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Persisting at the Edge of Ecological Collapse: The Impact of Urbanization on Fish and Amphibian Communities From Lake Xochimilco

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ABSTRACT

Freshwater ecosystems are globally threatened by habitat loss, pollution, and invasive species, all of which are particularly acute in urban areas. To assess the impacts of urbanization on freshwater biodiversity—specifically the effects of alien species on native primary aquatic vertebrates—we investigated the World Heritage Site, Lake Xochimilco in Mexico City. Focusing on fishes and amphibians, we applied environmental DNA metabarcoding using primer pairs targeting mitochondrial 12S and 16S across the remnant lake and collected 14 aquatic environmental variables for sampled sites. Our survey recovered ca. 60% of Lake Xochimilco's historically recorded fish and amphibian species, including rare species and novel taxa not detected by past traditional surveys. However, our findings imply a severely degraded wetland, with alpha diversity indices indicating a low-diversity ecosystem dominated by alien fishes. Beta diversity analysis revealed a heterogeneous ecosystem that may be driven partially by the presence of alien fish, particularly cyprinids. Environmental variables linked to pollution predicted the presence of non-native fish families. We also found evidence that some species prefer to occupy different water bodies within the lake remnant. Despite the ongoing degradation of this ecosystem, native and endemic fauna are persisting, although detections were typically rare. We found no evidence of the Critically Endangered axolotl salamanders (*Ambystoma* sp.) from wild sites; however, we detected their presence in one wildlife refuge, highlighting the potential of refuges to prevent complete extinction in the wild. We also found evidence of cryptic taxonomic diversity in *Lithobates* frogs and evidence of endemic genera, including the threatened mexclapique fish (*Girardinicthys viviparus*). These fishes are considered extirpated, suggesting remnant populations persist undetected by traditional surveys. Despite clear evidence of an ecosystem under extreme decline compared to historical biological records, our study demonstrates the potential for restoration, given the presence of native freshwater species and the success of wildlife refuges.

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1 | Introduction

Freshwater environments contain some of the most altered ecosystems on the planet (Carpenter et al. 2011; Reid et al. 2019). At a global scale, freshwater habitats are estimated to have lost 64% of their total area in the last 30 years (Costanza et al. 2014), with a combination of environmental factors presenting a substantial threat to their biodiversity (Collen et al. 2014; Tickner et al. 2020). Land conversion associated with the growth of urban areas has been shown to have the most severe impacts on freshwater biodiversity (e.g., Petsch et al. 2021; Yang et al. 2024) and urban freshwaters typically suffer from increased pollution load, silting, and homogenization of habitat structure (Dudgeon et al. 2006). These disturbances have led to dramatic negative biotic responses, including simplified animal communities, with generalist tolerant species dominating (Faeth et al. 2011), and the increased invasive potential of alien species (Marques et al. 2020). Notably, alien species have become a strong driver of the decline in native fish and amphibian populations by means of predation (Strayer 2010), competition for resources (Falaschi et al. 2020; Kats and Ferrer 2003), and as vectors of diseases (Forero Rodríguez et al. 2024; Kiesecker et al. 2001). Moreover, deliberately introduced alien species, such as cichlids (e.g., *Oreochromis* sp.) and carps (e.g., *Cyprinus carpio*, *Carassius auratus*), are difficult to control due to their adaptability and resilience to new environments (Hartel et al. 2007; Nunes et al. 2019). Even species translocations of native species within the same region can severely affect the ecology of ecosystems (Kats and Ferrer 2003). Additionally, climate change and harmful algal blooms have been identified as drivers of the freshwater biodiversity decline (Reid et al. 2019).

To assess the impacts of urbanization on biodiversity, efficient and rapid monitoring is needed. Environmental DNA (eDNA) methods are increasingly being used across different environments, due to their noninvasive ease of implementation and ability to detect biota at any life stage (e.g., Garlapati et al. 2019; Ruppert et al. 2019). This molecular tool has also been critical to the detection of elusive, cryptic, rare, or threatened species that are difficult to survey using traditional means (e.g., Andres et al. 2023; Bessey et al. 2020; Cilleros et al. 2019; Dufresnes et al. 2019; Saenz-Agudelo et al. 2022). There is considerable evidence that eDNA datasets and traditional surveys are often congruent (e.g., Keck et al. 2022 and refs therein), and in some cases eDNA outperforms traditional methods for both fishes (e.g., Fediajevaite et al. 2021; Hallam et al. 2021; Valentini et al. 2016) and amphibians (e.g., Lopes et al. 2017; Smart et al. 2015). Inferences made using eDNA can be coupled with management actions (Reid et al. 2019) making it a key tool in the conservation of freshwater ecosystems (Turak et al. 2017). Despite public genetic sequence databases generally being considered reliable, (Leray et al. 2019), there are still gaps in species representation, particularly from tropical and subtropical ecosystems (e.g., Doble et al. 2020; Jerde et al. 2021; Marques et al. 2021) and some data may be poorly curated.

Here, to assess the degree to which urbanization impacts subtropical freshwater biodiversity, we studied Lake Xochimilco, Mexico. This central Mexican wetland is one of the few remnants of the endorheic Basin of the Valley of Mexico (Alcocer-Durand and Escobar-Briones 1992) historically containing 20 fish and three amphibian species, several of which are endemic to this lake (Alcocer-Durand and Escobar-Briones 1992; García-Vázquez et al. 2016; Huidobro-Campos et al. 2016; Montero-Rosado et al. 2022). Despite its status as a UNESCO World Heritage Site, Ramsar Wetland of International Importance, and as a Natural Protected Area by the Government of Mexico City (Montero-Rosado et al. 2022), Lake Xochimilco is situated in one of the largest metropolitan areas on the planet. Unsurprisingly, given its location, Lake Xochimilco has been severely impacted by the growth of Greater Mexico City (~22.5 million inhabitants, UN World Urbanization Prospects 2024), and is now a highly modified aquascape of narrow canals, six small lakes, and two flood plains (Mazari-Hiriart et al. 2019; Zambrano et al., 2007) (Figure 1).

This severe modification of Lake Xochimilco has reduced the sensitivity of traditional survey methods by decreasing population sizes of native fauna and making some heavily urbanized areas inaccessible for surveys (Zambrano et al. 2009). Like many freshwater ecosystems in central Mexico (Gesundheit and Garcia 2018), the pressures on Lake Xochimilco's ecosystem have worsened since the 2000s due to accelerated urbanization, land use change, water pollution, and aquifer exploitation (Jiménez et al. 2020; Mazari-Hiriart et al. 2019). Several species have been introduced for aquaculture programs (Espinosa-Pérez 2014; Espinosa-Pérez and Ramírez 2015; Huidobro-Campos et al. 2016; Miller et al. 2009) such as old-world carps (*Carassius aureus* and *Cyprinus carpio*) and African cichlids (*Oreochromis aureus* and *O. niloticus*). Ornamental fish species translocated from other Mexican basins for mosquito biocontrol programs (Espinosa-Pérez and Ramírez 2015; Mendoza et al. 2015; Miller et al. 2009) have also been reported to be thriving in Lake Xochimilco. These species include *Goodea atripinnis*, *Xiphophorus* sp., and *Pseudoxiphophorus* sp.; however, there is a lack of consensus on whether any are established in the system (Huidobro-Campos et al. 2016).

Due to these biological and environmental pressures, Lake Xochimilco has experienced significant biodiversity losses in the last 100 years leaving local native biodiversity critically threatened. The endemic cyprinid fish genus *Evarra* that included only three species has been considered extinct for decades (Alcocer-Durand and Escobar-Briones 1992; Huidobro-Campos et al. 2016). The culturally significant axolotl (*Ambystoma mexicanum*), and Tlaloc's leopard frog (*Lithobates tlaloci*) are species both at risk of extinction in the wild and considered Critically Endangered (IUCN SSC Amphibian Specialist Group 2020a, 2020b). The status and distribution of many remaining native freshwater animals, as well as alien and translocated fish species is uncertain. Of critical concern is the low population density for native fish and amphibians. Based on traditional survey methods (weighted nets "*atarrayas*" that are thrown by hand), axolotl population densities were last estimated to be <35 individuals per km^2 in 2015 (Voss et al. 2015). The current status of axolotl populations in the Lake is unknown.

Given the ongoing degradation of Lake Xochimilco and the urgent need to determine the presence of Critically Endangered (possibly Extinct) endemics, we used eDNA metabarcoding to assess diversity levels among aquatic vertebrate

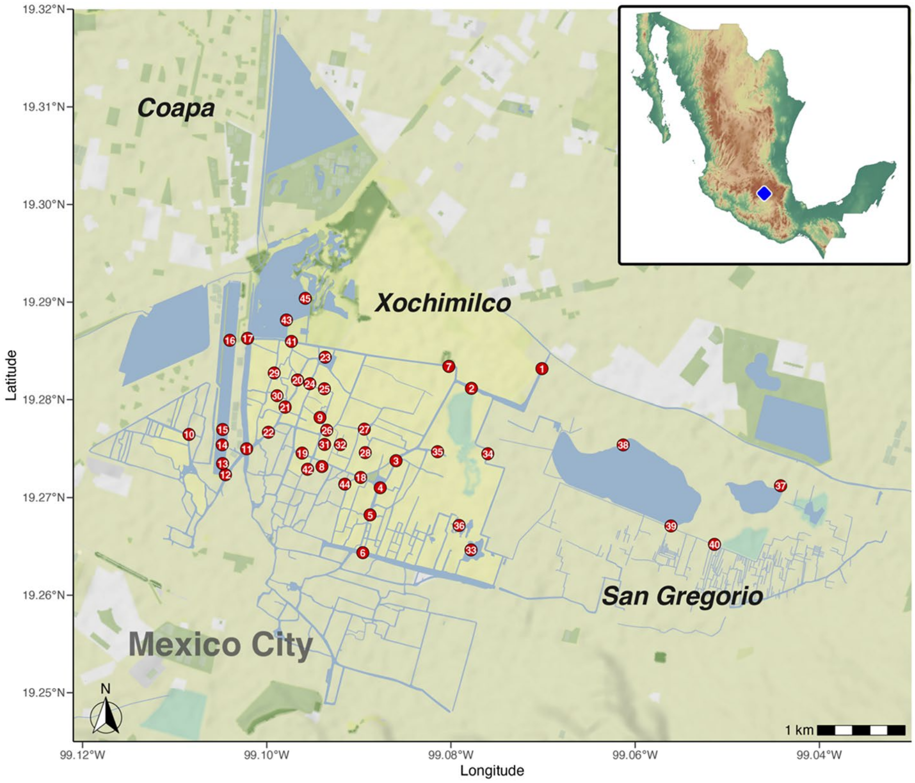

FIGURE 1 | Top: Study site of Lake Xochimilco and its location within Mexico (blue square). Lake Xochimilco, (19°17′ 6′′ N, 99°6′ 2′′ W, 2200 m.a.s.l.) covers an area of 26.72 km^2 and average shallow depth of < 2 m (Montero-Rosado et al. 2022; Zambrano et al. 2010b). Sampling localities across Lake Xochimilco are indicated by red circles, with numbers indicating their Site ID. Sites 8 and 9 are on-site wildlife refuges. Made with packages *ggplot2* and ggmaps, with Stamen Terrain layers from StadiaMaps.

communities—specifically fishes and amphibians. Based on comprehensive sampling across the Lake Xochimilco system and the application of two mitochondrial DNA markers (12S and 16S) our aims were to obtain a contemporary understanding of biodiversity via the presence/absence of species and estimate relative species abundances from eDNA read counts. From these aims, we addressed the following questions: (1) To what extent have alien species colonized the Lake Xochimilco ecosystem, and what impacts have they had on native fish families? (2) How widely do native fishes and amphibians persist across the remnant lake? (3) Are species composition and relative abundances of fishes and amphibians associated with different water bodies? (4) How do anthropogenic environmental stressors influence the presence/absence of native and non-native fish families? and (5) How effective are the selected eDNA metabarcoding primers at detecting fish and amphibian species in this system?

2 | Methods

2.1 | Sampling Design

Field sampling was conducted from November 2020 to April 2021 at 43 wild sites along canals and lagoons of Lake Xochimilco to account for the site heterogeneity reported by Zambrano et al. (2009). Two wildlife refuges for the axolotl were also sampled, for a total of 45 sampling sites (Figure 1). Highly urbanized areas in the lake's southern portion were not included (for personal safety) and some canals on previous maps no longer exist. Water bodies were classified here based on physical characteristics: artificial (the *Virgilio Uribe* rowing pond); large canals (canals large enough for motorized boats); small canals (narrow canals that can only be traversed by compact rowing boats (called *coyucos*)); lagoons (zones that function as water reservoirs connecting canals); and wildlife refuges – two constructed water bodies in the chinampas that are delimited by physical (volcanic rocks) and biological (aquatic plants that function as biofilters) barriers from exterior water currents to function as protected areas for native freshwater wildlife.

2.2 | eDNA And Environmental Sampling

Water was sampled three times at each site without disturbing sediments using three different 2 L Nalgene containers and filtered for 20 min or until the filters were clogged entirely (following Hallam et al. 2021). Field negative controls (17 samples, 11 sequenced) were also taken using distilled water. Total water volume filtered for biological samples was not statistically significant (see Supporting Information, for further details of water sampling, and filtered water volume). Filtration was conducted off-site using a portable GeoPump series II (Geotech) and silicone Masterflex Platinum L/S 15 tubing (Masterflex-Avantor) using 0.45 μm pore PVDF Sterivex filters (Millipore-Merck).

Water environmental characteristics, including pH, temperature, total dissolved solids (TDS), and electroconductivity (EC), were measured with a portable Combo meter (Hanna

Instruments). Organic (ammonia, chlorine, nitrates, nitrites, phosphates, sulfates, and sulfides) and metallic (aluminum, copper, iron) pollutant concentrations were measured using an eXact Micro20 portable photometer (Industrial Test Systems).

