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Original research article

Historic and contemporary selection define conservation units for a short-range endemic within an anthropogenically-altered riverscape

Zachery D. Zbinden [a,1], Tyler K. Chafin [a,2,3], Jeremy S. Tiemann [b,4], David R. Edds [c,5], Bradley T. Martin [a,6,7], Jordan Hofmeier [d,8], Michael E. Douglas [a,*,9], Marlis R. Douglas [a,10]

[a] Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA
[b] Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign-Urbana, 61820, USA
[c] Department of Biological Sciences, Emporia State University, Emporia, KS 66801, USA
[d] Ecological Services, Kansas Department of Wildlife and Parks, Pratt, KS 67124, USA

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ABSTRACT

Numerous selective forces act upon allelic frequencies of broad-ranging biodiversity, yielding well-represented conservation units (CUs). Yet these are much less apparent in range-restricted forms where anthropogenic impacts, topography, and population structure are minimized. Here, short-range-endemics (SREs) are noteworthy for having historic habitats abridged, temporal recolonizations curtailed, and anthropogenic extinctions accelerated. Their CUs/intermediate stages contrast with conventional perceptions regarding evolution/natural history and thus are often overlooked. Herein we evaluate both for an SRE (Madtom catfish). We do so by deriving/ evaluating 2725 genomic DNA loci/SNPs (one/read) from N = 178 non-lethally sampled individuals (plus N = 57 outgroups) from the Neosho River Basin (KS/OK/MO; USA). Six significantly different populations were sequentially identified, with dispersal significantly constrained by downstream impoundments (N = 14 low-head dams; N = 2 reservoirs; timespan >100 years). Flow regulation/fragmentation were identified as the most strongly associated of eight environmental variables. Genotype-environment analyses (GEA) revealed localized adaptive differences among populations, with N = 61 loci significantly associated with the environment. Redundancy analyses (RDA) identified strong correlations between genetic and environmental variances across two axes: Hydrologic-physiographic (N = 20); Landcover

* Corresponding author.
E-mail address: med1@uark.edu (M.E. Douglas).
[1] https://orcid.org/0000-0002-3709-224X
[2] Present Address: Biomathematics and Statistics Scotland, Edinburgh, UK
[3] https://orcid.org/0000-0001-8687-5905
[4] https://orcid.org/0000-0001-7635-1403
[5] https://orcid.org/0000-0001-9475-7378
[6] Present Address: Biological Sciences, Seton Hall University, South Orange, NJ USA 07079
[7] https://orcid.org/0000-0002-3014-4692
[8] https://orcid.org/0000-0002-9975-0551
[9] https://orcid.org/0000-0001-9670-7825
[10] https://orcid.org/0000-0001-6234-3939

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(N = 18). Stream reach and location juxtaposed with local adaptation along the longitudinal and directional gradients of important environmental variables. The 61 loci associate with N = 30 genes: Anatomical-developmental (head, eyes, brain); Response to stimuli (stress, chemical, hypoxia); and Metabolism. Our results not only demonstrate CUs/intermediates can be identified within SREs, but also characterize for NMT an apparent response to long-term, anthropogenically-induced habitat perturbation. Long-term persistence for CUs/intermediates can be promoted via spatial conservation (SCP).

1. Introduction

The term 'conservation unit' has scientific name-recognition but is often employed anecdotally in many non-scientific evaluations (i.e., geographic, legislative, jurisdictional, etc.). A general definition would be a population of organisms considered distinct for purposes of conservation (Funk et al., 2012), whereas a more specific interpretation would incorporate three components: (1) A biological entity (species, subspecies, population); (2) Associated cultural/political issue(s); and (3) Evaluations subsequently translated into geographic units (Breitenmoser et al., 2012).

The designation of appropriate CUs is the first objective for species conservation, and includes a focus on diversity and distinctness as metrics for 'intermediate stages' (e.g., differentiated population groups; Hausdorf, 2021). These have been documented both globally, (Convention on Biological Diversity; https://www.cbd.int) and regionally (COSEWIC: Committee on the Status of Endangered Wildlife in Canada (2018) (https://cosewic.ca/index.php/en-ca/reports/preparing-status-reports/guidelines-recognizing-designatable-units); U.S. Endangered Species Act (ESA)) (see Allendorf et al., 2022, Box 20.1) for CUs so defined).

While neutral genetic markers serve to diagnose diversities and demographic connectivities, adaptive markers identify intermediate CU stages (Flanagan et al., 2018). Yet, the latter are often problematic in their diagnoses, particularly given rapid shifts in climate (Capblancq et al., 2020; Bernatchez et al., 2024), or an inherently weak population structure. The latter may instead shift the CU-diagnosis towards landscape-induced allele frequencies (Wang and Bradburd, 2014; Ruiz-Gonzalez et al., 2015; Turbek et al., 2023). Yet, one benefit in searching for intermediate groups is the potential for locating them despite a lack of overall structure (Whitlock, 2014), often noted within broad geographic distributions (Bernatchez et al., 2019; Canales-Aguirre et al., 2022; Rougemont et al., 2023; Miller et al., 2024). In this sense, historically separated (and genomicaly identified) populations are more apparent when intraspecific CUs span a broader geographic distribution (Barbosa et al., 2018; Fernandez-Fournier et al., 2021; Schweizer et al., 2021).

We broaden and extend the search for CUs and their applicability by establishing a protocol by which appropriate assays can be attempted within a relatively untouched category of biodiversity (i.e., the short-range endemic, SRE). Our approach supports three (of 17) UN 2030 Sustainable Development Goals (https://sdgs.un.org/goals), as well as eight (of 23) targets in the Kunming-Montreal Global Biodiversity Framework (https://www.cbd.int/gbf/targets/). As such, it contributes substantially towards an approach developing but slowly on the global stage (Heuertz et al., 2023), due primarily to its reduced biogeographic template.

1.1. Short-range endemics (SREs)

These represent range-limited biodiversity with distributions that approximate 10,000 km^2 (Harvey, 2002). Of note, we consider our study species an SRE in that its distribution is riverine (i.e., linear) rather than lacustran (i.e., area-based). SREs are particularly vulnerable to anthropogenic degradation, with active modification of both occupancies and phenologies (Lavergne et al., 2004, Mason et al., 2018). This, in turn, exacerbates the sampling biases already inherent for biodiversity elements within constrained geomorphic environments (Eberhard et al., 2009; Mentges et al., 2021; Nuñez-Penichet et al., 2022).

SREs also possess an ecologically restricted natural history, to include a specialized niche, small population size, low reproductive output, and limited dispersal capacity (Botts et al., 2013; Davis et al., 2015; Gretgrix et al., 2023). The latter is particularly concerning, given the global prevalence of ongoing environmental change (Newbold et al., 2018; Arana et al., 2023). In addition, standardized sampling protocols are ill-suited for such specialized environments, such that the limited monitoring capacities of federal and state agencies become greatly over-extended (Leidy and Moyle, 2021; Wangmo et al., 2022). Importantly, when SREs become targets for intense habitat modification (as herein), the juxtaposition of niche and life history renders them increasingly susceptible to anthropogenic extirpation (Tomlinson et al., 2020).

Unfortunately, the limited ranges and restricted natural histories of SREs (Dubos et al., 2022) also act synergistically to impose upon agencies the additional burden of a shifting baseline (i.e., where acceptable protocols are gradually forgotten over time and those more contemporary but less efficient are instead employed; Pauly, 1995; Jönsson et al., 2021). Shifting baselines can also promote extinction vortices which are more pronounced in small-bodied vertebrates where declines are comparatively more predisposed (Williams et al., 2021).

Management plans for SREs must therefore be continually readjusted on a serial basis to recalibrate those natural history components impacted by the small population paradigm (which underscores the liabilities of small populations that facilitate decline-to-extinction: Caughley, 1994; Fig. 4 of Mussmann et al., 2017). This can be best accomplished through a broad-scale, non-lethal program based on collection/analysis of genomic DNA (Barbosa et al., 2018). The coupling of genomic variability with features of the landscape/riverscape allows both dispersal and local adaptation to be effectively quantified (Nielsen et al., 2023). A non-lethal approach also fosters the capacity for such data to be collected iteratively (i.e., addressing conservation objectives that shift temporally as

climate impacts relentlessly advance; Miller et al., 2024).

