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Environmental DNA mitochondrial markers to assess potential occupancy of Endangered Yaqui catfish in the Yaqui River basin, Mexico

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ABSTRACT: Acquiring data on rare and threatened species can be challenging, particularly in remote areas. Environmental DNA (eDNA) offers a less effort-intensive method for detecting species compared to physical fish sampling methods. In our study, we focused on the Endangered Yaqui catfish *Ictalurus pricei*, a freshwater fish endemic to the Sonoran desert in Arizona, USA, and Sonora, Mexico, and the non-native channel catfish *I. punctatus*. We developed and employed mitochondrial eDNA markers to sample 35 locations in the Yaqui River basin in Mexico and employed a hierarchical Bayesian formulation of a co-occurrence model to investigate the interactions between the species while accounting for the effects of covariates on species occupancy and detection. Our best model included the influence of channel catfish mitochondrial eDNA on detecting Yaqui catfish mitochondrial eDNA, and we found that channel catfish mitochondrial eDNA detection was negatively related with water temperature and elevation but positively related to substrate size. Yaqui catfish occupancy, as determined with mitochondrial eDNA detection, was best explained by stream permanence and the presence of forested areas, while channel catfish mitochondrial eDNA occurrences were also associated with stream permanence, as well as conifer and shrub-dominated landscapes. Non-native channel catfish mitochondrial eDNA was found in all but 5 locations where Yaqui catfish mitochondrial eDNA was detected, indicating a high likelihood of interaction and hybridization. This potential for hybridization poses a significant threat to the already Endangered Yaqui catfish, emphasizing the need to protect and secure remaining populations for their long-term survival.

KEY WORDS: *Ictalurus pricei* · *I. punctatus* · Co-occurrence occupancy · Non-native channel catfish · eDNA

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1. INTRODUCTION

The Endangered Yaqui catfish *Ictalurus pricei* (Rutter, 1896) (NatureServe & Lyons 2019), a species native to the Pacific slope of southern North America, has recently become extinct in the USA, making it critical to find remaining population segments in Mexico for conservation efforts (Minckley 1971, Varela-Romero et al. 2011, Stewart et al. 2017). The desert rivers that it calls home have long been threatened by human-caused disturbances and development (Hendrickson et al. 1980). Hybridization with non-native, congeneric channel catfish *I. punctatus* (Rafinesque, 1818) also poses a threat to the species' existence (Gutiérrez-Barragán et al. 2021). Records are lacking to document when channel catfish were first introduced into the basin, but this species was rare prior to 1980 (Hendrickson et al. 1980). Approximately 30 yr later, channel catfish were considered 'well documented and widespread' in the Yaqui River basin (Varela-Romero et al. 2011), indicating their rapid spread. However, very little is known about the Yaqui catfish; basic life history parameters such as survival, growth, and longevity have only recently been described in the USA (Stewart et al. 2017). A few studies have conducted phylogenetic analysis in Sonora, Mexico (Hendrickson et al. 2007, Castañeda-Rivera et al. 2014, Ballesteros-Córdova et al. 2016, Varela-Romero et al. 2020, Pérez-Rodríguez et al. 2023), but only 1 study assessed their distribution based on historical habitat conditions in the Yaqui River basin using landscape-scale historical data (Hafen et al. 2021). Varela-Romero et al. (2011) reviewed the historical records for the species in the Río Yaqui and Fuerte basins, and their findings indicate a reduction in Its historical distribution concomitant with a spread of channel catfish.