2.3 | eDNA Extraction, PCR Amplification, and Mock Community

Filters were extracted following Hallam et al. (2021) and references therein, but here we used the DNeasy PowerWater Kit (QIAGEN) for DNA extraction following manufacturer's protocol for small water filters with minor modifications. An extraction blank was included in each extraction round (12 in total, 4 sequenced). Extracted DNA was quantified using a Qubit 2.0 fluorometer and the dsDNA Broad Range Assay Kit (Invitrogen-Thermo Fisher) and stored at −80°C.

Two primer pairs were selected, targeting the 12S and 16S rRNA mitochondrial genes based on primer pair performance (Mitani et al. 2009, Vences et al. 2012, Yang et al. 2014). The 12S-V5 primer pair (F: TAGAACAGGCTCCTCTAG, R: TTAGATACCCCACTATGC) (Riaz et al. 2011) targets a small region of ~110 bp in the 12S rRNA gene and targets vertebrates. The hypervariable region of ~250 bp in the 16S rRNA gene amplified by the Meta16S primer pair (F: ACGAGAAGACCCTATGGARCT, R: TCCTGATCCAACATCGAGGTC) is based on the Vert-16S-eDNA primer pair (Vences et al. 2016) and was modified for this study to target amphibians. Library building and metabarcoding were conducted at the Natural Environment Research Council Environmental Omics Facility (NEOF), University of Sheffield, UK.

Sequencing of 160 samples (135 eDNA samples, 11 field negative controls, 4, extraction blanks, 4 PCR negatives, and 4 positive controls by triplicate = 480 total samples) was conducted at the NEOF Environmental Omics Facility, the University of Liverpool, U.K. on the MiSeq system (Illumina, United States). The Meta16S and 12S-V5 libraries were sequenced using 2 × 250 bp and 2 × 150 bp paired-end reads chemistries, respectively, on separate MiSeq runs. Samples were then demultiplexed for downstream analysis.

2.4 | Reference Databases

A biological inventory for the federal entity of Mexico City, which contains Lake Xochimilco in the southern portion of the city and River Magdalena on the western side of Sierra de Las Cruces, was created from a literature survey. Tissue samples from amphibians and fish specimens from this inventory that were not available as sequences on GenBank were obtained and sequenced (Table S2, Supporting Information). Sampling was conducted under permit SPARN/DGVS/08010/24 from Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT).

The final reference database (comprising GenBank and our new sequence data) contained 12S and 16S sequences for 81.6% of the total fish and herpetofauna species (76 species) recorded for Mexico City. While three amphibian species (*Ambystoma altamirani, Anaxyrus compactilis*, *Pseudoeurycea tlilicxitl*) had missing records, we instead included sequences belonging to species from the same genera. Only three fish species (*Evarra bustamantei*, *E. eigenmanni*, and *E. tlahuacensis*) had no records in the reference database. See Supporting Information for further details on reference database construction.

2.5 | Sequencing Data Analysis

Demultiplexed files were analyzed using RStudio v2023.06.0 + 421 (Posit Software, 2023) with primer removal using the Python package *cutadapt* v3.7 (Martin 2011) as implemented in R (R Core Team 2024) with a low minimum length of 10 bases with an adapter removal setting of 2 for Meta16S and 3 for 12S-V5. Subsequent steps used the R package *dada2* v1.22.0 (Callahan et al. 2016). Read quality was assessed using the quality plots function to determine the parameters of the second filtering step. The filtering parameters for the Meta16S sequences were a truncated length of 205 with read truncation at the first instance of a quality score of 2, and a maximum number of expected errors was set at 8. We used the default option (pooled) for the ASV inference step as the computationally intensive pseudo-pooled option did not affect results despite being more sensitive to rare ASVs (Callahan et al. 2016). For the 12S-V5 sequences, the filtering parameters were a truncated length of 105. The subsequent merging step was set at a maximum mismatch of 0 bases and a minimum overlap of 12 bases. Chimaera removal was conducted using the "consensus" method. The final ASV list file was exported to FASTA format.

The corrected taxonomy assignment table was merged with the ASV counts and sample metadata tables using the package *phyloseq* v1.46.0 (McMurdie and Holmes 2013). A filtering step to remove unassigned ASVs and analyze sequences from the positive and negative control samples was conducted. Detection of contaminant sequences was conducted using the R package *decontam* v1.0 (Davis et al. 2018) while cross-contamination events were analyzed using tables created by R package *metagMisc* v0.5.0 (Mikryukov 2022). All downstream analyses were carried out using the Meta16S dataset due to the superior performance of this primer regarding taxa detection and taxonomic resolution over 12S-V5.

2.6 | Statistical and Ecological Analyses

Community analyses were conducted using *vegan* v2.6.4 (Oksanen et al. 2019). Alpha diversity analyses (to obtain observed richness, Shannon, and Simpson indexes) were conducted on nontransformed data. Beta diversity analyses were conducted using a Hellinger-transformed dataset. Pairwise PERMANOVA analysis was performed using *pairwiseAdonis* v0.4.1 (Martinez-Arbizu 2017). A differential abundance analysis was conducted to assess if the focal taxa were more abundant at specific water body types using *DESeq2* v.1.44.0 (Love et al. 2014). Partial correlations to investigate the interactions of native and non-native fish families were performed using the R package *ppcor* v1.1 (Kim 2015). Correlation analysis and matrixes were built using *corrplot* v0.92 (Wei and Simko 2024) and *psych* v2.3.9 (Revelle 2024).

2.7 | Multivariate Model Analysis

Multivariate model analyses of eDNA count data, water parameters, and contaminant readings were conducted using *mvabund* v4.2.1 (Wang et al. 2012). Several water parameters and contaminant data differed significantly from a normal distribution according to the Shapiro–Wilk test ($p < 0.05$). Therefore, the correlation matrix was built using Spearman's rank test with Bonferroni correction. Additionally, Variance Inflation Factor values (VIFs, a multicollinearity measurement) were analyzed, and those variables with values of 5.0 and above were considered for removal from the analysis. Based on this criterion, electroconductivity (EC), nitrites, and sulfur were not included in the multivariate model. Given the large differences between the eDNA counts of the five different fish families, these values were normalized using log transformation. After correcting for multiple tests, the diagnostics (residuals, normal q-q, and scale-location) of the multivariate model indicated that the total dissolved solids (TDS) variable was an outlier and was therefore removed from the model. Following the diagnostics and AIC values, the best model was the following:

Fish families eDNA counts (log transformed) ~ pH + water temperature + dissolved oxygen + nitrates + ammonia + phosphate + sulphate + chlorine (log transformed)

The model was subjected to both multivariate and univariate tests. To check water parameters and contaminant correlation, *corrplot* was used, while collinearity between variables was checked using *car* v3.1.2 (Fox and Weisberg 2019).

3 | Results

3.1 | Sequencing Data

The Meta16S and 12S-V5 metabarcoding library sequencing runs obtained 8,828,887 and 12,321,416 raw reads, respectively. After raw reads were processed through filtration, denoising, and merging steps, final sets of 7,142,851 and 10,316,654 reads were retained, respectively. From the 12S-V5 pipeline, a total of 657 ASVs were inferred, while from the Meta16S pipeline, a total of 1590 ASVs were inferred (Table S3). For further information on sequencing data (taxonomic assignment, mock community, contamination, and sequencing depth) see Supporting Information.

3.2 | Fish and Amphibian Species Inventory Detected by eDNA

In total, 31 primary aquatic vertebrate taxa (identified to species or generic level) were detected for 28 fishes and three amphibians across both sequencing libraries (Table S4). Of these taxa, the 12S-V5 primer pair detected 23 taxa (22 fishes and 1 amphibian), and the Meta16S primer pair detected 21 taxa (18 fishes and 3 amphibians). Although the primers showed reasonable overlap with each other, and the historical data (Figure 2), there were taxa uniquely identified by each primer, as well as by method (i.e., traditional vs. eDNA surveys). The Meta16S primer pair typically showed better taxonomic resolution than the 12S-V5 primer pair, with the latter not able to distinguish fish taxa such as Cichlidae, Cyprinidae, and Atherinopsidae beyond the family level.

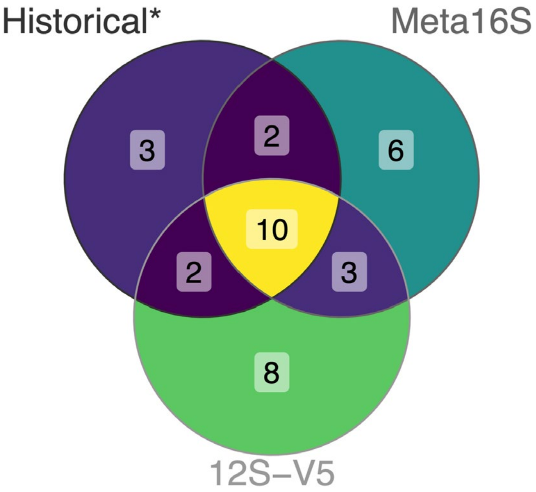

FIGURE 2 | Venn diagram representing the number of amphibian and fish species detected by the two eDNA metabarcoding primer pairs—12S-V5 and Meta16S. The historical* (traditional survey data) was taken from published data from Huidobro-Campos et al. (2016). In combination, the metabarcoding approach detected more species than traditional survey methods (Huidobro-Campos et al. 2016; Vázquez-Silva et al. 2017) at Lake Xochimilco.

The 28 fish taxa identified by eDNA belong to six different families of which five (Atherinopsidae, Cichlidae, Cyprinidae, Goodeidae, and Poeciliidae) have been previously recorded. One family, Sisoridae (Asian catfishes), is a first record for Lake Xochimilco. Along with detecting *ca.* 60% of the previously recorded species for Lake Xochimilco, the primers detected fish species that had not been reported using traditional surveying methods for the area (Table S4). Most of these newly recorded taxa were translocated species (defined here as native to other Mexican basins). All three native fish species (*Chirostoma jordani*, *Chirostoma humboldtianum*, and *Girardinichthys viviparus*) previously recorded through traditional surveys were identified, although only the 12S-V5 marker detected *C. humboldtianum*. While *Chirostoma* species were found to be widely distributed, *G. viviparus* (Chapultepec splitfin) was only detected at site 35 (Figure 1). Alien species belonging to *Carassius*, *Cyprinus* (carps), and *Oreochromis* (cichlids) were detected by Meta16S, while only *Cyprinus* could be clearly identified by 12S-V5.

However, some reported native species from the most recent inventories based on traditional surveying (Huidobro-Campos et al. 2016; Vázquez-Silva et al. 2017) such as *Algansea tincella* and *Aztecula sallaei* could not be detected by either marker. In addition, some alien (*Ctenopharyngodon idella*) and translocated species (*Lepomis macrochirus*, *Micropterus salmoides*) were not identified either. Although the genus *Lepomis* was

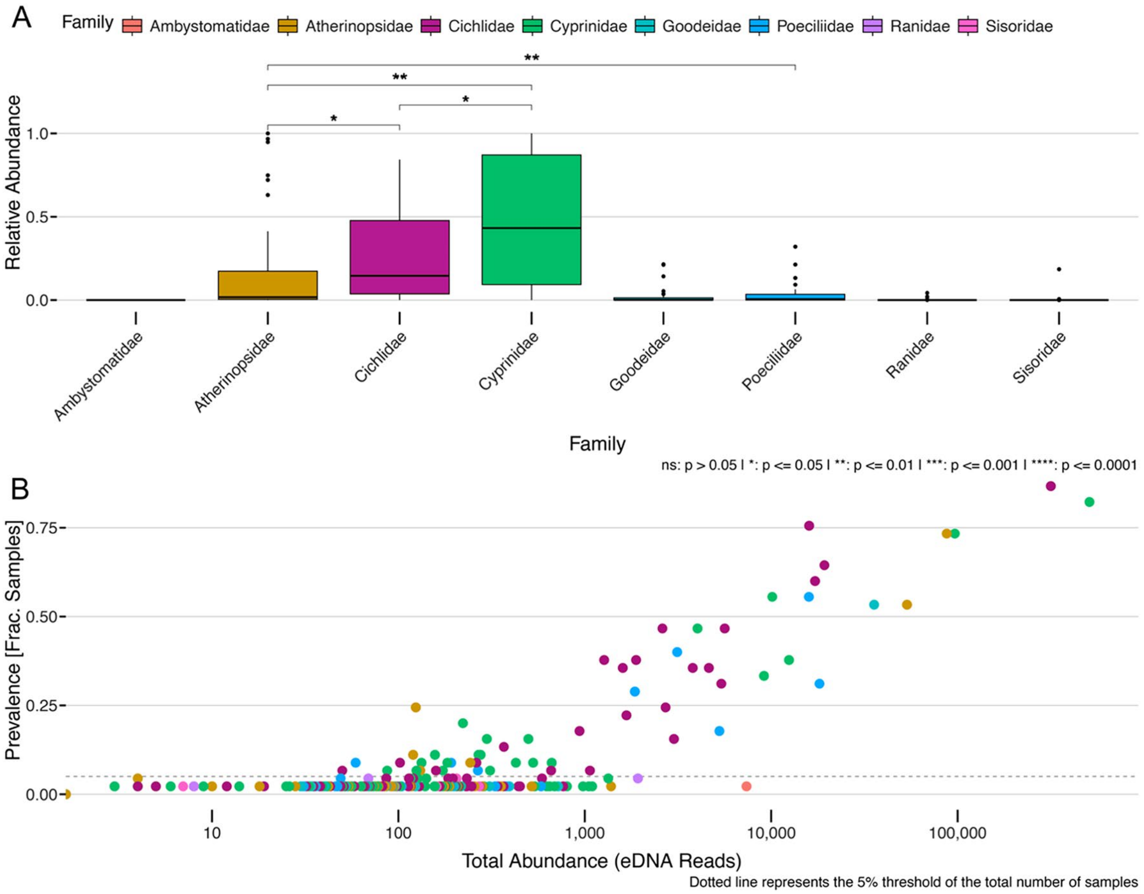

FIGURE 3 | Relative read abundance plots from the Meta16S metabarcoding library. (A) shows the proportional abundances from the identified amphibian and fish families at Lake Xochimilco with comparisons made using the nonparametric Wilcoxon test; (B) describes the prevalence of the identified amphibian and fish families by total eDNA read counts across the total number of samples of the metabarcoding library.

identified, this was from a different species (*L. cyanellus*) to the one recorded from historical records (*L. macrochirus*). Moreover, there was no detected sequence that could be associated or partially associated with the native genus *Evarra* (Cyprinidae).