1.2. Study species

The Neosho Madtom (NMT: *Noturus placidus*; Fig. 1) is a diminutive catfish (<75 mm TL) endemic to the Neosho River system (SE Kansas, SW Missouri, NE Oklahoma, NW Arkansas). It is found within medium-sized streams, most often beneath rocks in clear-water riffles with moderate-to-strong current (Wildhaber et al., 2000). It has a short life-span (1–2 years), reproduces within a single season, and deposits eggs in cavities beneath larger substrate (Bulger et al., 2002). Juveniles contrast twith adults by inhabiting shallower habitats with slower flows with more moveable substrates (Bulger and Edds, 2001).

NMT has lost ~33 % of its original range primarily due to habitat loss and impoundment-induced fragmentation (USFWS, 1991). Its current range encompasses 20,374 km^2 (with 10,114 km^2 in Kansas; Parenthetically, we recognize NMT as an SRE given that its linear (i.e., riverine) distribution does not occupy the entirety of the two-dimensional topographic estimates presented above). Its distribution encompasses: (i) Neosho and Cottonwood rivers upstream of John Redmond Reservoir (KS); (ii) Neosho River downstream of John Redmond (KS, OK); and (iii) Spring River (MO/KS/OK/AR; Fig. 2) (USFWS, 1991). Anthropogenic activities have heavily impacted the system via heavy metal contamination, gravel mining, and impoundments, with the latter consisting of 20 dams (four substantial and federal; 20 low–head) (Fencl et al., 2015). The latter were established 1860–1995, with nine in-place for over a century (Table S1; Figs. 2, 4).

Abundance of NMT is significantly, positively associated with mean annual stream flow (Davis and Paukert, 2008) and significantly reduced above/below low-head dams (Tiemann et al., 2004b). NMT status was evaluated (USFWS, 2013), with greatest impacts (i.e., timing and periodicity of flows) recorded below John Redmond Reservoir. These, in turn, impact habitat quality, quantity, reproductive activity, recruitment, and the invertebrate food base.

Herein, we provide a case study for the adaptive management of a threatened (or data-deficient) SRE (Hogg et al., 2022). We do so by exploring the extent and distribution of its CUs (and intermediates) then testing if these juxtapose with various isolating factors driven by topography and environment. We accomplish this by quantifying thousands of SNP markers (Vaux et al., 2023) which allows the interpretation of key natural history components: (i) Population structure (i.e., presence of conservation units); (ii) Genetic diversity (i.e., potential for persistence); and (iii) Eco-evolutionary drivers (i.e., capacity for long-term persistence). These, in tandem, successfully define short- and long-term management goals which focus on sustaining SREs as essential components of regional biodiversity.

2. Methods

2.1. Sampling and tissue acquisition

Sampling was conducted from 8–13 August 2021 at 19 sites across the Neosho River System (Appendix A). Collections were performed within a 4.5 m^2 area at each site by agitating the substrate 3 m upstream from a stationary seine (1.5 m wide; 3 mm mesh). Captured *Noturus* were temporarily retained within an aerated container of stream water. Fish were non-lethally processed, with upper caudal fin lobes excised/secured for molecular analysis (University of Illinois IACUC protocol #20123).

Fig. 1. Neosho Madtom (Ictaluridae; *Noturus placidus*. (Photo: Greg Sievert, Emporia, KS 66801).

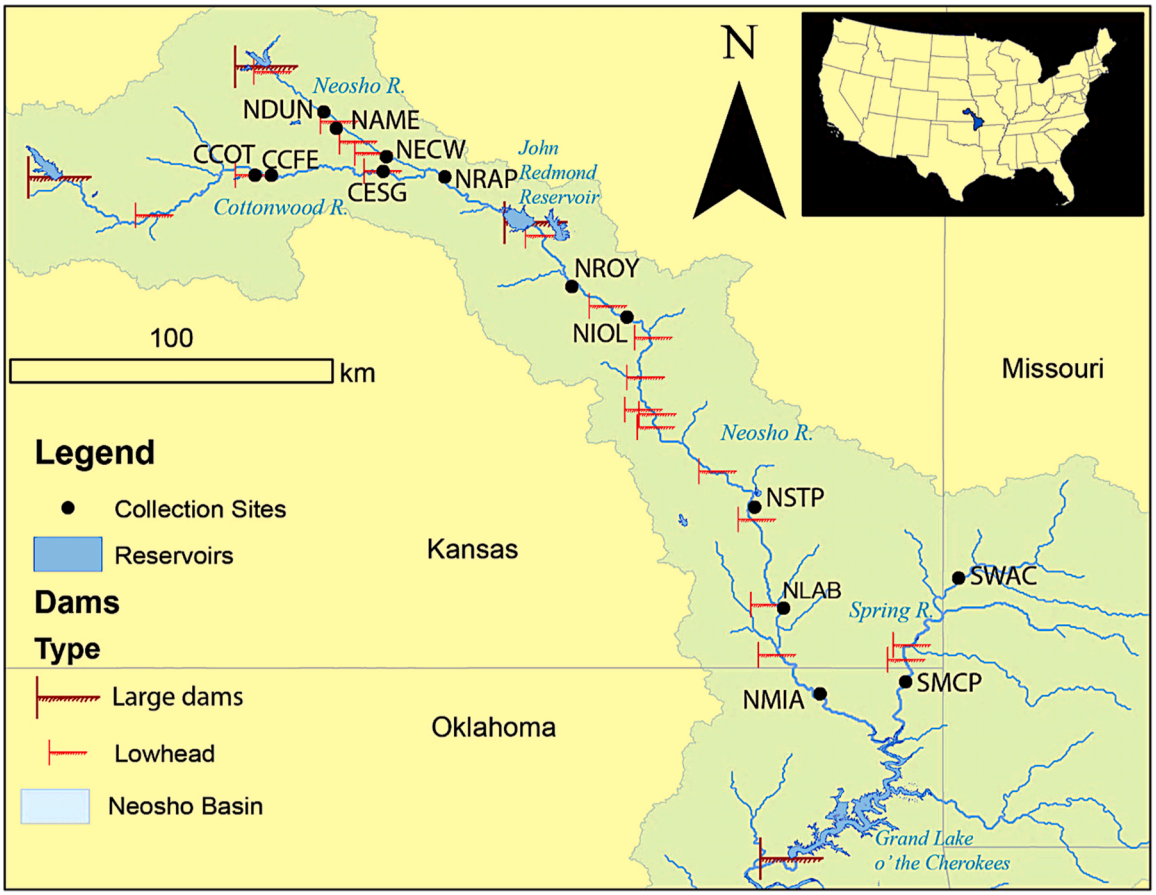

Fig. 2. Map of the Neosho River system and major tributaries (Cottonwood and Spring rivers) with sampling locations for Neosho Madtom (N = 14; Closed circles with site ID). Light green highlighted area encompasses the Neosho River watershed. The system is highly impacted, with dams denoted by transverse red symbols. John Redmond Reservoir (below NRAP) demarcates northern and southern sites on the Neosho River. Grand Lake o' the Cherokees separates Spring River sites from mainstem.

2.2. Genomic data acquisition

DNA was extracted using the QIAamp Fast DNA Tissue Kit protocol (Seufi and Galal, 2020), with quantity/ quality assessed via fluorometry and gel electrophoresis. We employed double digest restriction-site associated DNA sequencing (ddRAD; Peterson et al., 2012) to subsample DNA regions from each individual (see Appendices B, C). Our initial data processing employed standardized bioinformatic screening across individuals and study sites to eliminate low-quality sequences (IPYRAD; Eaton and Overcast, 2020; Appendix B), optimize accuracy, and minimize missing data (Chafin et al., 2017; Eaton et al., 2017). However, there are no universally accepted guidelines for data filtering (Hemstrom et al., 2024). Given our study species is a short-range endemic, a minimally stringent filtering criterion was employed to remove only anomalous loci (e.g., those off-target or contaminants). In this sense (per Huang and Knowles,2016), overly restrictive filtering truncates the observed mutation spectrum, biasing against high mutation rates. Our 50 % within-species threshold represented the observed upper quartile-bound for our study species, and its application removed only the observed 'long tail' which likely represented contamination or off-target loci (e.g., DaCosta and Sorenson, 2014). This procedure has also been previously employed as a precedent by our lab (Bangs et al., 2020).