The Yaqui catfish once inhabited several basins in Mexico, including the Río Casas Grandes, Mayo, Sonora, Yaqui, and Fuerte basins (Miller et al. 1991). However, recent data indicate that it is now only found in the Río Yaqui and Fuerte basins (Hendrickson et al. 2007, Varela-Romero et al. 2011, 2020). The non-native channel catfish, which can be found in the same areas as Yaqui catfish (Varela-Romero et al. 2011), is very similar in appearance, making it difficult to accurately identify pure Yaqui catfish in the field, especially if hybrids are present. These differences include body size, shape of the caudal fork, number of anal fin rays, and fusion of the supraoccipital process and dorsal supraneurals (Minckley 1971, Minckley & Marsh 2009, Stewart et al. 2017). However, environmental DNA (eDNA) analysis can be a powerful tool for distinguishing different fish species in streams, providing a rapid assessment of the presence and distribution of both Yaqui catfish and channel catfish. This technique has limitations when hybridization occurs, as species-specific mitochondrial haplotypes could detect hybrids at different filial generations (introgression) and not pure species (Gutiérrez-Barragán et al. 2021), and the rate of detection of hybrids has not yet been evaluated.

The conservation of wild areas within the natural distribution of the Yaqui catfish is crucial for the recovery of the species (Varela-Romero et al. 2011, 2020, Stewart et al. 2017). However, a lack of environmental data has hindered conservation efforts for this species. To effectively protect the Yaqui catfish, a landscape-scale analysis is needed to understand its space use and identify potential refuge areas. A study by Hafen et al. (2021) used historical data from Arizona State University, the Global Biodiversity Information Facility (GBIF), and peer-reviewed literature (Hendrickson et al. 1980, 2007) to find associations between Yaqui catfish presence and small, intermittent streams. The study identified some locations with temporally consistent occurrences of Yaqui catfish, such as Cajon Bonito, south of the international border (Hafen et al. 2021). However, it is important to note that the study relied on historical data and its conclusions indicate potential distribution rather than current distribution. Additionally, examination of historical data alone cannot account for the effects of non-native species, such as the channel catfish, which can displace native species from formerly occupied areas (Hendrickson et al. 2007, Varela-Romero et al. 2011). Furthermore, the historical data collection methods used broad-based ichthyofaunal surveys and relied on opportunistic collections (Hendrickson et al. 1980, 2007, Varela-Romero et al. 2011), which may have biased past findings and limit the extrapolation of any results, given that they are not from a random sample, and this prevents generalization to the population at large.

To better estimate the current distribution of Yaqui catfish, especially in relation to non-native channel catfish, a robust statistical framework using a probabilistic sampling design is needed to reduce selection bias. The remote and rugged desert environment of the Yaqui River basin makes access difficult, particularly for standard fish-sampling gear such as electrofishing and nets. However, eDNA sampling is altogether labor-efficient, time-efficient, and cost-efficient (Qu & Stewart 2019). Markers can be species-specific, making positive species identification more accurate. However, hybrids cannot be identified using mitochondrial DNA (mtDNA) because of its maternal in-

heritance, potentially overestimating the occurrence of species from a species-specific marker. This issue only arises if the incidence of hybridization between Yaqui catfish and channel catfish is high. The incidence of hybridization and variable hybridization outcomes for these 2 species throughout the Yaqui River basin has not been quantified, but could be substantial. When conducted within an occupancy modeling framework, eDNA sampling can help identify which local and regional factors influence habitat occupancy of the mitochondrial genes of the target species' (Jerde et al. 2011, Pilliod et al. 2013, Laramie et al. 2015, Thomsen & Willerslev 2015). This approach can streamline field data collection and provide a robust framework from which mitochondrial eDNA species interactions and habitat use can be generalized across a landscape (Long et al. 2011). Although physical sampling would allow collection of DNA from individual fish to assess the prevalence of hybridization at these sites, the efficiency of water collection for eDNA is a good first step to gauge the scope and scale of the potential for hybridization. The goal of this study is to develop species-specific mitochondrial eDNA markers for Yaqui catfish and channel catfish and apply a landscape-scale approach to investigate factors affecting occupancy and detection probability of mitochondrial genes of Yaqui catfish and the occurrence of channel catfish based on non-invasive sampling of water for eDNA signatures.