The three amphibian taxa detected by the markers belong to two families (Ambystomatidae and Ranidae) (Table S4), which have been previously recorded. Both markers detected the salamander *Ambystoma* sp., but only in one of the two sampled wildlife refuges (Site 8, Figure 1), where it was recorded in one of the three biological replicates. The frog genus *Lithobates* was detected only by the Meta16S marker that recovered three ASVs (1927, 69, 8 reads, respectively), where it was identified from three wild site samples (Sites 32, 44, and 45, Figure 1). None of the ASVs matched the endemic Lake Xochimilco *L. tlaloci*, and although two of the ASVs (1 and 3) matched the sequence we generated of a specimen listed as *L. montezumae* (UNAM-CNAR A5-47A4, Table S2) with high similarity (> 99%), the match was much lower (~94%) to the sequence of this taxon on Genbank (collected at Lagunas de Zempoala, ~50 km south of Mexico City, Hillis and Wilcox 2005; Chambers et al. 2025). However, these ASVs also matched with a high similarity GenBank sequences recorded as an undescribed species (99%, AY779224, collected 375 km to the northwest, Hillis and Wilcox 2005) and unpublished sequences (> 99%, MT372907.1, MT372908.1, MT372909.1, sampled from Queretaro ~150 km also to the northwest, García- Solís et al. unpub.). The *Lithobates* ASV2 did not match the aforementioned sequences with high similarity and had a lower similarity score (~95%) with other *Lithobates* species on Genbank.

3.3 | Relative Abundances From eDNA Reads and Interactions of Native and Alien Fishes

Relative abundances (Figure 3A) indicated that eDNA reads from the two alien fish families, Cichlidae and Cyprinidae, are by far the most abundant across Lake Xochimilco (~20% and ~45% by average proportional abundance, respectively). In contrast, the most abundant native fish family, Atherinopsidae (< 10% average proportional abundance), was significantly lower than the alien fish families (Figure 3A). The only other fish family containing native species, Goodeidae (< 5% by average proportional abundance), is the least abundant. Read counts of translocated species from the Poeciliidae family (< 5% by average proportional abundance) were also scarce. The widespread presence of the alien families is further supported by their detection across the metabarcoding library (Figure 3B). ASVs from Cichlidae and Cyprinidae were found in over 60% of total samples. In contrast, ASVs from Atherinopsidae and Goodeidae were significantly less common, detected in fewer than 40% and 30% of samples, respectively (Figure 3A). ASVs from Poeciliidae also showed limited presence, appearing in less than

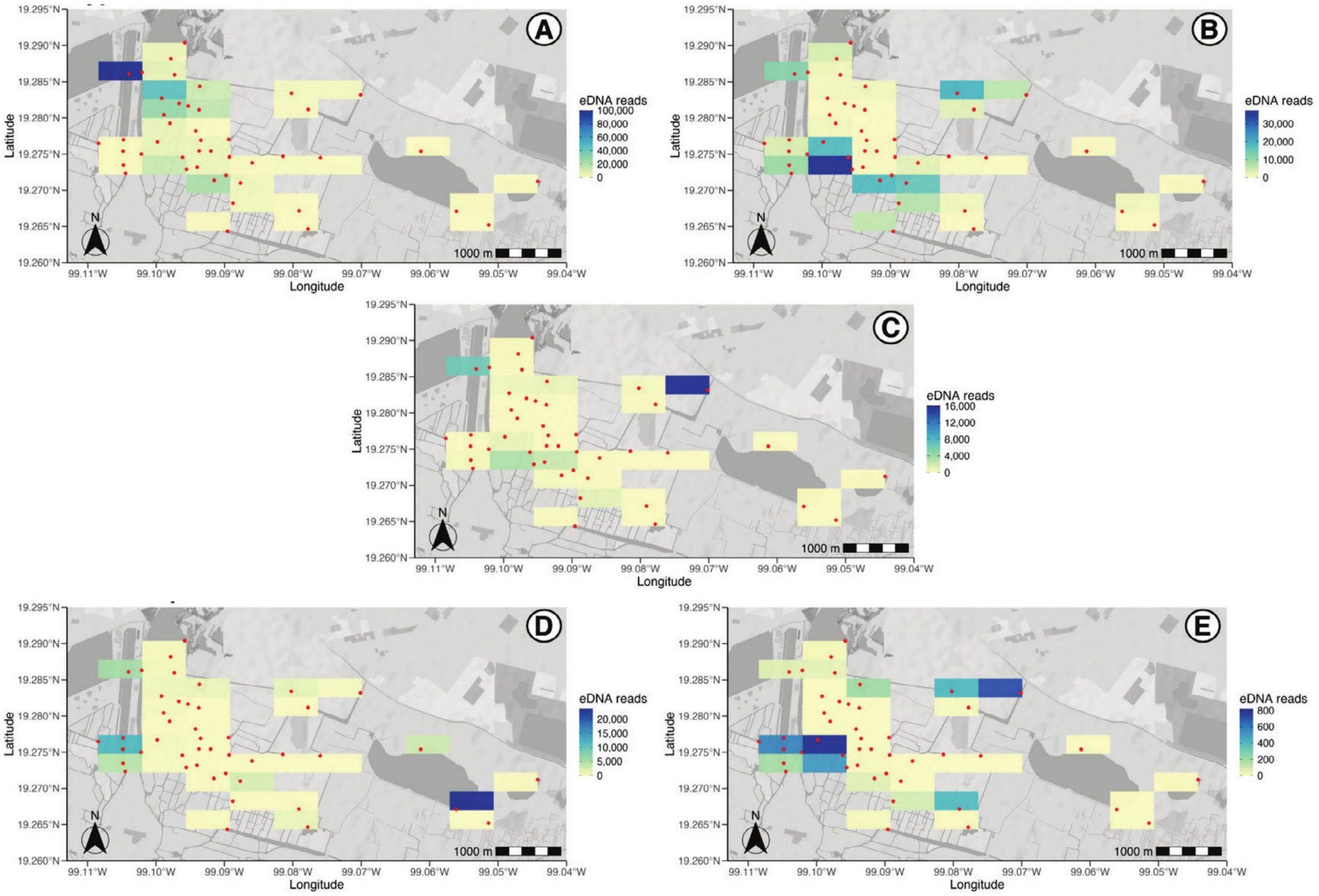

FIGURE 4 | Distribution map of the abundance eDNA read counts (Meta16S metabarcoding library) from the five fish families currently found at Lake Xochimilco: (A) Cyprinidae, (B) Cichlidae [Alien], (C) Poeciliidae [Translocated], (D) Atherinopsidae, (E) Goodeidae [Native]. Grid cells are colored based on the total amount of eDNA reads found in the sampling points that belong to that particular grid cell. Red circles indicate sampling point location. Made with *ggplot2* and *ggmap*.

30% of samples. Amphibian reads were the scarcest overall, detected in less than 5% of total samples (Figure 3B).

To explore possible associations between fish families, proportional data from the total number of reads per site were used to test the partial correlation between eDNA detections of native and alien fish families (Figure S3). Spearman's rank partial correlation analysis revealed significant negative associations between eDNA from the alien species, *Carassius* sp. and *Cyprinus* sp. (Cyprinidae) and that of native fish families Atherinopsidae and Goodeidae, as well as with the alien *Oreochromis* sp. (Cichlidae). A significant negative association was also observed between Cichlidae and the native Atherinopsidae. The only significant positive association detected was between eDNA from Cichlidae and Poecillidae. All the other relationships were nonsignificant.

3.4 | Spatial Distributions and Community Diversity Across Water Bodies Based on eDNA

The distributions of the previously recorded five fish families (Atherinopsidae, Cichlidae, Cyprinidae, Goodeidae, and Poeciliidae) known to occur at Lake Xochimilco presented some differences based on their eDNA count abundances (Figure 4). Both alien fish families (Cichlidae and Cyprinidae) were more abundant in quadrants located in areas dedicated to tourism or with high levels of human activity and development (such as the eastern part of the lake, Figure 4A,B). The artificial pond (located in the northeastern part of the area, separated from the lake) is an exception; the eDNA samples from this area indicated high eDNA abundance of native fish families. Additionally, both native fish families, Atherinopsidae and Goodeidae, were more abundant in quadrants located to the center and west of Lake Xochimilco (Figure 4D,E), belonging to the core zone of the Natural Protected Area where human activity is scarce or of low impact, such as traditional family agriculture.

The differential abundance analysis (base expression level = 2.900) showed that some taxa also showed a significant preference for water body type (Figure S2). *Oreochromis* cichlids showed the largest abundance in the large canal category (8.032, p adjusted <0.005), while *Cyprinus* carps showed the largest abundance in the small canal category (8.196, p adjusted <0.005). In contrast, *Carassius* carps showed very similar abundances for both small (5.969) and large canals (5.806). Native charal (*Chirostoma jordani*) abundance was the highest (7.913, p adjusted <0.005) in the artificial body (rowing pond). The rest of the taxa (including alien, translocated, and native categories) showed significantly lower levels of differential abundances across the different water body types. Overall, the large canal water body type was the category

TABLE 1 | Alpha diversity indexes (using number of observed taxa) from the different water body types found at Lake Xochimilco using data from Meta16S metabarcoding library. Values show the median (25%,75%), while comparisons were done using the nonparametric Kruskal–Wallis rank sum test.

α-Diversity index	Artificial body	Lagoon	Large canal	Refuge	Small canal	*p*
	N = 36	*N* = 90	*N* = 144	*N* = 18	*N* = 117	
Observed	4.00 (3.75, 5.00)	2.00 (1.00, 4.75)	5.00 (3.00, 7.25)	0.00 (0.00, 1.00)	3.00 (1.00, 5.00)	< 0.001
Shannon	0.76 (0.67, 1.09)	0.17 (0.00, 0.86)	0.89 (0.49, 1.14)	0.00 (0.00, 0.00)	0.38 (0.00, 0.81)	< 0.001
Simpson	0.44 (0.38, 0.64)	0.37 (0.02, 0.63)	0.45 (0.31, 0.59)	1.00 (0.00, 1.00)	0.46 (0.13, 0.71)	0.3

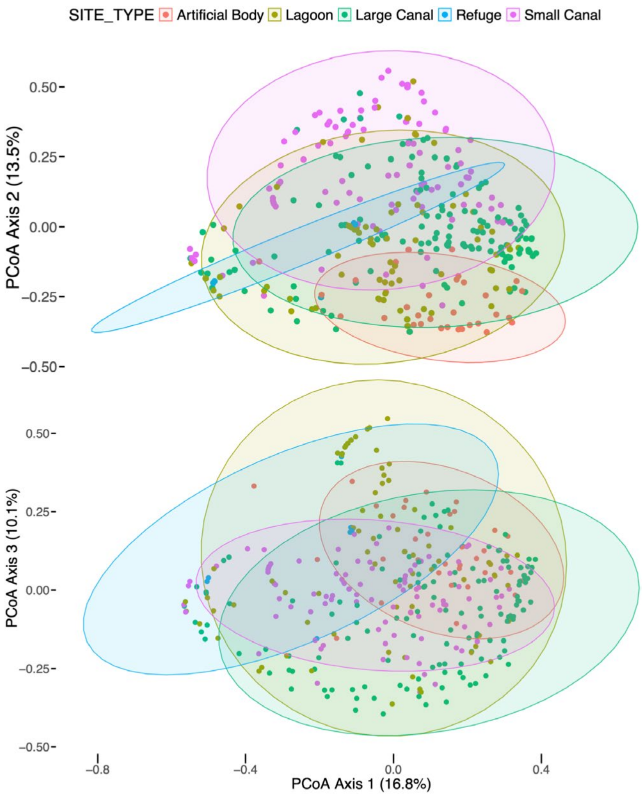

FIGURE 5 | PCoA of Bray–Curtis distances comparing the effect of water body type on the amphibian and fish communities (using number of species). The first three axes of beta diversity analysis explain 40.4% of the total variation. While visually, the differentiation between the different water body types is not obvious, the PERMANOVA analysis indicates a significant degree of species community differentiation. Ellipses made with a 95% confidence interval.

with the largest abundance values across all analyzed taxa, although this was driven by alien cichlid and carp taxa.

Large canals seem to be the most taxonomically diverse water body in the wetland (based on the observed number of identified species) compared to lagoons, small canals, and the artificial body. Nevertheless, both Shannon and Simpson indices revealed an overall low species diversity ecosystem, with an important degree of commonality (Table 1). Such effects may be driven by the abundant and constant presence of alien fish species (*Carassius*, *Cyprinus*, and *Oreochromis*) as seen in the abundance estimates (Figure 2).

Analysis of beta diversity using the PCoA method with Bray–Curtis distances indicated that the community composition of fish and amphibian diversity also differed depending on the water body type (Figure 5). Results of a PERMANOVA analysis indicated a significant difference between the centroids (using the Bray distance method) of the different clusters according to water body type ($p < 0.001$). Additional pairwise PERMANOVA analysis found that all comparisons between water body types had significant differences ($p < 0.01$). PCR variability from samples and replicates showed no significant effect on the model ($p > 0.05$).