For the among-species data, our standard was relaxed to 75 %, given that exactly 24 % of samples represented outgroup species. Here, missing data tends to be both phylogenetically structured and associated with mutation-disruption (Eaton et al., 2017). The goal was to characterize potential hybridization within N. placidus, and to ensure this, filtering our multi-species data at 75 % meant that if a locus is present in all outgroups, it must also be observed in 1–2 *N. placidus* to be retained. Again, our lab has previously replicated this process (Mussmann et al., 2020), but using 67 % versus 75 %, as the former more specifically fit ingroup versus outgroup size.

We then employed an NMT genome (Whitacre et al., 2022) as a reference to align our data. The result was a comprehensive panel of N = 2725 loci genotyped across individuals. We assessed fine-scale population structure, explored environmental factors correlating with genetic divergence, and assayed for signs of local adaptation (Appendix B).

2.3. Inferring population structure

We employed three clustering methods to ascertain whether NMT persists as a single or multiple genetic populations (Appendix C). These were: (i) *K*-means clustering paired with Discriminant Analysis of Principal Components (DAPC; Jombart et al., 2010), which groups individuals into a specified number of distinct clusters (*K*); (ii) t-distributed Stochastic Neighbor Embedding (t-SNE; van der Maaten & Hinton, 2008), which highlights local structure in two dimensions by visualizing the non-linear dimensionality reduction of the data; and (iii) ADMIXTURE: A biogeographic ancestry analysis (Alexander et al., 2009), which infers genetic populations using a model-based estimation of individual ancestry.

Once genetically discrete clusters were identified, two approaches were used to evaluate within and among genetic diversity. We first quantified variation at three hierarchical levels: Among populations; Among sites within populations; and Among individuals within site (analysis of molecular variation, AMOVA; Excoffier et al., 1992). We could then gauge if differences existed among major groups (e.g., river regions or populations) or were instead more localized (e.g., sites). We refrained from significance testing and instead reported only variance components (Meirmans, 2015). We then calculated pairwise genetic divergences using an unbiased F_{ST} estimate (G''_{ST}; Meirmans and Hedrick, 2011), with values ranging from 0 (no difference) to 1 (complete separation). We employed bootstrap re-sampling and Bonferroni correction to ascertain if differences were significantly greater than zero. As a cautionary note, testing for significant differences among groups defined a priori by their differences—as with AMOVA—is clearly circular (although widely done).

2.4. Riverscape genomics

We hypothesized that heightened levels of genetic divergence would be manifested between populations when an environmental feature (e.g., a dam) was a barrier to dispersal. This inference could only be accomplished indirectly by examining how observed patterns of genetic divergence relate to environmental characteristics (Appendix C).

To do so, we first utilized autoStreamTree (Chafin et al., 2023a) to map pairwise genetic divergence (F_{ST}) onto a graph network of stream segments. Genetic divergence was thus estimated for each segment within the NRS. Next, we employed the standardized genetic fragmentation index (F_{INDEX}: Prunier et al., 2020) to assess if genetic divergence was promoted by presence of dams. We generated a pairwise standardized index of impact for populations immediately above and below each barrier, expressed as the ratio of observed Fst to the maximum theoretical divergence predicted under simulations (see Prunier et al., 2020 for more information on parameterization and prior specification). Prunier et al. (2020) also established the following from their simulations: (a) Confidence intervals overlapping with Findex < 0.20 indicated no impact on gene flow, and; (b) A Findex > 0.90 designated total cessation. These are the thresholds we adopted herein.

Finally, we employed ResistNet (Chafin et al., 2023b) to model effects of river segments on genetic divergences among samples. A comprehensive array of environmental features ($N = 281$) was derived from HydroATLAS v.0.1 (Linke et al., 2019), then augmented by calculating variables reflecting dam effects: (i) Barrier density per river segment; (ii) Age of oldest barrier within a segment; and (iii) Indices of river fragmentation and connectivity (Grill et al., 2019). We used a random forest regression approach (Pedregosa et al., 2011) to ascertain the association of each variable with genetic divergence (F_{ST}). We then modeled resistance to dispersal by employing the retained environmental features as input to ResistNet.

2.5. Estimating population genomic parameters

Genetic diversity in a population underscores its viability, resilience, and adaptability to changing environmental conditions. We subsequently derived several such estimates from our genotype data, including: (i) Mean allelic richness per locus; (ii) Enumeration of unique alleles (private alleles); (iii) Magnitude of inbreeding (F_{IS}); (iv) Expected/observed heterozygosities; and (v) Evolvability (Shannon Index).

We also calculated genome-wide heterozygosity (H_{GW}) for comparison with similar studies by retaining polymorphic and non-polymorphic loci from our unfiltered alignment. Genome-wide heterozygosity is a more appropriate cross-study comparison as heterozygosity based solely only on polymorphic loci will be biased upwards (Schmidt et al., 2021). Finally, we also estimated N_e (NeEstimator v.2; Do et al., 2014), which determines rates of genetic drift, inbreeding, and loss of genetic variability in the context of natural selection (Charlesworth, 2009) (Appendix C).

2.6. Loci under selection and local adaptation

We assessed signals of local adaptation via genotype-environment association analysis (GEA; Lotterhos and Whitlock, 2015). We compiled a comprehensive set of environmental variables ($N = 281$) via HydroATLAS v.0.1 and categorized them into five groups: (i) Hydrologic-physiographic; (ii) Climate; (iii) Landcover; (iv) Geology-soils; and (v) Anthropogenic characteristics. To mitigate collinearity, variables within each category were consolidated into composite variables using Robust Principal Component Analysis (ROBPCA; Reynkens, 2018).

We evaluated the relationships between loci and composite environmental variables using Redundancy Analysis (RDA), a multivariate extension of multiple linear regression (Forester et al., 2018). We inferred adaptive loci as those deviating more than ± 3 standard deviations from mean loadings on the canonical axes predicting genotype-environment correlations. The expectation for most loci is neutrality (i.e., non-adaptive), as they either occur in non-coding genomic regions or yield synonymous mutations that do not

alter amino acids (Kimura, 1991). Variation due to population structure (e.g., via partial RDA) was also retained. The simple RDA approach has a superior combination of false-positive/negative rates when selecting outlier loci using a conservative threshold (3 sd ± mean) and relatively weak global population structure ($F_{ST} \approx 0.05$; Forester et al., 2018), as found in NMT ($F_{ST} = 0.054$).

Our next objective was to discern whether distinct populations or areas exhibited variability in local adaptation (i.e., diverse adaptations to different environments). To do so, we first repeated our population structure analysis but only with loci identified in the RDA as potentially adaptive. We then employed t-SNE, given its proficiency in rapidly and accurately depicting hierarchical structure. We hypothesized that geographic regions with different local adaptations would form separate clusters within the t-SNE ordination.

Finally, we validated the biological importance of those loci by exploring the biological processes that might drive local adaptation. To do so, we first matched functional annotations (i.e., known genes via the Channel Catfish (*Ictalurus punctatus*) genome; Liu et al., 2016) against the NMT genome (Whitacre et al., 2022) which lacks functional annotations. This was done via an iterative alignment procedure (Shumate and Salzberg, 2021) that maps exons and transcripts. Functional effects and gene associations of the adaptive loci were then predicted using SnpEff (Cingolani et al., 2012), with gene ontologies compiled (QuickGO; Binns et al., 2009) (Appendix C).