2. MATERIALS AND METHODS

2.1. Study site

The Yaqui River basin is located in the states of Chihuahua and Sonora, Mexico, with a small portion in southwestern New Mexico and southeastern Arizona in the USA (Fig. 1), but we focused our efforts in Mexico because the species is functionally extirpated in the wild in the USA (Stewart et al. 2017). Temperature and precipitation vary throughout the basin, with an average air temperature of 24°C (Hudson et al. 2005). The Sierra Madre Occidental, a mountain range that runs north—south along the southeastern portion of the basin, has a lower average air temperature (15°C). Basin-wide average precipitation is 50 cm, but lowlands average 5 cm yr^{-1}. The North American Monsoon, which occurs between June and September, accounts for 50 to 80% of precipitation in the area (Nicholas & Battisti 2008, Munoz-Hernandez et al. 2011). Major sub-basins are the Rio Yaqui, Rio Moctezuma, Rio Bavispe, Rio Aros, and Rio Sirupa, which combine to flow into the Gulf of California. There are 3 large reservoirs, the largest of which, Presa Oviachic, provides water for agriculture in the southern portion of the basin (Matson et al. 2005, Hudson et al. 2005, Matson 2012). Vegetation in the western, more arid part of the basin is dominated by creosote bush

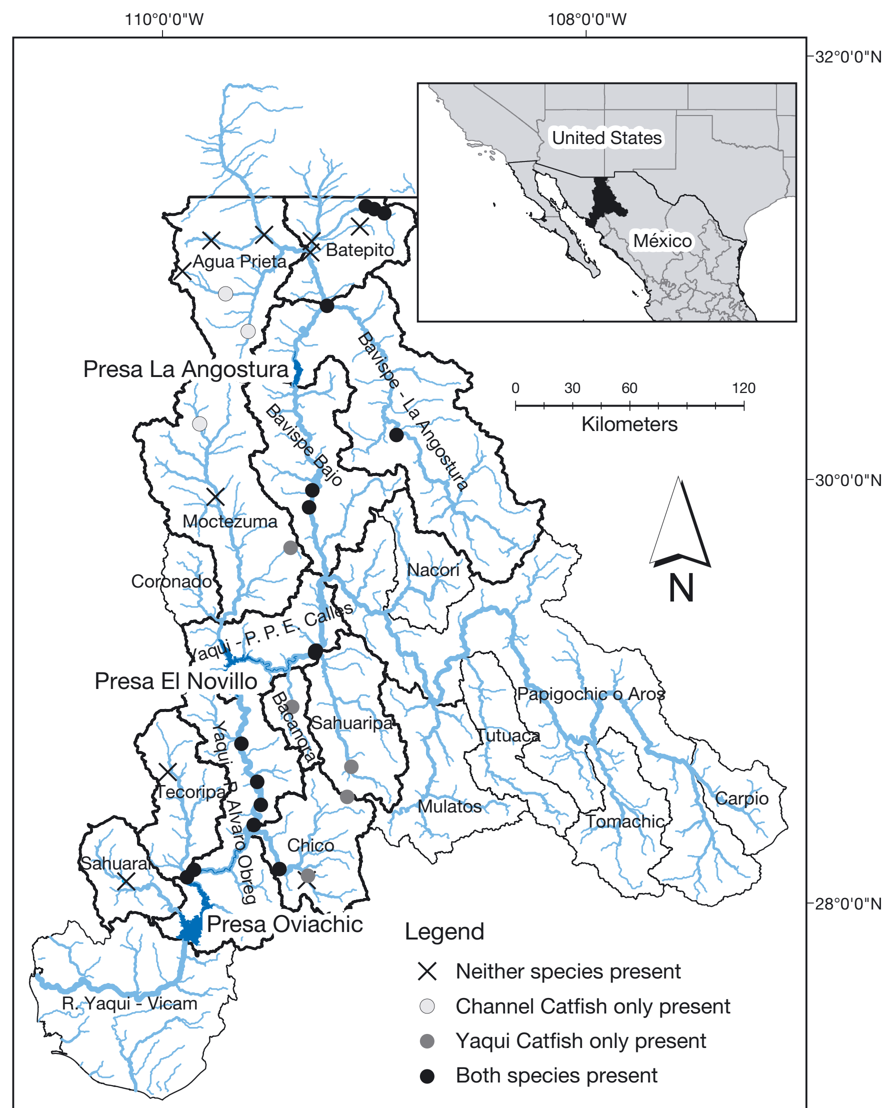

Fig. 1. Yaqui River basin, Mexico, with major sub-basins, reservoirs, and rivers indicating site occupancy for mitochondrial environmental DNA (eDNA) of Yaqui catfish and channel catfish

Larrea tridentata, *Yucca* spp., and various cactus species (Family Cactaceae; Hudson et al. 2005). The portion of the basin in Chihuahua is mountainous, with sparse human occupation in deciduous and coniferous forests at higher elevations, switching to grassland as elevation decreases (Gentry 1942, Abarca et al. 1995). Drug cartels are known to cultivate crops in mountainous areas, making them less accessible and dangerous to travel (Le Cour Grandmaison et al. 2019), so we restricted our sampling to the state of Sonora.

2.2. eDNA assay development

We developed a mitochondrial DNA assay specific to Yaqui catfish by examining partial sequences of the cytochrome *b* (*cytb*) gene available from GenBank (n = 10), as well as from 34 non-target species that are potentially sympatric (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/n053p569_supp/; all supplements). We aligned all sequences in MEGA 7 (Kumar et al. 2016) and used