3.5 | Effects of Environmental Stressors on eDNA Detection of Native and Alien Fishes

According to the multivariate analysis, only water temperature (Wald = 4.302, $p = 0.016$), dissolved oxygen (Wald = 4.349, $p = 0.016$), and nitrates (Wald = 6.323, $p = 0.002$) had significant effects on overall eDNA counts. Univariate tests showed that the variables of significant effect varied by fish family.

For alien families, nitrate levels had a positive effect on the presence of Cichlidae (Coef = 0.03, Wald = 3.609, $p = 0.006$) and Cyprinidae (Coef = 0.041, Wald = 3.841, $p = 0.003$) eDNA counts. Dissolved oxygen had a positive effect only on Cyprinidae eDNA counts (Coef = 0.041, Wald = 3.754, $p = 0.001$), but showed no significant effect on other families. Among native Mexican families, pH had a positive significant effect on the presence of eDNA counts from Atherinopsidae (Coef = 0.40, Wald = 2.667, $p = 0.029$), while water temperature registered a significant negative effect on the eDNA counts from Poeciliidae (Coef = −0.14, Wald = 2.746, $p = 0.037$). Only the Goodeidae eDNA counts were not significantly influenced by any of the analyzed environmental variables.

Analysis of the R^2 values indicated that the most influential variables were nitrate (model fit 1, $R^2 = 0.1714$), pH (model fit 2, $R^2 = 0.2256$), and water temperature (model fit 3, $R^2 = 0.2770$). The sum of the R^2 values of all the variables included in model fit 1 resulted in a global R^2 value of 0.4027.

4 | Discussion

Freshwater ecosystems are severely threatened worldwide (Dudgeon et al. 2006; Reid et al. 2019; Strayer and Dudgeon 2010), and their primary freshwater vertebrates, amphibians, and fishes, are among the most threatened animal groups on the planet (Hughes et al. 2021; World Wildlife Fund

(WWF) 2022; Luedtke et al. 2023). The situation of Mexican ecosystems is no different, particularly in central Mexico, where anthropogenic pressures stemming from urban centers have greatly impacted freshwaters (Contreras-MacBeath, Brito-Rodríguez, et al. 2014; Domínguez-Domínguez et al. 2007; Lira-Noriega et al. 2015; Lyons et al. 2000; Metcalfe et al. 1989; O'Hara et al. 1994). Lake Xochimilco illustrates the severe pressures urban ecosystems face from land use change, alien species, pollution, and climate change (e.g., Alcocer-Durand and Escobar-Briones 1992; Pérez-Belmont et al. 2019; Zambrano et al. 2010a). The anthropogenic impact observed at our study site is not unique, and Lake Xochimilco's ecological condition mirrors that of other wetlands that have been engulfed by expanding metropolitan areas worldwide (Brinkmann et al. 2020; Burgin et al. 2016; Pauchard et al. 2006; Rojas et al. 2022; Yang et al. 2024).

We showed that environmental DNA metabarcoding detected greater overall fish and amphibian diversity compared to historical data from traditional net-based surveys. However, our findings in combination with water parameter records indicate a severely degraded wetland. Alpha diversity metrics revealed a low-diversity ecosystem dominated by alien fish species. Despite the degraded condition of Lake Xochimilco, native and endemic fauna persist, and we found some evidence of potential cryptic amphibian diversity. Additionally, our results support the effectiveness of on-site wildlife refuges in conserving species such as the Critically Endangered axolotl.

4.1 | Domination of Alien Fish Species in Lake Xochimilco

It is now accepted that the introduction of species into new habitats is a phenomenon occurring globally, with minimal signs of successful control or regulation (Seebens et al. 2017). Malpractices in global trade, lack of monitoring and climate change are becoming important drivers behind biological invasions (Meyerson and Mooney 2007; Pyšek et al. 2020; Walther et al. 2009). Our data confirmed the continued presence of alien fish species at Lake Xochimilco, with the most abundant and commonly found eDNA reads assigned to the Old-World cyprinids *Cyprinus* and *Carassius*. Additionally, African cichlids (*Oreochromis* sp.) were detected consistently across the wetland area. The presence of these fish species (which are typically used in aquaculture and commercial trade) has been an ongoing occurrence in Lake Xochimilco since the last decades of the 20th century (Voss et al. 2015), where they have been noted to have detrimental effects, such as predation and competition with native species, and speeding up the eutrophication process (Alcocer-Durand and Escobar-Briones 1992; Contreras et al. 2009; Huidobro-Campos et al. 2016; Zambrano et al. 2007; Zambrano et al. 2010a).

The distribution of alien fish families in Lake Xochimilco (Figure 4) supports previous studies showing how urbanization and alteration of aquatic habitats may enhance their colonization while negatively affecting the diversity and abundance of native aquatic animals (Hermoso et al. 2011; Larentis et al. 2022; Marques et al. 2020; Riley et al. 2005; Stranko et al. 2010). Additionally, species distribution models have shown that abundance of native fish species is a significant variable to determine the abundance of alien fish species, as in the case of the topmouth gudgeon (*Pseudorasbora parva*) in Belgium (Verhelst et al. 2016). Therefore, the high abundance of eDNA from Cichlidae and Cyprinidae (taken as a proxy for biomass) may be an indicator of a low abundance of native fish family biomass. Moreover, the models developed by Verhelst et al. (2016) indicated that predator abundance also has a strong effect on alien species. In the case of Lake Xochimilco, the axolotl was considered the aquatic apex predator, due to the smaller size of native fish from the genera *Chirostoma*, *Evarra*, and *Girardinichthys* (Alcaraz et al. 2015; Alcocer-Durand and Escobar-Briones 1992; Griffiths et al. 2003; Smith 1989; Zambrano et al. 2006; Zambrano et al. 2010a). Therefore, with axolotl populations either reduced or absent, it is unlikely that native fish species could predate the eggs and fry of alien cichlids and cyprinids (Zambrano et al. 2010a).

Our findings from Lake Xochimilco reflect the global ecological trend of alien species colonisations (Toussaint et al. 2016) and the homogenization of fish species assemblages within central Mexico's (Gesundheit and Garcia 2018) freshwater ecosystems. The commonality and abundance of alien species indicate a colonized ecosystem, following the results from predicted establishment models for *Oreochromis* and *Cyprinus* species in North American freshwater ecosystems (Zambrano et al. 2001; Zambrano et al. 2006; Zambrano et al. 2010a). The impacts on ecosystem services are one of the critical consequences of alien species invasions (Charles and Dukes 2007), with species such as *Cyprinus carpio* shown to have a large impact on community composition and ecosystem processes (Matsuzaki et al. 2009).

Biological processes of alien fish species, including bioturbation, excretions, and consumption, have likely led to rapid ecosystem collapse to a turbid state while delaying ecosystem recovery (Reynolds and Aldridge 2021). Our findings showed a negative correlation between alien species and native species (although we cannot infer the direction of the interactions (Blanchet et al. 2020)) and may indicate a similar scenario for the current ecological state of Lake Xochimilco.

Translocated fish species identified in Lake Xochimilco are native to the broader biogeographical zone but are not native to the lake (Matsuzaki et al. 2013). Species that fit this description were detected at several sampling sites and include Goodeidae and Poeciliidae, both common in the aquarium trade. Several studies have suggested the lack of controls, monitoring, and human-mediated release as the origin of Lake Xochimilco populations (Contreras-Balderas et al. 2008; Contreras-MacBeath, Gaspar-Dillanes, et al. 2014; Huidobro-Campos et al. 2016; Zambrano et al. 2010a). Although there have been fewer studies on the effects of translocated species on new habitats compared to alien species, translocated species are increasing worldwide (Arthington 1991; Gesundheit and Garcia 2018; Lenhardt et al. 2011; Xiang et al. 2021). Several studies have found translocated fish to be detrimental to native fish diversity and richness (Maceda-Veiga et al. 2022; Matsuzaki et al. 2013). In contrast, our results indicated that translocated Poeciliidae may not be abundant in the Lake Xochimilco ecosystem. Partial correlation results also showed no significant relationship with the native families Atherinopsidae or Goodeidae, suggesting that these taxa likely have a limited effect on each other, which is in marked contrast to the alien fish families (Figure S3). The difference between translocated versus alien species may reside in the ecological characteristics of the identified poeciliid genera (*Xiphophorus*, *Pseudoxiphophorus*, *Peociliopsis*, and *Poecilia*) as Neotropical species from southern Mexico (Miller et al. 2009). Therefore, our results support previous studies that found translocated species to only have a significant impact on native fish assemblages when they originate from ecosystems with similar environmental characteristics (McKinney 2005; Toussaint et al. 2016). Nevertheless, despite alien fish species having a stronger impact on native fishes, the effects of translocated species should not be understated as they may disrupt the ecosystem by being disease vectors (Salgado-Maldonado and Pineda-López 2003; Vázquez-Silva et al. 2017), or by hybridizing with native species (e.g., Gesundheit and Garcia 2018; Shechonge et al. 2019).

In addition to previously reported species in both past surveys and the latest inventory of the area (Huidobro-Campos et al. 2016), we detected novel introduced species for the area (e.g., the Mexican cichlid *Thorichthys aureus* from the aquarium trade) that may indicate recent introductions that could lead to further invasions (Strayer 2010). Particular attention should be given to our detection of *Glyptothorax*, a catfish from Asia, as it has not been reported previously as occurring in the Valley of Mexico or even central Mexico (Contreras-MacBeath, Gaspar-Dillanes, et al. 2014; Huidobro-Campos et al. 2016). eDNA is a particularly sensitive tool to detect these events with spatial precision when applying distribution models (Itakura et al. 2019; Jo et al. 2020; Tillotson et al. 2018; Wood et al. 2021). However, care should be taken with the interpretation of these types of findings regarding their short-, mid-, and long-term prospects. While detections from a single eDNA study may act as a warning for potential problems, it does not provide data on the species invasiveness/colonization potential which may be subject to environmental abiotic characteristics (Dejean et al. 2012; Hunter et al. 2015). Confirmation of the species presence in the ecosystem should be analyzed through multiple eDNA analyses using species-specific approaches (Chucholl et al. 2021; Ruppert et al. 2019; Xia et al. 2018). However, to ensure detections are not false positives from wastewater (e.g., food fish and/or aquarium species), other data sources are needed to validate detections.

4.2 | Detected, but Scarce: Native Fish and Amphibian Diversity Across Lake Xochimilco

Although native fish and amphibian species were detected from eDNA metabarcoding at Lake Xochimilco, detections were typically rare. Of particular importance is the detection of the rare endemic fish species Chapultepec splitfin (*Giradinicthys viviparus*), known locally as mexclapique (Alcocer-Durand and Escobar-Briones 1992) considered extirpated in the wild by recent surveys (Huidobro-Campos et al. 2016) and listed as Endangered by the IUCN Red List (Koeck 2019). While most ASVs from the Neotropical Silversides (Atherinopsidae) were identified as the native species *Chirostoma jordani*, only one was identified as the endemic species *C. humboldtianum*, currently listed as Vulnerable in the IUCN Red List (Soto-Galera 2019). However, we failed to detect the cyprinid genus *Evarra* (endemic to the Valley of Mexico) supporting the consensus that all three species (*E. bustamantei*, *E. eigenmanni*, and *E. tlahualensis*) are now extinct (Alcocer-Durand and Escobar-Briones 1992; Huidobro-Campos et al. 2016).

Environmental DNA metabarcoding also detected native amphibian ASVs in Lake Xochimilco (*Ambystoma* and *Lithobates*), although specific species could not be reliably identified. However, these detections were incredibly rare, representing only a single refuge site for *Ambystoma*, and three wild sites in the case of *Lithobates*. The *Ambystoma* eDNA was detected in only one of the two on-site wildlife refuges, with no further detection from any of the 43 wild sampling points. A severe decline in *Ambystoma mexicanum* population densities has been recorded since the late 1990s (Griffiths et al. 2004) and has continued in recent years (Ayala et al. 2019; Contreras et al. 2009; Valiente et al. 2010; Zambrano et al. 2010a), with the last estimation as low as 35 individuals km^2 (Voss et al. 2015). As validation of *Ambystoma* eDNA amplification was conducted through three different stages (in silico, in vitro and in situ sampling), our results suggest that the population decline has continued since the 2015 estimate, presenting two potential scenarios: (1) the wild population density is extremely low (few eDNA molecules being resuspended in the water column to allow metabarcoding detections) or (2) the wild population is now extinct (Zambrano pers. obs.). The refuge where our single detection was made is a pilot for an on-site conservation program. These refuges likely contain wild-type *Ambystoma mexicanum*, as opposed to captive-bred ones, as no individuals were introduced to these sites (Zambrano pers. obs).

Our study also identified three ASVs assigned to the anuran genus *Lithobates* from nonrefuge sampling sites. Although the sequences successfully passed the bioinformatic filtering steps, the read count was exceptionally low. This may be due to the low population density of these frogs and/or lower binding affinity of the primer. These ASVs could not be assigned to the Critically Endangered Tlaloc's leopard frog (*L. tlaloci*) or reliably to the co-occurring common Moctezuma's leopard frog (*L. montezumae*). It is likely that there is undescribed diversity of leopard frogs in the region (Ochoa-Vázquez et al. 2019), although further taxonomic work is required. While our findings may indicate possible cryptic diversity in *Lithobates* at Lake Xochimilco, these should be interpreted with caution. Although data recovered by eDNA metabarcoding must be integrated into a multidisciplinary framework to be used to describe a species new to science, it can be an important aid in discovering previously unknown genetic diversity from severely impacted ecosystems, such as Lake Xochimilco (Ayala et al. 2019; Chaparro-Herrera et al. 2013; Contreras et al. 2009; Griffiths et al. 2004; Merlín-Uribe et al. 2013; Pérez-Belmont et al. 2019; Voss et al. 2015; Zambrano et al. 2009; Zambrano et al. 2010a; Zambrano et al. 2020), and act as an early warning sign to focus taxonomic attention.