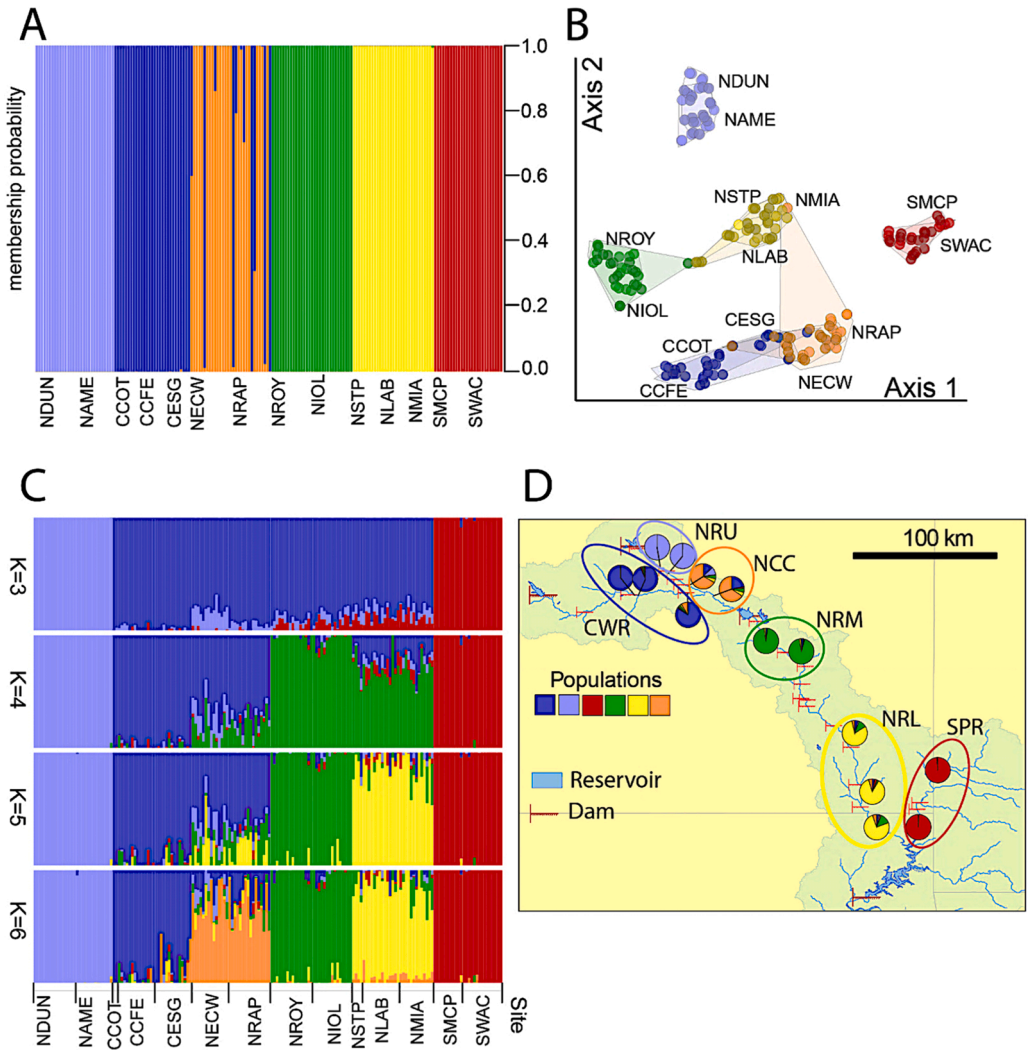

Fig. 3. Multiple clustering approaches were employed to group Neosho Madtom (N = 178) genotyped across N = 2725 loci These are: (A) Membership probabilities based on Discriminant Analysis of Principal Components (DAPC) as defined using *K*-means clustering; (B) Clusters based on t-distributed stochastic neighbor embedding (t-SNE) with sites colored per inferred populations; (C) Four models of hierarchical population structure via ADMIXTURE, from three (*K*=3) to six (*K*=6); and (D) Potential Management Units (and three-letter codes) based on population structure of Neosho Madtom, with genetic composition at each sampling site depicted as a pie chart representing proportion of population ancestry based on ADMIXTURE analysis (*K*=6).

3. Results

3.1. Sampling and genomic data

We sampled N = 19 NRS sites and captured NMT at 14, yielding N = 192 tissue samples (Appendix A). Genotypes for N = 185 were subsequently incorporated as an alignment (Ipyrad) consisting of 9345 SNPs across 5584 reads (100 base-pair regions). After filtering (Appendix B), our genetic panel contained 2725 loci/SNPs (one SNP/read) across N = 178 individuals (Appendix D). To guarantee accurate estimates of genetic divergence, next-generation sequencing data should encompass eight or more individuals per location (Nazareno et al., 2017).

Mean sequencing depth (i.e., how many times a particular locus was represented by a sequence; Peterson et al., 2012) was elevated (=78.5x), thus supporting the accuracy of our genotype data. The mean of missing-data-per-individual was low (11.1 %), again supporting the validity of our SNP dataset (Zbinden et al., 2023a,b).

3.2. Population structure

NMT clustered into six genetic populations (Fig. 3). Genetic structure was hierarchical, with upper Neosho River (NDUN & NAME) and Spring River (SMCP & SWAC) relatively more diverged (Fig. 3C). Admixture validation indicated both K= 2 and K= 6 were better populations models than K= 1 (Appendix E). Given that not all populations were equally divergent, and two differed much more, the optimal, cross-validation error (a measure of fit) indicated K= 3, with log-likelihood (a second measure) failing to plateau as population numbers increased (Appendix E).

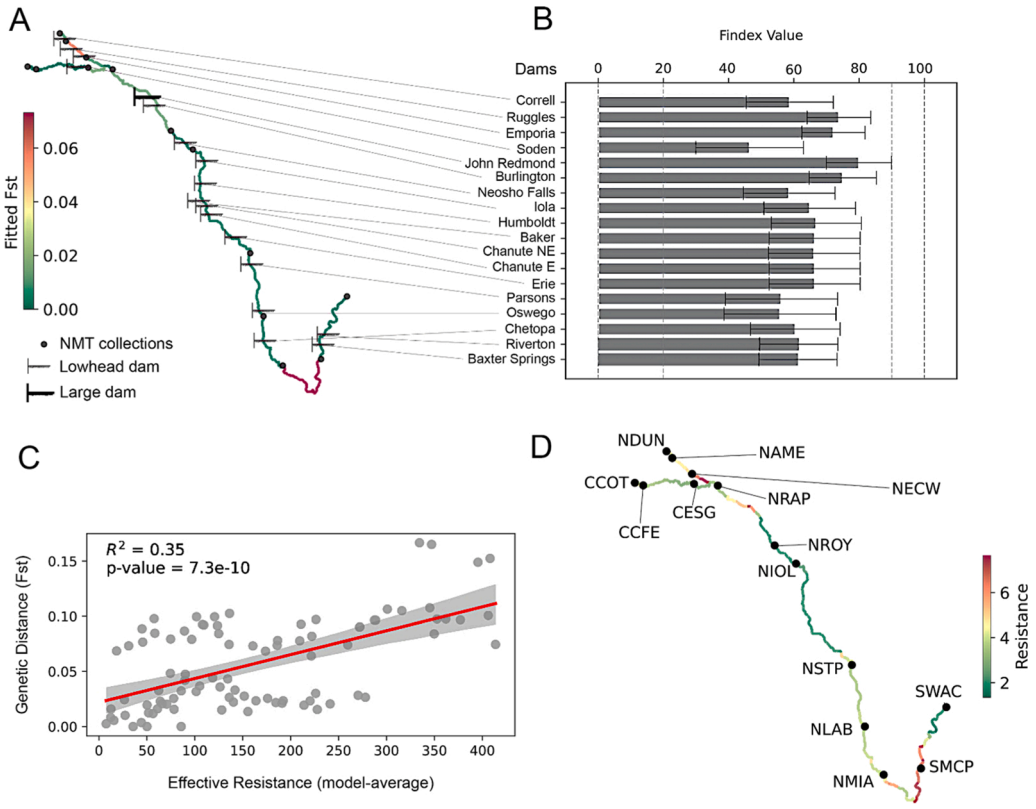

Fig. 4. Riverscape genetic analysis of population connectivity for Neosho Madtom. Results are inferred from an analysis of SNP data for N = 178 individuals collected across 14 sites and genotyped across N = 2725 loci. (A) Neosho River system with NMT collections and dams. River segment colors reflect the level of potential isolation for each river segment, based on fitted genetic divergence (F_{ST}) per river reach segment using the StreamTree model, with estimates ranging from low (green) to high genetic divergence (red). Note: Only N = 18 dams (of 24 listed in Table S1) are positioned between NMT sampling locations and were analyzed here. (B) Dam effects on population connectivity (gene flow): Normalized index of genetic fragmentation (F_{INDEX}) where 0 =no barrier effect and 100 =complete reduction in gene flow (95 % confidence intervals are indicated). (C) Linear regression of pairwise effective resistance based on river network environmental factors, expressed as the sum of model-averaged reach-wise values along the least-cost distance path between samples, compared to their observed genetic divergence (F_{ST}). (D) Model-averaged effective resistance attributed to environmental factors within each stream reache, ranging from low (=0) to high (=10) resistance to individual movement. Six genetic populations: Cottonwood River (CWR), Upper Neosho River (NRU), Neosho-Cottonwood Confluence (NCC), Middle Neosho River (NRM), Lower Neosho River (NRL), and the Spring River (SPR).

