-aquatic species, such as the Neotropical otter (*Lontra longicaudis*), similarly were found to have different

TABLE 3 | Results from maximum-likelihood populations effect models (MLPE) indicating environmental variables associated with gene flow in platypus (*Ornithorhynchus anatinus*) in south-east Queensland, Australia. Environmental resistances were analysed at (a) a fine-scale resolution for the topographic and vegetation features and (b) a coarse resolution for the climatic landscape resistance variables.

Predictor variable	Resistance	Log likelihood	AIC	Δ AIC	AIC weight	$R^2_{(m)}$	$R^2_{(c)}$
<i>(a) Fine-scale 100 m</i>							
NDVI	3.60	-1440.22	2888.42	0.00	0.81	0.13	0.64
Null	3.37	-1442.23	2892.45	4.03	0.11	0.11	0.65
TWI	3.48	-1442.59	2893.17	4.75	0.07	0.12	0.65
Elevation	5.64	-1458.54	2925.08	36.66	0.00	0.18	0.68
<i>(b) Coarse-scale 1 km</i>							
Antecedent rainfall	3.97	-1439.79	2887.59	0.00	0.59	0.16	0.65
Coldest month (July)	3.56	-1440.84	2889.67	2.08	0.21	0.12	0.66
Hottest month (January)	3.45	-1441.40	2890.80	3.21	0.12	0.12	0.65
Null	3.43	-1441.85	2891.71	4.12	0.07	0.12	0.65

Note: Candidate models are ranked by AIC. All models had an intercept of 88.44 and four degrees of freedom. The response variable represents genetic distance for both candidate model sets. The null model had no predictor variation and was included as a baseline against which to compare the parametrized models. The models better than the null are indicated in bold.

Abbreviations: AIC = Akaike Information Criterion. NDVI = Normalised Difference Vegetation Index, $R^2_{(c)}$ = Conditional R^2 , $R^2_{(m)}$ = Marginal R^2 , TWI = Topographic Wetness Index.

genetic groups represented by river basins (Latorre-Cardenas et al. 2020).

The landscape resistance analysis at the habitat scale (pRDA) demonstrated that genetic diversity was structured by antecedent rainfall prior to breeding season and Topographic Wetness Index. This reflects the high dependency of platypuses on water for connectivity within catchments (Serena et al. 1998, 2001, 2014; Grant and Fanning 2007; Coleman et al. 2022). Water availability is key to in-stream connectivity from a management perspective, particularly in times when platypus activity increases during the breeding season (July–October) and juvenile dispersal (February–March).

Isolation by resistance between sites using MLPE was explained by antecedent rainfall, and this model also demonstrated the influence of NDVI on gene flow in the platypus. Suitable habitat for platypus connectivity is evidently represented by both vegetation and water variables. These variables are similarly important for movement in many freshwater species which rely on vegetation cover for shelter and protection during dispersal and home range movements (Catterall, Lynch, and Jansen 2007; Baguette et al. 2013). For the platypus, vegetated riparian zones are important for the integrity of their habitat because stable banks allow burrowing, while vegetation cover conceals burrow entrances and provides in-stream shelter and habitat for their invertebrate food sources (Ellem, Bryant, and O'Connor 1998; Grant and Temple-Smith 1998; Serena et al. 1998). Urbanisation reduces the density of riparian vegetation cover and diminishes the protection it creates from noise and light pollution (Perkin et al. 2011; Newport, Shorthouse, and Manning 2014). A vegetation barrier can also conceal individuals from common predators known to consume platypus, such as foxes (*Vulpes vulpes*), cats (*Felis catus*) and dogs (*Canis lupus*) (Serena et al. 1998; Musser, Grant, and Turak 2024). Research from Victoria has found that platypus preferred stream reaches with higher forest

cover (Coleman et al. 2022). Therefore, a low NDVI indicating impervious areas or less dense and lower-quality habitats like open grasslands may reduce the opportunity for platypus to disperse in-stream and overland to nearby waterways. Overall, improving riparian vegetation may help increase movement because they are concealed as they swim, forage and disperse through the waterways, especially in an urban environment where threats intensify.