As monitoring of the two Critically Endangered amphibian species was mixed, we suggest temporal surveys should be a priority to ensure sampling across breeding seasons when we might expect more eDNA to be shed. Although our sampling coincided with one of the axolotl breeding seasons (January–February, the other being August–September), the breeding season of Tlaloc's leopard frog is unknown to the best of our knowledge. The notable absence of this species warrants further investigation. Processing a greater volume of water to collect more eDNA may help in this regard, as has been shown with rare and endangered populations (Peixoto et al. 2021; Schabacker et al. 2020; Sepulveda et al. 2019), as they are likely contributing less eDNA to the water column. In our study, filtering volumes varied greatly due to suspended particle matter in the water column, as Lake Xochimilco has significant turbidity levels (Gayosso-Morales et al. 2017). A potential future workaround for filter clogging would be using high-capacity filters that have been proven to successfully collect eDNA while processing considerable filtered volumes (> 5 L) of muddy water (Peixoto et al. 2021).

4.3 | Different Water Bodies Across Lake Xochimilco Influence Relative Abundances of eDNA Reads and Community Diversity

Alpha diversity metrics varied between sites, suggesting that waterbody type (small canals, larger canals, and lagoons) is an ecological characteristic playing a significant role in the differentiation of species community composition. Beta diversity analysis also suggests a heterogenous ecosystem, potentially driven by the presence of alien fish species—particularly cyprinids—with evidence that some species prefer to occupy different water bodies within the remaining remnant lake. The findings support previous studies (Zambrano et al. 2009; Zambrano et al. 2010b) demonstrating Lake Xochimilco as a highly heterogenous wetland due to severe habitat fragmentation and waterway layout.

While alien fish families such as Cyprinidae and Cichlidae were detected throughout the system (see Section 4.1), they were more abundant in areas with high levels of human activity and development. Variation in their eDNA read abundances suggests that some water bodies may also be more prone to colonization or proliferation, although this varied across these taxa, and could potentially be due to water chemistry and/or habitat structure.

We suggest that future conservation management should focus efforts on managing water bodies within the remnant lake that revealed higher eDNA abundances of native fish families, such as the artificial pond, and areas belonging to the core zone of the Natural Protected Area (Figure 1). In addition to the on-site refuges, these areas may serve as functional refugia (see Chester and Robson 2013; Deacon et al. 2018; Zamora-Marín et al. 2021) and are therefore potentially less affected by the ecological impacts of alien fish dominance, such as bioturbation and nutrient cycling, which can lead to ecosystem homogenization and collapse (see Lougheed et al. 2008; Petsch 2016; Stendera et al. 2012).

Inferring direct biomass or species abundance from eDNA read counts has been considered a potential outcome from eDNA methods (Lodge et al. 2012) but is currently disputed with studies showing supporting (e.g., Bylemans et al. 2019; Doi et al. 2017; Li et al. 2021; Muri et al. 2020; Skelton et al. 2023) or contradictory results (e.g., Carvalho et al. 2022; Fonseca 2018; Nakagawa et al. 2022). Therefore, while we treat our results based on read abundance with caution, they are consistent with reported populations from traditional surveys (Zambrano pers. obs.) and present a reasonable suggestion on the state of the ecosystem.

4.4 | Anthropogenic Environmental Stressors Showed Varied Effects on Native and Alien Fish Families

Multivariate analysis indicated that eDNA read counts from the fish families are influenced by physical (water temperature) and chemical characteristics (pH and nitrates), but the effect varied by fish family. Assuming this reflects some indicator of biomass or abundance, this may reflect the evolutionary history of the fish family. For the Neotropical silversides (Atherinopsidae) eDNA counts had a positive coefficient (0.40) with pH, which may reflect their adaptation to the alkaline water of the Basin of the Valley of Mexico (Alcocer-Durand and Escobar-Briones 1992). Both fish families represented by alien genera, Cichlidae (*Oreochromis*) and Cyprinidae (*Carassius*, *Cyprinus*) had a positive coefficient with nitrates. These alien genera are used in aquaculture due to their resilience and adaptability to thrive in altered freshwater ecosystems (Zambrano et al. 2007; Zambrano et al. 2010a), and alien carp species have been found to alter the water chemistry as a by-product of their feeding activities (Weber and Brown 2009; Zambrano et al. 2001; Zambrano et al. 2006). There is a contrasting result in the case of Poeciliidae, as their eDNA read counts had a negative coefficient with water temperature, which is not surprising, given these species can thrive at warmer temperatures (20°C–25°C) (Fishbase, 30 October 2024).

Water at Lake Xochimilco has shown milder temperatures between 11°C and 22°C through the year during surveys conducted in 2000–2001 (Zambrano et al. 2009) and in 2002–2003 (Nandini et al. 2005) due to the lake's high altitude. In the present study, temperatures ranged from 15°C to 25.4°C across sampling (November to April), similar to more recent records of 19.5°C to 22°C (Nandini et al. 2016), 19°C to 26°C (Gayosso-Morales et al. 2017) and 16°C to 22.5°C (Pérez-Belmont et al. 2019) through different parts of the lake. While it has been established that Lake Xochimilco is a highly heterogeneous system with yearly aquatic temperature variations (Zambrano et al. 2007, 2010b), the new records may indicate the water temperature range is shifting upwards and thus may present an additional factor of change in the fish species assemblages.

In future, we suggest additional factors should be investigated; for example, water turbidity and water current may influence these values and it is important to consider that PCR variability and competition of targets with different initial sequence counts during multiplex reactions could have an impact on the overall trends and effects analyzed. Furthermore, the multivariate analysis could be better fitted to eDNA single-species approaches as the primer set would not be competing over different targets (Ruppert et al. 2019) due to the optimization of the reaction.

4.5 | Performance of eDNA Metabarcoding Primers

Inclusion of multiple primer pairs in metabarcoding studies can be critical (e.g., Doble et al. 2020) for maximizing taxonomic recovery through differential binding affinity. Here, our primers both amplified significant species diversity across the vertebrate groups and though the coverage was not completely overlapping, this provided greater taxonomic coverage (Garlapati et al. 2019; Ruppert et al. 2019). The 12S-V5 generated the highest number of reads in the library (> 12 million reads vs. ~8 million reads by the Meta16S) but lacked taxonomic resolution for some taxa such as Cichlidae and Cyprinidae compared to the Meta16S (Table S5). Despite some limitations in taxonomic assignment, the 12S-V5 detected rare species, such as *Girardinichthys viviparus*, in higher read numbers. This may be due to the shorter amplicon size (~110 bp), which is more suitable for amplifying potentially degraded eDNA molecules in the water column (Barnes et al. 2014; Harrison et al. 2019). This highlights the potential for using short universal markers (such as the 12S-V5) as complementary markers to longer, more informative ones.

Although both eDNA markers did amplify human and domestic animals (~5% for 12S-V5 and ~2% for Meta16S), they also amplified rare species in the lake, such as the leopard frogs (*Lithobates* sp.) and the mexclapique fish (*G. viviparus*) at least to genus. Nevertheless, recently evolved species (such as those belonging to *Ambystoma*, *Oreochromis*, *Cyprinus*, and *Carassius*) proved to be difficult to differentiate at the species level (Table S5), and may require more specific markers (e.g., Doble et al. 2020; Vences et al. 2016), and detection for anurans seemed to depend on the eDNA marker selection. Care should therefore be taken to ensure that the specific markers are optimized to obtain reliable species information (Xia et al. 2021), particularly for Critically Endangered species such as the axolotl.

5 | Conclusions

Here, we report on the ecology of the high-altitude subtropical Lake Xochimilco, an urban wetland that is highly fragmented with a high degree of eutrophication caused by anthropogenic pressures. Based on eDNA metabarcoding and the recording of water parameters and pollutant readings, our findings indicate that Lake Xochimilco is a collapsed ecosystem dominated by alien fish species. Yet, despite the degraded environment and the prevalence of alien taxa, some native fish species (such as *G. viviparus* and *Chirostoma* sp.) were detected along with *Ambystoma* salamanders, and *Lithobates* frogs that could represent a new cryptic species. This highlights that even severely impacted aquatic ecosystems may harbor hidden diversity, including species of conservation concern. The detection of native rare species, which may be undetectable by traditional methods due to their low population densities, indicates their resilience to survive in degraded ecosystems and the importance of limiting further declines and mitigating past damage. As our findings support the value of on-site wildlife refuges for the culturally and ecologically important axolotl, we recommend establishing managed refuges to safeguard this and other native species.

Author Contributions

A.M.-O., E.L.C., and J.J.D. were involved in conceptualization. A.M.-O. conducted fieldwork, laboratory work, bioinformatics, and downstream analysis. L.Z. acquired permits and provided field expertise. G.J.H., P.P., and D.A.D., advised on laboratory work. D.J.M. provided analytical advice. O.D.-D., V.H.R., B.T., C.J.M., and J.W.S. contributed samples. A.M.-O., E.L.C., and J.J.D. wrote the manuscript, and all authors edited the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study is openly available on the UCL Data Research Repository at https://doi.org/10.5522/04/c.7886462.v1. All sequences generated for the reference databases are available on NCBI (see Supporting Information).

References

Alcaraz, G., X. López-Portela, and C. Robles-Mendoza. 2015. "Response of a Native Endangered Axolotl, *Ambystoma mexicanum* (Amphibia), to Exotic Fish Predator." *Hydrobiologia* 753, no. 1: 73–80. https://doi.org/10.1007/s10750-015-2194-4.

Alcocer-Durand, J., and E. G. Escobar-Briones. 1992. "The Aquatic Biota of the Now Extinct Lacustrine Complex of the Mexico Basin." *Freshwater Forum* 2, no. 3: 171–183.

Andres, K. J., T. D. Lambert, D. M. Lodge, J. Andrés, and J. R. Jackson. 2023. "Combining Sampling Gear to Optimally Inventory Species Highlights the Efficiency of eDNA Metabarcoding." *Environmental DNA* 5, no. 1: 146–157. https://doi.org/10.1002/edn3.366.

Arthington, A. H. 1991. "Ecological and Genetic Impacts of Introduced and Translocated Freshwater Fishes in Australia." *Canadian Journal of Fisheries and Aquatic Sciences* 48, no. S1: 33–43. https://doi.org/10.1139/f91-302.

Ayala, C., A. G. Ramos, Á. Merlo, and L. Zambrano. 2019. "Microhabitat Selection of Axolotls, *Ambystoma mexicanum*, in Artificial and Natural Aquatic Systems." *Hydrobiologia* 828, no. 1: 11–20. https://doi.org/10.1007/s10750-018-3792-8.

Barnes, M. A., C. R. Turner, C. L. Jerde, M. A. Renshaw, W. L. Chadderton, and D. M. Lodge. 2014. "Environmental Conditions Influence Edna Persistence in Aquatic Systems." *Environmental Science & Technology* 48, no. 3: 1819–1827. https://doi.org/10.1021/es404734p.

Bessey, C., S. N. Jarman, O. Berry, et al. 2020. "Maximizing Fish Detection With eDNA Metabarcoding." *Environmental DNA* 2, no. 4: 493–504. https://doi.org/10.1002/edn3.74.

Blanchet, F. G., K. Cazelles, and D. Gravel. 2020. "Co-Occurrence Is Not Evidence of Ecological Interactions." *Ecology Letters* 23, no. 7: 1050–1063. https://doi.org/10.1111/ele.13525.

Brinkmann, K., E. Hoffmann, and A. Buerkert. 2020. “Spatial and Temporal Dynamics of Urban Wetlands in an Indian Megacity Over the Past 50 Years.” *Remote Sensing* 12, no. 4: 662. https://doi.org/10.3390/rs12040662.

Burgin, S., M. J. M. Franklin, and L. Hull. 2016. “Wetland Loss in the Transition to Urbanisation: A Case Study From Western Sydney, Australia.” *Wetlands* 36, no. 6: 985–994. https://doi.org/10.1007/s13157-016-0813-0.

Bylemans, J., D. M. Gleeson, R. P. Duncan, C. M. Hardy, and E. M. Furlan. 2019. “A Performance Evaluation of Targeted eDNA and eDNA Metabarcoding Analyses for Freshwater Fishes.” *Environmental DNA* 1, no. 4: 402. https://doi.org/10.1002/edn3.41.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. “DADA2: High-Resolution Sample Inference From Illumina Amplicon Data.” *Nature Methods* 13, no. 7: 581–587. https://doi.org/10.1038/nmeth.3869.

Carpenter, S. R., E. H. Stanley, and M. J. V. Zanden. 2011. “State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes.” *Environment and Resources* 36, no. 1: 75–99. https://doi.org/10.1146/annurev-environ-021810-094524.

Carvalho, C. S., M. E. Oliveira, K. G. Rodriguez-Castro, B. H. Saranholi, and P. M. Galetti. 2022. “Efficiency of eDNA and iDNA in Assessing Vertebrate Diversity and Its Abundance.” *Molecular Ecology Resources* 22, no. 4: 1262–1273. https://doi.org/10.1111/1755-0998.13543.

Chambers, E. A., C.-G. Lara-Tufiño, J. D. G. A. Y. Cisneros-Bernal, et al. 2025. “Distinguishing Species Boundaries From Geographic Variation.” *Proceedings of the National Academy of Sciences of the United States of America* 122, no. 19: e2423688122. https://doi.org/10.1073/pnas.2423688122.

Chaparro-Herrera, D. de J., S. Nandini, and S. S. S. Sarma. 2013. “Effect of Water Quality on the Feeding Ecology of Axolotl *Ambystoma mexicanum*.” *Journal of Limnology* 72, no. 3: 46. https://doi.org/10.4081/jlimnol.2013.e46.