Based on population structure (Fig. 3D), sampling sites were grouped into six potential Management Units (MUs): CWR (Cottonwood River); NRU (Upper Neosho River); NCC (Neosho-Cottonwood Confluence); NRM (Middle Neosho River); NRL (Lower Neosho River); and SPR (Spring River). Genetic variation was hierarchical, with AMOVA indicating 12.7 % attributed among populations, 1.5 % among sites within populations, and 85 % among individuals within sites.

Estimates of pairwise genetic divergence (F_{ST}) reflected isolation. Values were significantly different from zero (Bonferroni adjusted $p < 0.0014$), and ranged from 0.02 (CWR versus NCC) to 0.18 (NRU versus SPR) (Appendix F). Both NRU and SPR showed greatest divergence ($\bar{x}$ =0.12), with the remaining four less so ($\bar{x}$ =0.03). Results were consistent with the hierarchical structure observed via Admixture (Fig. 3C). We also note that John Redmond dam CIs overlapped with the 0.90 threshold established by Prunier et al. (2020). We interpret this as representing a 'complete' barrier,' a result also independently verified by our ResistNet analysis which identified that segment as demonstrating very high resistance.

3.3. Riverscape genomics

Mapping pairwise genetic divergences onto stream segments (autoStreamTree) revealed patterns consistent with our analysis of population structure (Fig. 4A). Divergence values reflect limited gene flow among populations, underscoring reduced connectivity due to dispersal barriers. The largest F_{ST} values (>0.05) were on segments separating SPR and NRU (the two most distinct populations) from the remainder (Fig. 4A). Intermediate F_{ST} values (>0.02) separated CWR from NCC, as well as NCC from downstream NRM and NRL.

Abundance of NMT is significantly lower immediately below low–head dams (Tiemann et al., 2004b) and this reduced gene flow and dispersal between river sections, with genetic divergence clearly impacted (F_{INDEX}; Fig. 4B). John Redmond Dam represents the most substantial barrier and is consequently associated with increased network resistance (via ResistNet). Two dam-related indices (i.e., degree of fragmentation and flow regulation) were among the eight most strongly associated in the network resistance model (relative importance >0.8; Appendix G). The degree of fragmentation approximates longitudinal resistance at the reach scale. In contrast, the second index (flow regulation) captures impoundment-driven fluctuations on a temporal basis (i.e., variability in flow plus a shift in timing of flow events). The remaining top-ranked variables relate to landscape features (soil types, landcover/vegetation, anthropogenic development, and extent of protected area; Appendix G).

3.4. Population genomic parameters

The most genetically distinct populations (NRU, SPR) also manifested lower overall genetic diversities (Table 1). However, SPR also

Table 1
Sample sizes, numbers of individuals evaluated, and local genetic diversity estimated per management unit (MU) of Neosho Madtom (*Noturus placidus*) in the Neosho River Basin, U.S.A.

MU	Ca/ FC /Seq /SNP	PA	AR	H_E	H_O	H_{GW}	F_{IS}	ENT	N_e
CWR	49/ 32/ 31 /30	21	1.72	0.206	0.206	0.00065	−0.006	0.321	2326
NCC	50/ 32 /31 /30	19	1.76	0.212	0.213	0.00067	−0.003	0.333	279
NRU	34/ 32 /31 /30	25	1.61	0.187	0.189	0.00061	−0.008	0.287	349
NRM	36 /32 /31 /31	20	1.54	0.291	0.209	0.00068	−0.01	0.29	1138
NRL	42/ 36 /34 /31	49	1.77	0.213	0.209	0.00067	0.015	0.336	1622
SPR	28 /28 /27 /26	77	1.5	0.255	0.193	0.00066	−0.005	0.273	404
	239/ 192/ 185/ 178								

All estimates were calculated based on $N = 2725$ genetic loci, except genome-wide heterozygosity (H_{GW}) based on the total unfiltered genomic alignment.
Ca: Numbers of individuals captured per MU.
FC: Number of individuals fin-clipped per MU.
Seq: Number of individuals sequenced per MU.
SNP: Number of individuals evaluated for SNPs per MU.
PA: Number of private alleles unique to each population.
AR: Mean allelic richness per locus (at most 2 for biallelic SNPs).
H_E: Expected heterozygosity across polymorphic sites.
H_O: Observed heterozygosity across polymorphic sites.
H_{GW}: Genome-wide observed heterozygosity (polymorphic and non-polymorphic sites).
F_{IS}: Mean inbreeding coefficient.
ENT: Shannon Information (entropy), a measure of evolvability.
N_e: effective population size.
CWR=Cottonwood River.
NCC=Neosho-Cottonwood Confluence.
NRU=Upper Neosho River.
NRM=Middle Neosho River.
NRL=Lower Neosho River.
SPR=Spring River.

reflected the greatest number of unique alleles (N = 77). By contrast, CWR, NCC, and NRL demonstrated relatively higher allelic richness and population evolvability (i.e., capacity to generate heritable, adaptive phenotypic variation). All populations showed low inbreeding (F_{IS}; Table 1). Values near zero indicate random breeding, positive values diagnose inbreeding as a deficit of heterozygotes, whereas negative values point to heterozygote-excess (outbreeding).

3.5. Selection and local adaptation

Our analyses indicated patterns consistent with local adaptation. Genotype-environment association analysis (GEA) revealed adaptive loci significantly associated with environmental variation (Fig. 5), but structured differently among populations, again emphasizing localized adaptive differences (Fig. 6).