Primer-BLAST (Ye et al. 2012) to identify candidate primer sites that would amplify a region in our alignment that was unique to Yaqui catfish (Table S2). To design an eDNA assay to detect channel catfish, we used publicly available sequences covering the NADH 4 (ND4) gene (Table S3). Our alignment included data from 9 channel catfish sequences from the US states of Pennsylvania, South Carolina, Mississippi, Michigan, and Oregon, and 24 closely related or sympatric species. We scanned the alignment visually to identify primer regions which delineated a 140 nucleotide amplicon unique to channel catfish. We maximized nucleotide mismatches between oligonucleotides and non-target sequences to avoid instances of primer competition and cross-amplification of DNA from non-target species (Wilcox et al. 2015). We used Primer Express 3.0.1 (Life Technologies) to adjust primer and probe lengths to optimize annealing temperatures and screened them for secondary structures using the IDT OligoAnalyzer web application (www.idtdna.com/calc/analyzer). Using the NCBI nucleotide BLAST tool, we further examined the specificity of the assay *in silico* to reduce the potential for detecting non-target taxa. Each oligonucleotide was examined individually in this manner before the complete assay was assessed using Primer BLAST and the full NCBI nucleotide collection. We modeled assay specificity *in silico* using the purpose-built eDNAssay classification tool (Kronenberger et al. 2022). We input the assay primer and probe sequences, melting temperatures, and a sequence from each member of Ictaluridae which was represented at the assay locus on GenBank (Supplement 2). Close genetic relatives typically represent the greatest challenge to assay specificity (Langlois et al. 2021, Thalinger et al. 2021), but we included sequences from other fishes known from the Yaqui basin. We used a <0.5 assignment probability threshold to classify species predicted not to amplify with the assays. For non-targets which are not known from the Yaqui basin, but which may co-occur in other waters with channel catfish, we also applied the <0.5 amplification threshold to indicate potential taxa for expansion of channel catfish assay validation. We assessed the Yaqui catfish assay with eDNAssay against 90% of extant ictalurids; we assessed the channel catfish assay against 34% as there was a greater degree of missingness at the ND4 gene which was used for assay design (Supplement 2).