Charles, H., and J. S. Dukes. 2007. “Impacts of Invasive Species on Ecosystem Services.” In *Biological Invasions*, 217–237. Ecological Studies. https://doi.org/10.1007/978-3-540-36920-2_13.

Chester, E. T., and B. J. Robson. 2013. “Anthropogenic Refuges for Freshwater Biodiversity: Their Ecological Characteristics and Management.” *Biological Conservation* 166: 64–75. https://doi.org/10.1016/j.biocon.2013.06.016.

Chucholl, F., F. Fiolka, G. Segelbacher, and L. S. Epp. 2021. “eDNA Detection of Native and Invasive Crayfish Species Allows for Year-Round Monitoring and Large-Scale Screening of Lotic Systems.” *Frontiers in Environmental Science* 9: 639380. https://doi.org/10.3389/fenvs.2021.639380.

Cilleros, K., A. Valentini, L. Allard, et al. 2019. “Unlocking Biodiversity and Conservation Studies in High-Diversity Environments Using Environmental DNA (eDNA): A Test With Guianese Freshwater Fishes.” *Molecular Ecology Resources* 19, no. 1: 27–46. https://doi.org/10.1111/1755-0998.12900.

Collen, B., F. Whitton, E. E. Dyer, et al. 2014. “Global Patterns of Freshwater Species Diversity, Threat and Endemism.” *Global Ecology and Biogeography* 23, no. 1: 40–51. https://doi.org/10.1111/geb.12096.

Contreras, V., E. Martínez-Meyer, E. Valiente, and L. Zambrano. 2009. “Recent Decline and Potential Distribution in the Last Remnant Area of the Microendemic Mexican Axolotl (*Ambystoma mexicanum*).” *Biological Conservation* 142, no. 12: 2881–2885. https://doi.org/10.1016/j.biocon.2009.07.008.

Contreras-Balderas, S., G. Ruiz-Campos, J. J. Schmitter-Soto, et al. 2008. “Freshwater Fishes and Water Status in México: A Country-Wide Appraisal.” *Aquatic Ecosystem Health & Management* 11, no. 3: 246–256. https://doi.org/10.1080/14634980802319986.

Contreras-MacBeath, T., M. Brito-Rodríguez, V. Sorani, C. Goldspink, and G. M. Reid. 2014. “Richness and Endemism of the Freshwater Fishes of Mexico.” *Journal of Threatened Taxa* 6, no. 2: 5421–5433. https://doi.org/10.11609/jott.o3633.5421-33.

Contreras-MacBeath, T., M. T. Gaspar-Dillanes, L. Huidobro-Campos, and H. Mejía-Mojica. 2014. “Peces Invasores en el Centro de México.” In *Especies Acuáticas Invasoras en México*, 1st ed., 413–424. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO).

Costanza, R., R. de Groot, P. Sutton, et al. 2014. “Changes in the Global Value of Ecosystem Services.” *Global Environmental Change* 26: 152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002.

Davis, N. M., D. M. Proctor, S. P. Holmes, D. A. Relman, and B. J. Callahan. 2018. “Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data.” *Microbiome* 6, no. 1: 226. https://doi.org/10.1186/s40168-018-0605-2.

Deacon, C., M. J. Samways, and J. S. Pryke. 2018. “Artificial Reservoirs Complement Natural Ponds to Improve Pondscape Resilience in Conservation Corridors in a Biodiversity Hotspot.” *PLoS One* 13, no. 9: e0204148. https://doi.org/10.1371/journal.pone.0204148.

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. “Improved Detection of an Alien Invasive Species Through Environmental DNA Barcoding: The Example of the American Bullfrog *Lithobates Catesbeianus*.” *Journal of Applied Ecology* 49, no. 4: 953–959. https://doi.org/10.1111/j.1365-2664.2012.02171.x.

Doble, C. J., H. Hipperson, W. Salzburger, et al. 2020. “Testing the Performance of Environmental DNA Metabarcoding for Surveying Highly Diverse Tropical Fish Communities: A Case Study From Lake Tanganyika.” *Environmental DNA* 2, no. 1: 24–41. https://doi.org/10.1002/edn3.43.

Doi, H., R. Inui, Y. Akamatsu, et al. 2017. “Environmental DNA Analysis for Estimating the Abundance and Biomass of Stream Fish.” *Freshwater Biology* 62, no. 1: 30–39. https://doi.org/10.1111/fwb.12846.

Domínguez-Domínguez, O., L. Boto, F. Alda, G. P.-P. de León, and I. Doadrio. 2007. “Human Impacts on Drainages of the Mesa Central, Mexico, and Its Genetic Effects on an Endangered Fish, *Zoogoneticus Quitzeoensis*.” *Conservation Biology* 21, no. 1: 168–180. https://doi.org/10.1111/j.1523-1739.2006.00608.x.

Dudgeon, D., A. H. Arthington, M. O. Gessner, et al. 2006. “Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges.” *Biological Reviews* 81, no. 2: 163–182. https://doi.org/10.1017/s1464793105006950.

Dufresnes, C., T. Déjean, S. Zumbach, et al. 2019. “Early Detection and Spatial Monitoring of an Emerging Biological Invasion by Population Genetics and Environmental DNA Metabarcoding.” *Conservation Science and Practice* 1, no. 9: e86. https://doi.org/10.1111/csp2.86.

Espinosa-Pérez, H. 2014. “Biodiversidad de Peces en México.” *Revista Mexicana de Biodiversidad* 85: S450–S459. https://doi.org/10.7550/rmb.32264.

Espinosa-Pérez, H., and M. Ramírez. 2015. “Exotic and Invasive Fishes in Mexico.” *Check List* 11, no. 3: 1–13. https://doi.org/10.15560/11.3.1627.

Faeth, S. H., C. Bang, and S. Saari. 2011. “Urban Biodiversity: Patterns and Mechanisms.” *Annals of the New York Academy of Sciences* 1223, no. 1: 69–81. https://doi.org/10.1111/j.1749-6632.2010.05925.x.

Falaschi, M., A. Melotto, R. Manenti, and G. F. Ficetola. 2020. “Invasive Species and Amphibian Conservation.” *Herpetologica* 76, no. 2: 216–227. https://doi.org/10.1655/0018-0831-76.2.216.

Fediajevaite, J., V. Priestley, R. Arnold, and V. Savolainen. 2021. “Meta-Analysis Shows That Environmental DNA Outperforms Traditional Surveys, but Warrants Better Reporting Standards.” *Ecology and Evolution* 11, no. 9: 4803–4815. https://doi.org/10.1002/ece3.7382.

Fonseca, V. G. 2018. "Pitfalls in Relative Abundance Estimation Using eDNA Metabarcoding." *Molecular Ecology Resources* 18, no. 5: 923–926. https://doi.org/10.1111/1755-0998.12902.

Forero Rodríguez, J. S., M. Ortega-Andrade, N. Urbina-Cardona, F. Villalobos, and O. R. Rojas-Soto. 2024. "The Decline of Harlequin Frogs in the Neotropics: Assessing the Silent Impact of Chytrid Fungus and Invasive Rainbow Trout on Extinction Risks." SSRN 10.2139/ssrn.4863463.

Fox, J., and S. Weisberg. 2019. *An R Companion to Applied Regression*. Third. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

García-Vázquez, U. O., M. Trujano-Ortega, and G. Casas-Andreu. 2016. *Anfibios*, 383–389. *La Biodiversidad en la Ciudad de México*. La Biodiversidad en la Ciudad de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Garlapati, D., B. Charankumar, K. Ramu, P. Madeswaran, and M. V. R. Murthy. 2019. "A Review on the Applications and Recent Advances in Environmental DNA (eDNA) Metagenomics." *Reviews in Environmental Science and Biotechnology* 18, no. 3: 389–411. https://doi.org/10.1007/s11157-019-09501-4.

Gayosso-Morales, M. A., S. Nandini, F. F. Martínez-Jeronimo, and S. S. S. Sarma. 2017. "Effect of Organic and Inorganic Turbidity on the Zooplankton Community Structure of a Shallow Waterbody in Central Mexico (Lake Xochimilco, Mexico)." *Journal of Environmental Biology* 38, no. 6: 1183–1196. https://doi.org/10.22438/jeb/38/6(si)/03.

Gesundheit, P., and C. M. Garcia. 2018. "The Role of Introduced Species in the Decline of a Highly Endemic Fish Fauna in Central Mexico." *Aquatic Conservation: Marine and Freshwater Ecosystems* 28, no. 6: 1384–1395. https://doi.org/10.1002/aqc.2927.

Griffiths, R. A., V. Graue, and I. G. Bride. 2003. "The Axolotls of Lake Xochimilco: The Evolution of a Conservation Programme." *Newsletter of the Ambystoma Genetic Stock Center, University of Kentucky* 30: 12–18.

Griffiths, R. A., V. Graue, I. G. Bride, and J. E. McKay. 2004. "Conservation of the Axolotl (*Ambystoma mexicanum*) at Lake Xochimilco, Mexico." *Herpetological Bulletin* 89: 4–11.

Hallam, J., E. L. Clare, J. I. Jones, and J. J. Day. 2021. "Biodiversity Assessment Across a Dynamic Riverine System: A Comparison of eDNA Metabarcoding Versus Traditional Fish Surveying Methods." *Environmental DNA* 3: 1247–1266. https://doi.org/10.1002/edn3.241.

Harrison, J. B., J. M. Sunday, and S. M. Rogers. 2019. "Predicting the Fate of eDNA in the Environment and Implications for Studying Biodiversity." *Proceedings of the Royal Society B: Biological Sciences* 286, no. 1915: 20191409. https://doi.org/10.1098/rspb.2019.1409.

Hartel, T., S. Nemes, D. Cogălniceanu, et al. 2007. "The Effect of Fish and Aquatic Habitat Complexity on Amphibians." *Hydrobiologia* 583, no. 1: 173–182. https://doi.org/10.1007/s10750-006-0490-8.

Hermoso, V., M. Clavero, F. Blanco-Garrido, and J. Prenda. 2011. "Invasive Species and Habitat Degradation in Iberian Streams: An Analysis of Their Role in Freshwater Fish Diversity Loss." *Ecological Applications* 21, no. 1: 175–188. https://doi.org/10.1890/09-2011.1.

Hillis, D. M., and T. P. Wilcox. 2005. "Phylogeny of the New World true frogs (Rana)." *Molecular Phylogenetics and Evolution* 34: 299–314. https://doi.org/10.1016/j.ympev.2004.10.007.

Hughes, K., I. Harrison, W. Darwall, et al. 2021. *The World's Forgotten Fishes*. WWF International.

Huidobro-Campos, L., X. Valencia-Díaz, N. Álvarez-Pliego, and H. Espinoza-Pérez. 2016. "Peces." In *La Biodiversidad en la Ciudad de México. México Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO)*, 376–382.

Hunter, M. E., S. J. Oyler-McCance, R. M. Dorazio, et al. 2015. "Environmental DNA (eDNA) Sampling Improves Occurrence and Detection Estimates of Invasive Burmese Pythons." *PLoS One* 10, no. 4: e0121655. https://doi.org/10.1371/journal.pone.0121655.

Itakura, H., R. Wakiya, S. Yamamoto, K. Kaifu, T. Sato, and T. Minamoto. 2019. "Environmental DNA Analysis Reveals the Spatial Distribution, Abundance, and Biomass of Japanese Eels at the River-Basin Scale." *Aquatic Conservation: Marine and Freshwater Ecosystems* 29, no. 3: 361–373. https://doi.org/10.1002/aqc.3058.

IUCN SSC Amphibian Specialist Group. 2020a. "Ambystoma mexicanum." *IUCN Red List of Threatened Species* 2020: e.T1095A53947343. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T1095A53947343.en.

IUCN SSC Amphibian Specialist Group. 2020b. "Lithobates tlaloci (errata version published in 2024)." *IUCN Red List of Threatened Species* 2020: e.T19182A254275657. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T19182A254275657.en.

Jerde, C. L., A. R. Mahon, T. Campbell, et al. 2021. "Are Genetic Reference Libraries Sufficient for Environmental DNA Metabarcoding of Mekong River Basin Fish?" *Water* 13, no. 13: 1767. https://doi.org/10.3390/w13131767.

Jiménez, M., P. Pérez-Belmont, M. Schewenius, A. M. Lerner, and M. Mazari-Hiriart. 2020. "Assessing the Historical Adaptive Cycles of an Urban Social-Ecological System and Its Potential Future Resilience: The Case of Xochimilco, Mexico City." *Regional Environmental Change* 20, no. 1: 7. https://doi.org/10.1007/s10113-020-01587-9.

Jo, T., A. Fukuoka, K. Uchida, A. Ushimaru, and T. Minamoto. 2020. "Multiplex Real-Time PCR Enables the Simultaneous Detection of Environmental DNA From Freshwater Fishes: A Case Study of Three Exotic and Three Threatened Native Fishes in Japan." *Biological Invasions* 22, no. 2: 455–471. https://doi.org/10.1007/s10530-019-02102-w.

Kats, L. B., and R. P. Ferrer. 2003. "Alien Predators and Amphibian Declines: Review of Two Decades of Science and the Transition to Conservation." *Diversity and Distributions* 9, no. 2: 99–110. https://doi.org/10.1046/j.1472-4642.2003.00013.x.

Keck, F., R. C. Blackman, R. Bossart, et al. 2022. "Meta-Analysis Shows Both Congruence and Complementarity of DNA and eDNA Metabarcoding to Traditional Methods for Biological Community Assessment." *Molecular Ecology* 31, no. 6: 1820–1835. https://doi.org/10.1111/mec.16364.

Kiesecker, J. M., A. R. Blaustein, and C. L. Miller. 2001. "Transfer of a Pathogen From Fish to Amphibians." *Conservation Biology* 15, no. 4: 1064–1070. https://doi.org/10.1046/j.1523-1739.2001.0150041064.x.