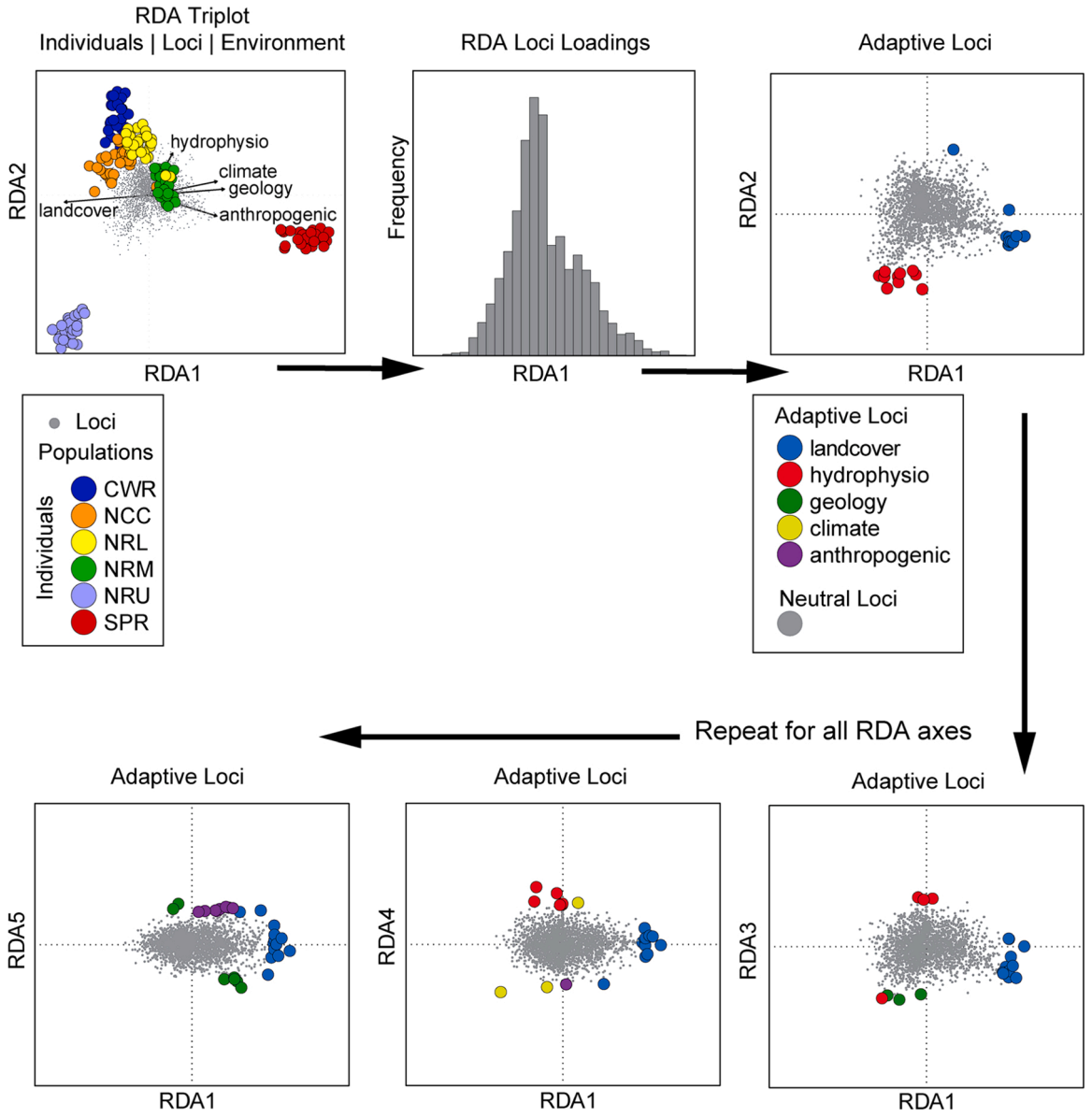

Fig. 5. Local adaptation of Neosho Madtom assessed using genotype-environment association (via redundancy analysis modeling of individual genetic variation versus environmental factors). Five canonical axes were produced, which represent associations between loci and environmental factors. The remainder are assumed neutral. For interpretation, loci are colored based on the environmental factor they are most correlated with. Six genetic populations are: Cottonwood River (CWR), Upper Neosho River (NRU), Neosho-Cottonwood Confluence (NCC), Middle Neosho River (NRM), Lower Neosho River (NRL), and Spring River (SPR).
(a) The distribution of loci loadings on each RDA axis was assessed (top middle), and outliers are locally adapted loci (±3 standard deviations from the mean). (b) Remaining plots (top right and bottom) identify locally adapted outlier loci (Appendices M and N).

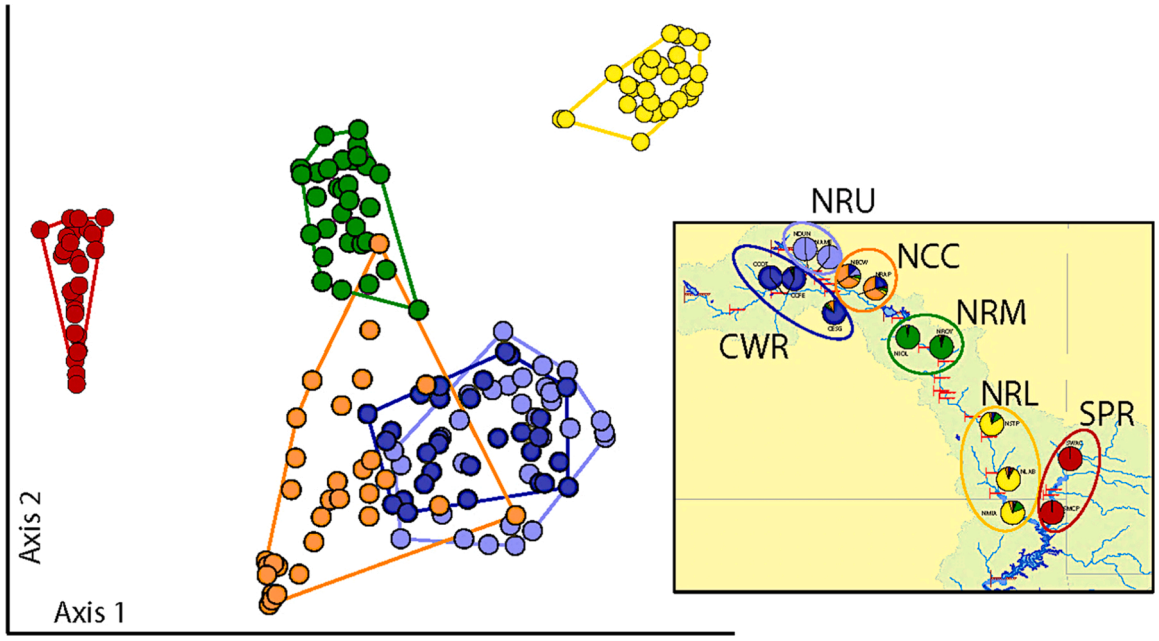

Fig. 6. Population structure of Neosho Madtom based on locally adapted genetic loci (see Appendices N,O, and Q). If populations have been adapting to the specifics of their local environment, we expect to see different groupings in the plot of genetic variation produced by t-distributed stochastic neighbor embedding (t-SNE). The northern populations (CWR, NRU, NCC) show much overlap in the plot, reflecting similarity in adaptation, whereas there is little (NRM) or no (NRL, SPR) overlap among the southern sites, pointing to possible differences in local adaptation along the longitudinal gradient of the river system. The southern sites appear distinct from each other, indicating unique local adaptation. Colors reflect six genetic populations: Cottonwood River (CWR), Upper Neosho River (NRU), Neosho-Cottonwood Confluence (NCC), Middle Neosho River (NRM), Lower Neosho River (NRL), and the Spring River (SPR).

Five robust principal components accounted for 87 % of the total environmental variance across N = 281 factors (Appendix H). For context, we provide system-wide summary statistics for those original environmental factors with the greatest loadings (Appendix I), with selective factors reported for each site/population (Appendix J).

Environmental factors were significantly related to individual genetic variation ($p = 0.001$), accounting for 6 % of the total genetic variance (Allocated as: Hydrologic-physiographic (39 %); Climate (27 %); Landcover (16 %); Total= 82 %; Appendix K). If most genetic loci are indeed neutral regarding environmental selection, then 6 % of the variance attributable to environmental variation is considerable (Meirmans, 2015; Brauer et al., 2018). The RDA canonical axes ($N = 5$) reflect the association of genetic and environmental variance (Appendix L), with RDA triplots visualizing those relationships (Fig. 5). Each environmental variable was correlated to some degree with each RDA canonical axis (Appendix M).

The analysis also estimated the loading for loci on each RDA canonical axis (Fig. 5). We screened for adaptive outlier loci that would indicate selection (local adaptation) by isolating those with loadings ±3 standard deviations from the mean on each axis ($N = 5$), with $N = 61$ representing locally adapted outliers. Most were strongly correlated with either hydrologic-physiographic ($N = 20$) or landcover ($N = 18$) (Appendix N). Factors most correlated with these loci (Appendix O) are varied, but point to the adaptive importance of characteristics both within stream reach (=size), and upstream (=erosion, forest cover).

Geographic differences were apparent in local adaptation to the environment, based on the clustering of adaptive loci (Fig. 6). Populations upstream of John Redmond Reservoir (CWR, NRU, NCC) overlapped in their variability regarding adaptive loci (Fig. 6). Those downstream (NRM, NRL, SPR) did as well (Axis 2 of t-SNE plot; Fig. 6), but with differences apparent (Axis-1 of t-SNE plot; Fig. 6). This suggests local adaptation is consistent with the directional gradient recorded by environmental variables (Appendix O; Population-level allele frequencies for adaptive loci in Appendix Q).

Patterns consistent with local adaptation were also apparent between adaptive loci and their genetic/biological functions. For adaptive loci, N = 6 were associated with amino acid changes within protein-coding regions; N = 2 with synonymous changes within protein-coding regions. The remainder (N = 53) are modifier mutations in non-coding regions (Appendix Q). These collectively associate with N = 30 genes with known biological functions: Anatomical development (head, eyes, brain); Response to stimuli (stress, chemical, hypoxia); and Metabolism (autophagy, ceramide, nitrogen, ubiquitin; Appendix Q).

4. Discussion

4.1. Conservation units

The most established of CUs is the 'evolutionary significant unit' (ESU, variously re-defined and edited: Barbosa et al., 2018; Table 1 in Hausdorf, 2021; Miller et al., 2024). It is the largest in magnitude, with a focus on broad-scale, range-wide conservation planning (Moritz, 1994). The 'adaptive unit' (AU: Differentiated via genomic loci) is most often intermediate, while the most reduced is the management unit (MU: Demographically independent with dynamics driven by birth/death rates rather than immigration) (CU decision tree in Turbek et al., 2023). An ESU can potentially encompass multiple AUs and MUs (respectively defined by adaptive or neutral genomic loci; Xuereb et al., 2021), with potential convergence depending upon definition. Landscape factors should be quantified to avoid mismanagement when CUs (and intermediate stages) are delineated (Hausdorf, 2021), but this can prove difficult, given the sampling strategy required to properly define ESUs (Moritz, 1994). We provide an example by employing as our study species the Neosho Madtom, categorized as an SRE (a topographically-limited biodiversity unit difficult to manage and oft-overlooked).