We tested the specificity of the assays *in vitro* using a QuantStudio 3 Real-Time PCR System (Life Technologies) in 15 µl reactions containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies), 900 nM each forward and reverse primer, 250 nM probe, 4 µl DNA template (~0.4 ng), and PCR-grade water for the remaining volume. Thermocycler conditions were 95°C for 10 min followed by 45 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. Pipettes, tube racks, and consumables were irradiated with UV light in a hood for 1 h prior to set-up. We screened DNA taken from 11 Yaqui catfish tissues obtained in Arizona and from 31 additional non-target species (Table S4). These 11 Yaqui catfish are descendants of fish collected in the early 1990s from the sub-basins of the Río Aros and Río Sirupa in east-central Sonora, Mexico, and transported to the Southwestern Native Aquatic Resources and Recovery Center (formerly Dexter National Fish Hatchery), New Mexico, USA, before being relocated to Uvalde National Fish Hatchery, Texas, USA. Following concern that introgressed Yaqui catfish might have been introduced unknowingly into the broodstock, the US Fish and Wildlife Service (USFWS) screened the broodstock in the early 1990s using 3 isozyme locus polymorphisms and morphometric counts to determine the extent and directionality of interspecific hybridization, with the objective of distinguishing Yaqui catfish forms from channel catfish to establish the founding broodstock (Jensen et al. 1996, Morizot et al. 1997, 1999). There were just 2 pure channel catfish in the population samples, and their introgression with Yaqui catfish was also confirmed. Therefore, any introgressed individuals or pure channel catfish were presumably eliminated to produce a purportedly pure

Yaqui catfish broodstock (Kelsch & Jensen 1997, Morizot et al. 1999). Following this, the US population was screened with 12 microsatellite loci derived from channel catfish, which found no evidence of introgression in the US Yaqui catfish population (Baker et al. 2008, USFWS 2009). Therefore, we assume that the US population and the 11 Yaqui catfish obtained in Arizona were pure. To validate the channel catfish assay, we screened DNA extracted from 7 channel catfish tissues and 24 non-target species (Table S5). Samples used for *in vitro* screening were obtained from archived materials, or from small fin clips collected from fish that were immediately released at the point of capture. Fin clips were stored in ⩾95% ethanol until DNA was extracted using the DNeasy Tissue and Blood Kit (Qiagen), following the manufacturer's protocol. Prior to extraction, we rinsed the tissues with a 10% sodium hypochlorite solution to remove DNA from co-occurring species that may have been on the tissue surface, then thoroughly rinsed each tissue with deionized water to minimize destruction of target DNA.

We optimized primer concentrations by testing a single DNA sample of the respective target species with concentrations of each primer at 100, 300, 600, and 900 nM for a total of 16 unique assay concentrations (Wilcox et al. 2015). We selected the assay concentration that displayed a high relative end-point fluorescence and the lowest C_t (cycle threshold) value for use in subsequent analyses. Using the optimal concentrations of 300 nM forward and 900 nM reverse primer and the same qPCR conditions as above, we tested the Yaqui catfish assay sensitivity and efficiency by analyzing a 7 level standard curve created from target qPCR product that was purified using a GeneJET™ PCR Purification Kit (Life Technologies), quantified on a Qubit 2.0 fluorometer (Thermo Fisher Scientific), and serially diluted in sterile Tris EDTA (TE) to 31 250, 6250, 1250, 250, 50, 10, and 2 copies per 4 µl. We analyzed the standard curve across 6 replicates of each concentration on a single 96 well qPCR plate. We performed the same standard curve experiment with the channel catfish assay, using the optimal concentration of 900 nM for both the forward and reverse primer.