Kim, S. 2015. "Ppcor: An R Package for a Fast Calculation to Semi-Partial Correlation Coefficients." *Communications for Statistical Applications and Methods* 22, no. 6: 665–674.

Koeck, M. 2019. "Girardinichthys viviparus, Chapultepec Splitfin." *IUCN Red List of Threatened Species* 2019: e.T9196A3150258. https://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T9196A3150258.en.

Larentis, C., B. C. K. Kliemann, M. P. Neves, and R. L. Delariva. 2022. "Effects of Human Disturbance on Habitat and Fish Diversity in Neotropical Streams." *PLoS One* 17, no. 9: e0274191. https://doi.org/10.1371/journal.pone.0274191.

Lenhardt, M., G. Markovic, A. Hegedis, S. Maletin, M. Cirkovic, and Z. Markovic. 2011. "Non-Native and Translocated Fish Species in Serbia and Their Impact on the Native Ichthyofauna." *Reviews in Fish Biology and Fisheries* 21, no. 3: 407–421. https://doi.org/10.1007/s11160-010-9180-8.

Leray, M., N. Knowlton, S.-L. Ho, B. N. Nguyen, and R. J. Machida. 2019. "GenBank Is a Reliable Resource for 21st Century Biodiversity Research." *National Academy of Sciences of the United States of America* 116, no. 45: 22651–22656. https://doi.org/10.1073/pnas.1911714116.

Li, W., X. Hou, C. Xu, et al. 2021. "Validating eDNA Measurements of the Richness and Abundance of Anurans at a Large Scale." *Journal of Animal Ecology* 90, no. 6: 1466–1479. https://doi.org/10.1111/1365-2656.13468.

Lira-Noriega, A., V. Aguilar, J. Alarcón, et al. 2015. "Conservation Planning for Freshwater Ecosystems in Mexico." *Biological Conservation* 191: 357–366. https://doi.org/10.1016/j.biocon.2015.07.004.

Lodge, D. M., C. R. Turner, C. L. Jerde, et al. 2012. "Conservation in a Cup of Water: Estimating Biodiversity and Population Abundance From Environmental DNA." *Molecular Ecology* 21, no. 11: 2555–2558. https://doi.org/10.1111/j.1365-294x.2012.05600.x.

Lopes, C. M., T. Sasso, A. Valentini, et al. 2017. "eDNA Metabarcoding: A Promising Method for Anuran Surveys in Highly Diverse Tropical Forests." *Molecular Ecology Resources* 17, no. 5: 904–914. https://doi.org/10.1111/1755-0998.12643.

Lougheed, V. L., M. D. Mcintosh, C. A. Parker, and R. J. Stevenson. 2008. "Wetland Degradation Leads to Homogenization of the Biota at Local and Landscape Scales." *Freshwater Biology* 53, no. 12: 2402–2413. https://doi.org/10.1111/j.1365-2427.2008.02064.x.

Love, M. I., W. Huber, and S. Anders. 2014. "Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data With DESeq2." *Genome Biology* 15, no. 12: 550. https://doi.org/10.1186/s13059-014-0550-8.

Luedtke, J. A., J. Chanson, K. Neam, et al. 2023. "Ongoing Declines for the World's Amphibians in the Face of Emerging Threats." *Nature* 622: 308–314. https://doi.org/10.1038/s41586-023-06578-4.

Lyons, J., A. Gutiérrez-Hernández, E. Díaz-Pardo, E. Soto-Galera, M. Medina-Nava, and R. Pineda-López. 2000. "Development of a Preliminary Index of Biotic Integrity (IBI) Based on Fish Assemblages to Assess Ecosystem Condition in the Lakes of Central Mexico." *Hydrobiologia* 418, no. 1: 57–72. https://doi.org/10.1023/a:1003888032756.

Maceda-Veiga, A., R. M. Nally, A. de Sostoa, and J. D. L. Yen. 2022. "Patterns of Species Richness, Abundance and Individual-Size Distributions in Native Stream-Fish Assemblages Invaded by Exotic and Translocated Fishes." *Science of the Total Environment* 838, no. Pt 1: 155953. https://doi.org/10.1016/j.scitotenv.2022.155953.

Marques, P. S., L. R. Manna, T. C. Frauendorf, E. Zandonà, R. Mazzoni, and R. El-Sabaawi. 2020. "Urbanization Can Increase the Invasive Potential of Alien Species." *Journal of Animal Ecology* 89, no. 10: 2345–2355. https://doi.org/10.1111/1365-2656.13293.

Marques, V., T. Milhau, C. Albouy, et al. 2021. "GAPeDNA: Assessing and Mapping Global Species Gaps in Genetic Databases for eDNA Metabarcoding." *Diversity and Distributions* 27: 1880-1892. https://doi.org/10.1111/ddi.13142.

Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet.Journal* 17, no. 1: 10–12. https://doi.org/10.14806/ej.17.1.200.

Martinez Arbizu, P. 2017. "pairwiseAdonis: Pairwise Multilevel Comparison Using Adonis. R Package Version 0.4.1."

Matsuzaki, S. S., T. Sasaki, and M. Akasaka. 2013. "Introductions and Future Extirpations Affect Freshwater Fish FD." *Global Ecology and Biogeography* 22, no. 9: 1071–1082. https://doi.org/10.1111/geb.12067.

Matsuzaki, S. S., N. Usio, N. Takamura, and I. Washitani. 2009. "Contrasting Impacts of Invasive Engineers on Freshwater Ecosystems: An Experiment and Meta-Analysis." *Oecologia* 158, no. 4: 673–686. https://doi.org/10.1007/s00442-008-1180-1.

Mazari-Hiriart, M., M. A. Tapia-Palacios, A. E. Zarco-Arista, and A. C. Espinosa-García. 2019. "Challenges and Opportunities on Urban Water Quality in Mexico City." *Frontiers in Environmental Science* 7: 169. https://doi.org/10.3389/fenvs.2019.00169.

McKinney, M. L. 2005. "Species Introduced From Nearby Sources Have a More Homogenizing Effect Than Species From Distant Sources: Evidence From Plants and Fishes in the USA." *Diversity and Distributions* 11, no. 5: 367–374. https://doi.org/10.1111/j.1366-9516.2005.00181.x.

McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8, no. 4: e61217. https://doi.org/10.1371/journal.pone.0061217.

Mendoza, R., S. Luna, and C. Aguilera. 2015. "Risk Assessment of the Ornamental Fish Trade in Mexico: Analysis of Freshwater Species and Effectiveness of the FISK (Fish Invasiveness Screening Kit)." *Biological Invasions* 17, no. 12: 3491–3502. https://doi.org/10.1007/s10530-015-0973-5.

Merlín-Uribe, Y., A. Contreras-Hernández, M. Astier-Calderón, et al. 2013. "Urban Expansion Into a Protected Natural Area in Mexico City: Alternative Management Scenarios." *Journal of Environmental Planning and Management* 56, no. 3: 398–411. https://doi.org/10.1080/09640568.2012.683686.

Metcalfe, S. E., F. A. Street-Perrott, R. B. Brown, P. E. Hales, R. A. Perrott, and F. M. Steininger. 1989. "Late Holocene Human Impact on Lake Basins in Central Mexico." *Geoarchaeology* 4, no. 2: 119–141. https://doi.org/10.1002/gea.3340040203.

Meyerson, L. A., and H. A. Mooney. 2007. "Invasive Alien Species in an Era of Globalization." *Frontiers in Ecology and the Environment* 5, no. 4: 199–208. https://doi.org/10.1890/1540-9295(2007)5[199:iasiae]2.0.co;2.

Mikryukov, V. 2022. "metagMisc: Miscellaneous Functions for Metagenomic Analysis." https://github.com/vmikk/metagMisc.

Miller, R. R., W. L. Minckley, and S. M. Norris. 2009. *Peces dulceacuícolas de México*. Comisión Nacional para el Uso y Conocimiento de la Biodiversidad (CONABIO), Sociedad Ictiológica Mexicana, El Colegio de la Frontera Sur, Consejo de los Peces del Desierto.

Mitani, T., A. Akane, T. Tokiyasu, S. Yoshimura, Y. Okii, and M. Yoshida. 2009. "Identification of Animal Species Using the Partial Sequences in the Mitochondrial 16S rRNA Gene." *Legal Medicine* 11: S449–S450. https://doi.org/10.1016/j.legalmed.2009.02.002.

Montero-Rosado, C., E. Ojeda-Trejo, V. Espinosa-Hernández, D. Fernández-Reynoso, M. C. Deloya, and G. S. B. Valdés. 2022. "Water Diversion in the Valley of Mexico Basin: An Environmental Transformation That Caused the Desiccation of Lake Texcoco." *Land* 11, no. 4: 542. https://doi.org/10.3390/land11040542.

Muri, C. D., L. L. Handley, C. W. Bean, et al. 2020. "Read Counts From Environmental DNA (eDNA) Metabarcoding Reflect Fish Abundance and Biomass in Drained Ponds." *Metabarcoding and Metagenomics* 4: 97–112. https://doi.org/10.3897/mbmg.4.56959.

Nakagawa, H., K. Fukushima, M. Sakai, L. Wu, and T. Minamoto. 2022. "Relationships Between the eDNA Concentration Obtained From Metabarcoding and Stream Fish Abundance Estimated by the Removal Method Under Field Conditions." *Environmental DNA* 4, no. 6: 1369–1380. https://doi.org/10.1002/edn3.346.

Nandini, S., P. R. García, and S. S. S. Sarma. 2016. "Water Quality in Lake Xochimilco, Mexico: Zooplankton Indicators and *Vibrio cholerae*." *Journal of Limnology* 75, no. 1: 91–100. https://doi.org/10.4081/jlimnol.2015.1213.

Nandini, S., P. Ramírez-García, and S. S. S. Sarma. 2005. "Seasonal Variations in the Species Diversity of Planktonic Rotifers in Lake Xochimilco, Mexico." *Journal of Freshwater Ecology* 20, no. 2: 287–294. https://doi.org/10.1080/02705060.2005.9664968.

Nunes, A. L., J. M. Fill, S. J. Davies, et al. 2019. "A Global Meta-Analysis of the Ecological Impacts of Alien Species on Native Amphibians." *Proceedings of the Royal Society B: Biological Sciences* 286, no. 1897: 20182528. https://doi.org/10.1098/rspb.2018.2528.

Ochoa-Vázquez, D., R. Rosas-Valdez, E. A. Martínez-Salazar, and O. Flores-Villela. 2019. "Identification of Leopard Frogs (Anura: Ranidae: *Lithobates*) Distributed in Some Localities of the Southern Mexican Plateau Using Mitochondrial DNA Sequences." *Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis* 30, no. 6: 739–748. https://doi.org/10.1080/24701394.2019.1634697.

O'Hara, S. L., S. E. Metcalfe, and F. A. Street-Perrott. 1994. "On the Arid Margin: The Relationship Between Climate, Humans and the Environment. A Review of Evidence From the Highlands of Central Mexico." *Chemosphere* 29, no. 5: 965–981. https://doi.org/10.1016/0045-6535(94)90163-5.

Oksanen, J., F. G. Blanchet, M. Friendly, et al. 2019. "vegan: Community Ecology Package." https://cran.r-project.org/web/packages/vegan/index.html.

Pauchard, A., M. Aguayo, E. Peña, and R. Urrutia. 2006. "Multiple Effects of Urbanization on the Biodiversity of Developing Countries: The Case of a Fast-Growing Metropolitan Area (Concepción, Chile)." *Biological Conservation* 127, no. 3: 272–281. https://doi.org/10.1016/j.biocon.2005.05.015.

Peixoto, S., C. Chaves, G. Velo-Antón, P. Beja, and B. Egeter. 2021. "Species Detection From Aquatic eDNA: Assessing the Importance of Capture Methods." *Environmental DNA* 3, no. 2: 435–448. https://doi.org/10.1002/edn3.130.

Pérez-Belmont, P., J. Alvarado, N. Vázquez-Salvador, E. Rodríguez, E. Valiente, and J. Díaz. 2019. "Water Quality Monitoring in the Xochimilco Peri-Urban Wetland: Experiences Engaging in Citizen Science." *Freshwater Science* 38, no. 2: 342–351. https://doi.org/10.1086/703395.

Petsch, D. K. 2016. "Causes and Consequences of Biotic Homogenization in Freshwater Ecosystems." *International Review of Hydrobiology* 101, no. 3–4: 113–122. https://doi.org/10.1002/iroh.201601850.

Petsch, D. K., S. A. Blowes, A. S. Melo, and J. M. Chase. 2021. "A Synthesis of Land Use Impacts on Stream Biodiversity Across Metrics and Scales." *Ecology* 102, no. 11: e03498. https://doi.org/10.1002/ecy.3498.

Pyšek, P., P. E. Hulme, D. Simberloff, et al. 2020. "Scientists' Warning on Invasive Alien Species." *Biological Reviews* 95, no. 6: 1511–1534. https://doi.org/10.1111/brv.12627.

Reid, A. J., A. K. Carlson, I. F. Creed, et al. 2019. "Emerging Threats and Persistent Conservation Challenges for Freshwater Biodiversity." *Biological Reviews* 94, no. 3: 849–873. https://doi.org/10.1111/brv.12480.

Revelle, W. 2024. *Psych: Procedures for Psychological, Psychometric, and Personality Research*. Northwestern University. https://personality-project.org/r/psych/.

Reynolds, S. A., and D. C. Aldridge. 2021. "Global Impacts of Invasive Species on the Tipping Points of Shallow Lakes." *Global Change Biology* 27, no. 23: 6129–6138. https://doi.org/10.1111/gcb.15893.

Riley, S. P. D., G. T. Busteed, L. B. Kats, et al. 2005. "Effects of Urbanization on the Distribution and Abundance of Amphibians and Invasive Species in Southern California Streams." *Conservation Biology* 19, no. 6: 1894–1907. https://doi.org/10.1111/j.1523-1739.2005.00295.x.