4.2. Sampling and genomic data

Our range-wide and population-dense sampling thus yielded robust statistical inferences, with 12(+) individuals in all but two (of 14) locations (Fig. 2; Appendix A). This contrasts sharply with a previous NMT study (Whitacre et al., 2022) where N = 10 individuals (5.2 % of our sample size) were collected across three sites approximating NRU, CWR, and NRM. The reported 'weak population structure' was subsequently interpreted as one panmictic NMT population, which contrasts markedly with our results.

However, whole-genome data extended our analyses by allowing loci to be assembed and linked with functional genes. Of note, however, is that when sample designs are similar, reduced representation genomic data (e.g., ddRAD; as herein) will yield the same perspectives on population structure as would whole genome sequencing (Martchenko and Shafer, 2023).

4.3. Population structure

Our genetically distinct gene pools (K=6) are consistent with structure estimates independently derived across subsidiary analyses (K-means clustering; DAPC; t-SNE; F_{ST}; Fig. 3; Appendix F). Aspects of our results also underscore the challenge of teasing apart and visualizing bioinformatics in a longitudinally-distributed species (i.e., 'the curse of dimensionality;' Schmidt et al., 2023).

A second example, represented by our ADMIXTURE analyses, suggested longitudinal variance in model fit. Here, K= 3 (Fig. 3C) yielded the lowest cross-validation assignment error (i.e., a good fit for NMT genetic structure; Appendix E), yet log-likelihood (a second indicator of model fit) failed to plateau as more populations were added (Appendix E). By explanation, we note that while K is flexible, it is most often reported as a single point (i.e., best K) within a continuous scale (Jombart and Collins, 2015; Verity and Nichols, 2016). We instead follow Meirmans (2015) and offer a range of K-models (Fig. 3C), where genetic populations and management units are augmented with biogeographic data.

Dispersal between sites can be obstructed by dams or soft barriers, ultimately diminishing gene flow and genetic variability (Hopken et al., 2013; Zbinden et al., 2023b). Given enough time, each isolated population will reflect unique variation, as detected by population genetic analyses, with genetically similar individuals from discrete populations subsequently clustering. Thus, the hierarchical population structure within the NRS is an amalgam of populations separated historically versus those recently isolated, whereas other SREs have display only long-term isolation (Arana et al., 2023; Gretgrix et al., 2023). The NRU and SPR are characterized by below-average discharge (Appendices J, O) and consequently represent more historic populations, as well as being 'tributaries' to the mainstem' (defined as the Cottonwood and Lower Neosho rivers).

Additionally, environmental characteristics seemingly differed longitudinally within the Neosho River, with adaptive differences subsequently emerging. Given the predilection of NMT for consistent flows over specific substrates, it is reasonable to hypothesize that dispersing individuals (pre-impoundment) would gravitate upstream towards increased discharge. This is consistent with its restrictive mainstem distribution, as well as the distinctiveness of its two largest tributaries (NRU and SPR). Our hierarchical distribution of genetic differences thus represents both processes: Stochastic (i.e., genetic drift in small populations); and Deterministic (i.e., anthropogenic selection in an altered environment).

Upstream populations (NRU, SPR) were minimally admixed, which was also true for CWR, save for its most downstream site (CESG) adjacent to NCC and without accompanying impoundments (Fig. 3C). NCC (at confluence of upper Neosho and Cottonwood rivers) reflects historic admixture, with individuals ancestal to CWR and NRU (upstream). Similarly, NRL (lowest stretch of the Neosho River) exhibits historic admixture with upstream populations (NRM, NCC; Fig. 3C). Again, contemporary (or first-generation) migrants were not found in any population (and if present, would appear in Fig. 3C as inconsistently-colored vertical bars). A one-year study of fine-scale inter-riffle movement in the CWR recorded but a single such occurrence (Fuselier and Edds, 1994).

4.4. Riverscape genomics

Our riverscape analyses highlighted environment impacts on NMT. Anthropogenic influences, as well as natural processes, modulated dispersal and population connectivity, thus acting as agents of selection (Xuereb et al., 2015; Benham et al., 2024). While difficult to distinguish, indirect inferences can be obtained from nuanced signals within genetic patterns. The separation of SPR and NRU from the remaining populations (Fig. 4A) potentially reflects historic (i.e., natural) processes occurring over an extended

pre-impoundment period. Conversely, those more moderate divergences among remaining populations (Fig. 4A), especially above/below John Redmond Dam, are more recent and anthropogenically-induced. This underscores our argument that artificial barriers have effectively blocked dispersal, and supports previous studies (Rasset et al., 2024; Tiemann et al., 2004b,a) implicating hydrologic modifications as drivers of variability in longitudinal fish assemblages. Dams, much like sub-optimal habitat, also act to reduce population connectivity and promote local isolation. John Redmond Dam emerged in our analyses as the most formidable barrier (greatest F_{INDEX}; Fig. 4B), as validated by elevated genetic divergences between adjacent populations.

In this study, the selective impacts of dams were further highlighted in our ResistNet analysis, where degree of fragmentation and flow regulation emerged as significant factors (Appendix G). These data underscored previous results (Fencl et al., 2015) that demonstrated low–head dams (N = 6 of 20; 30 %) impacting 47.3 km (~17 %) of the mainstem Neosho River, with nine (45 %) operational for over a century. Other landscape-level factors also impeded dispersal. ResistNet indicated soil types and landcover as potential proxies for in-stream environmental variability (Fig. 4C,D). In this sense, landscape-level differences in vegetation and soil affect runoff and thus impact fluctuations in turbidity and/or flows at the riverscape-level, which in turn mediate dispersal (Fox and Magoulick, 2019).

4.5. Population genomic parameters

The presence of rare genetic variants unique to each population contributed significantly to local, non-replicated genetic diversity among populations. This is particularly relevant for SREs such as NMT, as it underscores low levels of gene flow between populations as well as their unique anthropogenic selection pressures, as driven by low–head dams. Bluntnose Minnow (*Pimephales notatus*) within an Illinois stream also exhibited strong levels of population genetic differentiation, as driven by the altered hydrology of low–head dams (Smith et al., 2019).

Another relevant finding is that while short-term extinction risk is reduced (N_e>50), 50 % of populations reflect long-term risk, with N_e< 500 (i.e., '50/500' rule; Rieman and Allendorf, 2001; Jamieson and Allendorf, 2012). Only CWR, NRL, and NRM exceed N_e> 1000, in contrast to NCC, NRU, and SPR where N_e< 500 (Table 1). Although the '50/500' rule potentially categorizes conservation status, the unique contributions of demography, ecology, and life history must also be weighed when N_e estimates are interpreted (Waples, 2024). NMT is a narrow-niched aquatic species constrained by its habitat, a consideration reflected terrestrially by the Ridgenosed rattlesnake (an SRE in the sky-islands of southwestern North America; Davis et al., 2015), as well as a cadre of small-bodied South African anurans (Botts et al., 2013).

Genetic variability must also be considered within the context of within-population diversity and among-population distinctiveness. For example, NRU and SPR were genetically distinct yet manifested lower overall genetic diversities than did CWR, NCC, and NRL. The latter exhibited relatively higher diversities, as expressed by indicators of potential evolvability (i.e. allelic richness and Shannon entropy; Table 1). SPR reflected the greatest number of unique alleles, which contributed to its distinctiveness. The lack of inbreeding across populations is encouraging, save for a marginal positive F_{IS} for NRL (Table 1). Nevertheless, observed genetic divergences underscore the importance of maintaining fine-scale population structure. A prudent approach would obviously be to foster local genetic variants and adaptations.

4.6. Selection and local adaptation

Understanding the landscape of local adaptation is essential for managing populations or brood stocks (Schmidt et al., 2023). Individuals adapted to different environments but subsequently translocated may produce offspring ill-suited to newer conditions, resulting in lower survival, reduced fitness, and outbreeding depression. Alternatively, supplementation can erase locally adapted variation and cause genetic swamping (Flanagan et al., 2018; Hoffmann et al., 2021). Both are detrimental to population persistence, and each counters the intended management goal of promoting genetic viability.