Managing freshwater ecosystems requires an understanding of landscape features that will influence genetic connectivity, and our study suggests that water and vegetation management are critical for the platypus. In other studies, habitat and connectivity restoration has been recommended for species such as the yellow-bellied toad (*Bombina variegata*) (Pröhl et al. 2021) and the Neotropical otter (*Lontra longicaudis*) (Latorre-Cardenas et al. 2020). Restoration activities include modified drainage or vegetation tunnels or overpasses to avoid roads and urban areas (Pröhl et al. 2021; Musser, Grant, and Turak 2024) and establishing biological corridors for stream and terrestrial dispersal (Latorre-Cardenas et al. 2020). Our results similarly suggest that restoration of hydrological connections within freshwater systems and the surrounding native habitat should be a priority for riparian management. In another study, the construction of dams and weirs was found to restrict dispersal and gene flow and increase genetic differentiation in platypus (Mijangos et al. 2022). Regional assessments of instream barriers to maintain connectivity among platypus populations should be a priority for management.

While it would be desirable to have a larger number of samples, the platypus is notoriously difficult to catch due to their low abundance at some sites and sampling difficulty during periods of drought and flood. Sensitivity analyses have found that sample sizes of < 10 individuals pose a risk of low power and incorrect genetic population assignment, while sample sizes of 30

or more have relatively strong power and low assignment error rates (Wollstein and Lao 2015; Wang 2022). Thus, our results provide evidence that genetic connectivity is limited by the environment despite our limited sample size. With more samples, we might have uncovered a more complex genetic structure and the estimates of genetic connectivity in this paper could be considered conservative. Estimates of population density in other parts of the platypus range (1.3–2.1 platypus per km of stream in Victoria (Serena 1994) and 2.8 per km of stream in NSW (Bino, Grant, and Kingsford 2015)) suggest a higher population density than our sampling was able to cover (with an average of 72 km between samples). Thus, more dense sampling in future is likely to yield more accurate and higher resolution genetic structure.

Overall, our study highlights the need for innovation in water management, such as constructed wetland or waterway modification that will help sustain water in the landscape and reduce the pressures of water availability for all species, particularly in times of drought (Zhang et al. 2020). This would benefit other species conservation, such as fish and turtles which co-occur with platypus, because they similarly rely on water and deep connected pools. Threatened species such as Mary River Cod (*Maccullochella mariensis*) and Australian Lungfish (*Neoceratodus forsteri*) prefer areas with dense vegetation cover, overhanging vegetation and connected stream areas with pools (Simpson and Jackson 1996; Arthington 2009). It is also important to understand the management of environmental flows from dams and weirs during seasonal periods such as breeding and juvenile dispersal as increased water availability from dam release will help overall system connectivity (Hawke, Bino, and Kingsford 2021; Mijangos et al. 2022). The quality and density of riparian zones should also be assessed so that habitat integrity and vegetation cover can be increased. If water, food and shelter are protected at a catchment scale, platypus will not have to disperse into human-modified areas and risk their survival.

In conclusion, our findings emphasise the critical need for restoring hydrological connections within freshwater systems to maintain genetic connectivity among platypus populations. Additionally, evaluating the quality and density of riparian zones is essential to enhance habitat integrity and vegetation cover to help mitigate threats to platypus populations and promote their long-term conservation.

Author Contributions

T.B. and A.L.S. conceived and designed the study. T.B. performed the material preparation, data collection and analysis, with guidance from A.L.S. The manuscript draft was written by T.B. and both authors contributed to subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Ethics Statement

This research was conducted in accordance with the University of Queensland's Animal Ethics Unit—Native and exotic wildlife and marine animals (SAFS/139/18). We worked under permits from the Queensland Department of Environment and Science (WA0011257 and PTU19-001728), Queensland Department of Agriculture and Fisheries (202383), local council authorities, Brisbane City Council (PC-3579 and West region), Sunshine Coast Council (REG20/0053), Ipswich City Council (#14167), Moreton Bay Regional Council (A20995133) and SEQ Water (GCK20200831).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and/or analysed during the current study are available in the Zenodo public repository <https://doi.org/10.5281/zenodo.14188756>.

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Supporting Information

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