Riaz, T., W. Shehzad, A. Viari, F. Pompanon, P. Taberlet, and E. Coissac. 2011. "Ecoprimers: Inference of new DNA Barcode Markers From Whole Genome Sequence Analysis." *Nucleic Acids Research* 39, no. 21: e145. https://doi.org/10.1093/nar/gkr732.

Rojas, C., E. Sepúlveda, F. Jorquera, J. Munizaga, and J. Pino. 2022. "Accessibility Disturbances to the Biodiversity of Urban Wetlands due to Built Environment." *City and Environment Interactions* 13: 100076. https://doi.org/10.1016/j.cacint.2021.100076.

Ruppert, K. M., R. J. Kline, and M. S. Rahman. 2019. "Past, Present, and Future Perspectives of Environmental DNA (eDNA) Metabarcoding: A Systematic Review in Methods, Monitoring, and Applications of Global eDNA." *Global Ecology and Conservation* 17: e00547. https://doi.org/10.1016/j.gecco.2019.e00547.

R Core Team. 2024. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Saenz-Agudelo, P., E. Delrieu-Trottin, J. D. DiBattista, et al. 2022. "Monitoring Vertebrate Biodiversity of a Protected Coastal Wetland Using eDNA Metabarcoding." *Environmental DNA* 4, no. 1: 77–92. https://doi.org/10.1002/edn3.200.

Salgado-Maldonado, G., and R. F. Pineda-López. 2003. "The Asian Fish Tapeworm *Bothriocephalus acheilognathi*: A Potential Threat to Native Freshwater Fish Species in Mexico." *Biological Invasions* 5, no. 3: 261–268. https://doi.org/10.1023/a:1026189331093.

Schabacker, J. C., S. J. Amish, B. K. Ellis, et al. 2020. "Increased eDNA Detection Sensitivity Using a Novel High-Volume Water Sampling Method." *Environmental DNA* 2, no. 2: 244–251. https://doi.org/10.1002/edn3.63.

Seebens, H., T. M. Blackburn, E. E. Dyer, et al. 2017. "No Saturation in the Accumulation of Alien Species Worldwide." *Nature Communications* 8, no. 1: 14435. https://doi.org/10.1038/ncomms14435.

Sepulveda, A. J., J. Schabacker, S. Smith, R. Al-Chokhachy, G. Luikart, and S. J. Amish. 2019. "Improved Detection of Rare, Endangered and Invasive Trout in Using a New Large-Volume Sampling Method for eDNA Capture." *Environmental DNA* 1, no. 3: 227–237. https://doi.org/10.1002/edn3.23.

Shechonge, A., B. P. Ngatunga, S. J. Bradbeer, et al. 2019. "Widespread Colonisation of Tanzanian Catchments by Introduced *Oreochromis* Tilapia Fishes: The Legacy From Decades of Deliberate Introduction." *Hydrobiologia* 832, no. 1: 235–253. https://doi.org/10.1007/s10750-018-3597-9.

Skelton, J., A. Cauvin, and M. E. Hunter. 2023. "Environmental DNA Metabarcoding Read Numbers and Their Variability Predict Species Abundance, but Weakly in Non-Dominant Species." *Environmental DNA* 5, no. 5: 1092–1104. https://doi.org/10.1002/edn3.355.

Smart, A. S., R. Tingley, A. R. Weeks, A. R. van Rooyen, and M. A. McCarthy. 2015. "Environmental DNA Sampling Is More Sensitive Than a Traditional Survey Technique for Detecting an Aquatic Invader." *Ecological Applications* 25, no. 7: 1944–1952. https://doi.org/10.1890/14-1751.1.

Smith, H. M. 1989. *The Axolotl in Its Native Habitat*. Vol. 18. Newsletter of the Ambystoma Genetic Stock Center, University of Kentucky.

Soto-Galera, E. 2019. "Chirostoma humboldtianum, Shortfin Silverside." *IUCN Red List of Threatened Species* 2019: e.T191154A197132. https://doi.org/10.2305/IUCN.UK.2019-3.RLTS.T191154A1971329.en.

Stendera, S., R. Adrian, N. Bonada, et al. 2012. "Drivers and Stressors of Freshwater Biodiversity Patterns Across Different Ecosystems and Scales: A Review." *Hydrobiologia* 696: 1–28. https://doi.org/10.1007/s10750-012-1183-0.

Stranko, S. A., S. E. Gresens, R. J. Klauda, et al. 2010. "Differential Effects of Urbanization and Non-Natives on Imperiled Stream Species." *Northeastern Naturalist* 17, no. 4: 593–614. https://doi.org/10.1656/045.017.0406.

Strayer, D. L. 2010. "Alien Species in Fresh Waters: Ecological Effects, Interactions With Other Stressors, and Prospects for the Future." *Freshwater Biology* 55, no. s1: 152–174. https://doi.org/10.1111/j.1365-2427.2009.02380.x.

Strayer, D. L., and D. Dudgeon. 2010. "Freshwater Biodiversity Conservation: Recent Progress and Future Challenges." *Journal of the North American Benthological Society* 29, no. 1: 344–358. https://doi.org/10.1899/08-171.1.

Tickner, D., J. J. Opperman, R. Abell, et al. 2020. "Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan." *Bioscience* 70, no. 4: 330–342. https://doi.org/10.1093/biosci/biaa002.

Tillotson, M. D., R. P. Kelly, J. J. Duda, M. Hoy, J. Kralj, and T. P. Quinn. 2018. "Concentrations of Environmental DNA (eDNA) Reflect Spawning Salmon Abundance at Fine Spatial and Temporal Scales."

Biological Conservation 220: 1–11. https://doi.org/10.1016/j.biocon.2018.01.030.

Toussaint, A., O. Beauchard, T. Oberdorff, S. Brosse, and S. Villéger. 2016. "Worldwide Freshwater Fish Homogenization Is Driven by a Few Widespread Non-Native Species." *Biological Invasions* 18, no. 5: 1295–1304. https://doi.org/10.1007/s10530-016-1067-8.

Turak, E., I. Harrison, D. Dudgeon, et al. 2017. "Essential Biodiversity Variables for Measuring Change in Global Freshwater Biodiversity." *Biological Conservation* 213: 272–279. https://doi.org/10.1016/j.biocon.2016.09.005.

UN World Urbanization Prospects. 2024. https://population.un.org/wpp/.

Valentini, A., P. Taberlet, C. Miaud, et al. 2016. "Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding." *Molecular Ecology* 25, no. 4: 929–942. https://doi.org/10.1111/mec.13428.

Valiente, E., A. Tovar, H. González, D. Eslava-Sandoval, and L. Zambrano. 2010. "Creating Refuges for the Axolotl (*Ambystoma mexicanum*)." *Ecological Restoration* 28, no. 3: 257–259. https://doi.org/10.3368/er.28.3.257.

Vázquez-Silva, G., F. C. Arana-Magallón, L. G. Núñez-García, A. Martínez-Santiago, and J. R. Cruz-Aviña. 2017. "Contribución al estudio de la ictiofauna del Lago de San Gregorio Atlapulco y canales de Xochimilco." *Revista Latinoamericana el Ambiente y Las Ciencias* 8, no. 19: 33–46.

Vences, M., M. L. Lyra, R. G. B. Perl, et al. 2016. "Freshwater Vertebrate Metabarcoding on Illumina Platforms Using Double-Indexed Primers of the Mitochondrial 16S rRNA Gene." *Conservation Genetics Resources* 8, no. 3: 323–327. https://doi.org/10.1007/s12686-016-0550-y.

Vences, M., Z. T. Nagy, G. Sonet, and E. Verheyen. 2012. *DNA Barcodes, Methods and Protocols*, Methods in Molecular Biology. Vol. 858. Springer Nature, 79–107. https://doi.org/10.1007/978-1-61779-591-6_5.

Verhelst, P., P. Boets, G. V. Thuyne, H. Verreycken, P. L. M. Goethals, and A. M. Mouton. 2016. "The Distribution of an Invasive Fish Species Is Highly Affected by the Presence of Native Fish Species: Evidence Based on Species Distribution Modelling." *Biological Invasions* 18, no. 2: 427–444. https://doi.org/10.1007/s10530-015-1016-y.

Voss, S. R., M. R. Woodcock, and L. Zambrano. 2015. "A Tale of Two Axolotls." *Bioscience* 65, no. 12: 1134–1140. https://doi.org/10.1093/biosci/biv153.

Walther, G.-R., A. Roques, P. E. Hulme, et al. 2009. "Alien Species in a Warmer World: Risks and Opportunities." *Trends in Ecology & Evolution* 24, no. 12: 686–693. https://doi.org/10.1016/j.tree.2009.06.008.

Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. 2012. "Mvabund– An R Package for Model-Based Analysis of Multivariate Abundance Data." *Methods in Ecology and Evolution* 3, no. 3: 471–474. https://doi.org/10.1111/j.2041-210x.2012.00190.x.

Weber, M. J., and M. L. Brown. 2009. "Effects of Common Carp on Aquatic Ecosystems 80 Years After "Carp as a Dominant": Ecological Insights for Fisheries Management." *Reviews in Fisheries Science* 17, no. 4: 524–537. https://doi.org/10.1080/10641260903189243.

Wei, T., and V. Simko. 2024. "corrplot: Visualization of a Correlation Matrix." https://github.com/taiyun/corrplot.

Wood, Z. T., A. Lacoursière-Roussel, F. LeBlanc, et al. 2021. "Spatial Heterogeneity of Edna Transport Improves Stream Assessment of Threatened Salmon Presence, Abundance, and Location." *Frontiers in Ecology and Evolution* 9: 650717. https://doi.org/10.3389/fevo.2021.650717.

World Wildlife Fund (WWF). 2022. "Living Planet Report 2022 – Building a Nature-Positive Society."

Xia, Z., M. L. Johansson, Y. Gao, et al. 2018. "Conventional Versus Real-Time Quantitative PCR for Rare Species Detection." *Ecology and Evolution* 8, no. 23: 11799–11807. https://doi.org/10.1002/ece3.4636.

Xia, Z., A. Zhan, M. L. Johansson, et al. 2021. "Screening Marker Sensitivity: Optimizing eDNA-Based Rare Species Detection." *Diversity and Distributions* 27: 1981–1988. https://doi.org/10.1111/ddi.13262.

Xiang, T., X. Dong, and G. Grenouillet. 2021. "Ecological and Biological Traits of Non-Native Freshwater Fish Species Differentiate Them From Native Species in China." *Ecological Indicators* 131: 108218. https://doi.org/10.1016/j.ecolind.2021.108218.

Yang, B., X. Qu, H. Liu, et al. 2024. "Urbanization Reduces Fish Taxonomic and Functional Diversity While Increases Phylogenetic Diversity in Subtropical Rivers." *Science of the Total Environment* 908: 168178. https://doi.org/10.1016/j.scitotenv.2023.168178.

Yang, L., Z. Tan, D. Wang, et al. 2014. "Species Identification Through Mitochondrial rRNA Genetic Analysis." *Scientific Reports* 4, no. 1: 4089. https://doi.org/10.1038/srep04089.

Zambrano, L., V. Contreras, M. Mazari-Hiriart, and A. E. Zarco-Arista. 2009. "Spatial Heterogeneity of Water Quality in a Highly Degraded Tropical Freshwater Ecosystem." *Environmental Management* 43, no. 2: 249–263. https://doi.org/10.1007/s00267-008-9216-1.

Zambrano, L., E. Martnez-Meyer, N. Menezes, and A. T. Peterson. 2006. "Invasive Potential of Common Carp (*Cyprinus carpio*) and Nile Tilapia (*Oreochromis niloticus*) in American Freshwater Systems." *Canadian Journal of Fisheries and Aquatic Sciences* 63, no. 9: 1903–1910. https://doi.org/10.1139/f06-088.

Zambrano, L., M. I. Rivas, C. Uriel-Sumano, et al. 2020. "Adapting Wetland Restoration Practices in Urban Areas: Perspectives From Xochimilco in Mexico City." *Ecological Restoration* 38, no. 2: 114–123. https://doi.org/10.3368/er.38.2.114.

Zambrano, L., M. Scheffer, and M. Martínez-Ramos. 2001. "Catastrophic Response of Lakes to Benthivorous Fish Introduction." *Oikos* 94, no. 2: 344–350. https://doi.org/10.1034/j.1600-0706.2001.940215.x.

Zambrano, L., E. Valiente, and M. J. V. Zanden. 2010a. "Food Web Overlap Among Native Axolotl (*Ambystoma mexicanum*) and Two Exotic Fishes: Carp (*Cyprinus carpio*) and Tilapia (*Oreochromis niloticus*) in Xochimilco, Mexico City." *Biological Invasions* 12, no. 9: 3061–3069. https://doi.org/10.1007/s10530-010-9697-8.

Zambrano, L., E. Valiente, and M. J. V. Zanden. 2010b. "Stable Isotope Variation of a Highly Heterogeneous Shallow Freshwater System." *Hydrobiologia* 646, no. 1: 327–336. https://doi.org/10.1007/s10750-010-0182-2.

Zambrano, L., E. Vega, M. L. G. Herrera, E. Prado, and V. H. Reynoso. 2007. "A Population Matrix Model and Population Viability Analysis to Predict the Fate of Endangered Species in Highly Managed Water Systems." *Animal Conservation* 10, no. 3: 297–303. https://doi.org/10.1111/j.1469-1795.2007.00105.x.

Zamora-Marín, J. M., C. Ilg, E. Demierre, et al. 2021. "Contribution of Artificial Waterbodies to Biodiversity: A Glass Half Empty or Half Full?" *Science of the Total Environment* 753: 141987. https://doi.org/10.1016/j.scitotenv.2020.141987.

Supporting Information

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