Locally adapted differences can also be effectively leveraged by management (Weeks et al., 2011). Strategic mixing of different brood stocks can serve as a form of genetic rescue, bolstering genetic diversity, combating inbreeding depression, inducing hybrid vigor, and transferring locally adapted traits to those populations currently deficit (Whiteley et al., 2015). However, to distinguish a mere short-term demographic response (i.e., elevated population size due to the introduction of additional individuals) from an actual genetic rescue (i.e., an increase in genetic diversity that enhances fitness) requires a priori quantification of local genetic variation (as done herein), as well as assessment of fitness parameters via ecological data (Mussmann et al., 2017, 2020). Thus, estimates of local genetic diversity, as presented herein, are a valuable tool for supplementation or translocation as a potential management tool.

We inferred local adaptation along the NRS gradient based on GEA analysis, and the similarity of adaptive variation among populations (Figs. 5, 6). Both turbidity and NMT density have previously been linked to this gradient, and each is generally elevated upstream of John Redmond Reservoir (Wildhaber, 2011). Both could exert a subsidiary role in adaptation. Furthermore, upstream localities fall within smaller river reaches that receive less precipitation (Appendices J, O). NMT upstream of John Redmond Reservoir may also experience reduced predation pressure due to higher turbidity and optimal interstitial spaces within substrate (Wildhaber, 2011). The reverse may hold for populations below the reservoir, which retain sediment, have reduced turbidity, and offer more stable instream conditions, yet with limited shelter to deflect predation pressure (Wildhaber et al., 1999). The abundance of predatory Black Bass (*Micropterus* spp.; Branson, 1967) likely shifts along the longitudinal gradient as do more stable, preferred habitats (Johnson et al., 2009; Bruckerhoff et al., 2021).

GEA analyses are a standard for inferring locally adapted genotypes within environmental gradients (Forester et al., 2018). However, spurious results due to factors other than selection (such as population structure) can confound interpretations. There is no

correct answer to this dilemma, with conclusions based instead on the tolerance for false positives and a loss of statistical signal (Capblancq and Forester, 2021). Simple RDA (employed herein) has a superior combination of false-positive/false-negative rates when used to conservatively select outlier loci (3 sd ± mean) within the context of relatively weak global population structure ($F_{ST} \approx 0.05$; Forester et al., 2018; reflected as F_{ST}=0.054 for NMT). Furthermore, our recovery of environmental-linked outlier loci consistently juxtaposed with recognized genetic functions, thus reinforcing our interpretation of a genuine signal for local adaptation (Appendix Q).

4.7. Adaptive gene functions

Biological functions associated with our adaptive markers suggest NMT has adjusted to different stressors and resources throughout the NRS. An overarching concern is whether outlier loci are indeed biologically relevant, and thus, additional validation is required to promote confidence in candidate adaptive markers (Meirmans, 2015; Schmidt et al., 2023). We provide a first step by establishing consistent connections (i.e., biological functions) between genotypes and phenotypes. Our candidate markers are associated with amino acid changes in protein-coding regions and non-coding modifications that may affect upstream and/or downstream genes/complexes. Markers were collectively associated with identifiable biological functions in N = 30 genes: Anatomical development (head, eyes, brain); Response to stimuli (stress, chemical, hypoxia); and Metabolism (autophagy, ceramide, nitrogen, ubiquitin; Appendix Q).

4.8. CUs as viable components of SREs

Spatial conservation prioritization (SCP; Margules and Pressey, 2000; Kukkala and Moilanen, 2013) occurs when species/habitat distributions are employed in the development of cost-effective conservation networks/areas (Cas). However, ancillary complications often result (Nielsen et al., 2023). For example, when < 50 species are parsed, those that are very rare within elevated species-richness areas become markedly devalued, whereas the more widespread become over-represented as they emerge within any configuration (Kujala et al., 2018). However, narrowly-distributed taxa can still be informative in meeting conservation targets, although with variable cost-efficiency (Akasaka et al., 2022).

These perspectives juxtaposes with an elevated probability for diagnosis of CUs (and intermediate stages) within species broadly-distributed across a variable habitat (Bernatchez et al., 2019; Schweizer et al., 2021). Again, CUs most representative of biological diversity (and thus with greater propensity for long-term persistence) are reasonably parsed by intraspecific genomic data (Andrello et al., 2022). Importantly, our results demonstrate elevated habitat variance in SREs, as driven by anthropogenic impacts within a biogeographic framework. We recommend the integration of intraspecific genetic data into SCP delineation, thus allowing biodiversity to be more sharply defined while also promoting SREs as a viable (albeit oft-neglected) categorization requiring long-term conservation and management.

5. Conclusion

Our comprehensive evaluation of genetic diversity and population structure in NMT illuminates the complex interplay among genomic, environmental, and anthropogenic factors. Here we present several forward-focused strategies that safeguard species-integrity and facilitate long-term viability for SREs in general, and NMT in particular (Schmidt et al., 2023).

1. Conserving species integrity and genetic diversity
   Genetic diversity is essential to sustain adaptive capacity and promote resilience against anthropogenic environmental change. This is particularly true for SREs, with local persistence as a key natural history component (Lavergne et al., 2004). Yet, other well-established niche characteristics are more debilitating, such as elevated ecological specialization, reduced vagility, and pronounced habitat fidelity (Arana et al., 2023). Thus, a prerequisite for informed, science-based management in SREs is the designation of boundaries among (and differences between) populations (as herein).
2. Preserving local adaptation
   The retention of demographic process is fundamental to the evolutionary trajectory of NMT, in that the majority of its local adaptations are driven by anthropogenic habitat fragmentation. Low–head dams are a causative factor as their bank-to-bank format pools water and promotes habitat contractions within reaches. They most frequenly occur when ranges (such as the NRS) become highly transformed (Botts et al., 2013; Newbold et al., 2018). Importantly, the presence of many small low–head dams can have cumulative impacts that exceed those of a single larger dam (Consuegra et al., 2021).
3. Translocations for genetic or evolutionary rescue
   While all NMT populations appear genetically viable, their potential supplementation must be carefully balanced as outbreeding could ensue (Weeks et al., 2011). Genetic rescue for Rdgenosed Rattlesnake (an SRE) was rejected due to the rapidity of climate-driven habitat change in southwestern North America (Davis et al., 2015). Mixing populations could also jeopardize local adaptation, ultimately leading to demographic decline and loss of fitness. However, NMT supplementation (i.e., above–to–above and below–to-–below John Redmond Reservoir) could increase gene flow within species-triads (i.e., within CWR–NCC–NRU and NRL–NRM–SPR), thus reducing drift and stabilizing/elevating N_e.
4. Genetic monitoring and temporal tracking
   Establishing a long-term genetic monitoring program to track temporal changes is an adaptive management strategy for an SRE.

It would track an evolving genomic landscape and record subsequent impacts on demography (Mussmann et al., 2017). Here, an economical cost-per-sample approach to SCP would be a reduced marker panel of adaptive/neutral loci (e.g., GTseq; Campbell et al., 2015).

5. Retaining population structure

Identifying historic/anthropogenic processes that can potentially foster CUs in an SRE are a forward-thinking, yet oft-neglected endeavor. Equally important is an understanding of how local adaptation has sustained population persistence (Judson et al., 2024). For example, virtually every river within regional groups of Coho Salmon (*Oncorhynchus kisutch*) has distinctive, fine-scale structure, as driven by migration/elevation (Rougemont et al., 2023).

Genomic data are indispensable for conserving SREs but also for implementing their recovery. Our results establish that CUs (and intermediate steps) are both prevalent and quantifable in SREs, a biodiversity categorization often overlooked due to their severely reduced distributions and sampling difficulties.

Ethics Statement

Not applicable: This manuscript does not include human or animal research.

Article impact statement

Conservation units occcur not only in broad-ranging biodiversity but also short-ranged endemics which are problematic to manage and conserve

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marlis R. Douglas and Jordan Hofmeier reports financial support was provided by Kansas Department of Wildlife and Parks. Marlis R. Douglas reports a relationship with Kansas Department of Wildlife and Parks that includes: funding grants. Jordan Hofmeier reports a relationship with Kansas Department of Wildlife and Parks that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2025.e03783.

Data availability

Data available upon publication

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