Finally, we validated the assays *in vivo* by screening eDNA samples collected from 7 waterbodies in the southwestern USA with known patterns of occupancy by Yaqui catfish (Table 1) and 3 waterbodies which were known to be occupied by channel catfish (Table 2). The eDNA samples were collected by filtering up to 5 l of water or until 3 filters were clogged, as outlined in Carim et al. (2016). DNA was extracted from the filters with the DNeasy Tissue and Blood Kit following a modified protocol which included extraction controls (Franklin et al. 2019). Using the optimized qPCR conditions, the extracts were then analyzed along with a TaqMan Exogenous Internal Positive Control (IPC; 1.5 µl of 10× IPC VIC-labeled assay and 0.15 µl of 50× IPC DNA per reaction; Life Technologies) to screen for qPCR inhibition by environmental contaminants. A sample was considered inhibited if there was a >1 C_t shift in the IPC relative to the no-template control. All *in vivo* analyses were performed across 3 replicates and included a no-template control substituting molecular grade water for eDNA.

2.3. eDNA surveys

Sampling locations in Mexico were determined using a hydrologic data layer downloaded from the Instituto Nacional de Estadística y Geografía website (http://antares.inegi.org.mx/analisis/red_hidro/siatl/, accessed 8 December 2019) and a random stratified sampling system based on stream order for watersheds with historical observations of Yaqui catfish (Hafen et al. 2021). This protocol resulted in 35 target sampling sites in streams and reservoirs (Fig. 1). Up to 5 replicate samples, separated by 100 m, were taken at each of the 35 sites. Samples were collected from downstream to upstream. At each site, we filtered either 5 l of water through a glass microfiber filter (pore size 1.5 µm) or until we clogged 3 filters, whichever occurred first (Carim et al. 2016). Covariates measured at each site included feature type (stream or reservoir), water depth, flow velocity, Secchi tube depth, water temperature, large woody debris presence, habitat type (reservoir, riffle, run, pool), substrate size, and overhead canopy cover (Table 3). Substrate size was the modal diameter (mm) of 10 particles that were haphazardly picked up and measured as available. Canopy cover was measured with a densiometer at 4 points near the sample location and averaged (Hafen 2020).

After eDNA samples were collected, they were mailed to the National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT for laboratory processing. Samples were then transferred to a freezer (−20°C) upon arrival. Extractions were performed on half of each filter following a modified protocol using the DNeasy Blood and Tissue Kit including negative extraction controls and located in a dedicated DNA 'clean' extraction laboratory space (Franklin et al. 2019). The other half of each filter was archived in a −20°C freezer. If more than 1 filter was

Table 1. Collection information and detection results for *in vivo* testing of the Yaqui catfish assay. Water samples were collected from 7 waterbodies in the southwestern USA with known patterns of occupancy by Yaqui catfish. UNFH: Uvalde National Fish Hatchery; Y: yes; U: unknown; N: no. Dates are given as mo/d/yr

Waterbody	Site no.	Latitude	Longitude	State	Collection date (mo/d/yr)	Yaqui catfish expected	Yaqui catfish DNA detected	Mean DNA copies per liter
House pond Site 5	2	31.33735	−109.279	Arizona	12/12/2017	Y	N	0
House pond Site 5	3	31.33733	−109.279	Arizona	12/12/2017	Y	N	0
House pond Site 5	4	31.33715	−109.279	Arizona	12/12/2017	Y	Y	19.8
Big tank Site 6	6	31.87047	−109.402	Arizona	12/13/2017	Y	Y	2.5
Big tank Site 6	7	31.87053	−109.402	Arizona	12/13/2017	Y	N	0
Big tank Site 6	8	31.87042	−109.403	Arizona	12/13/2017	Y	N	0
Leslie Canyon	1	33.18586	−108.738	New Mexico	11/20/2017	N	N	0
House pond	1	31.33674	−109.279	Arizona	11/21/2017	U	N	0
North Pond	1	31.35488	−109.262	Arizona	11/21/2017	N	N	0
Oasis	1	31.34425	−109.261	Arizona	11/21/2017	Y	Y	3.4
Twin pond	1	31.34002	−109.265	Arizona	11/21/2017	Y	Y	1.8
Twin pond	2	31.33978	−109.265	Arizona	11/21/2017	Y	Y	2.0
Big tank	1	31.87095	−109.403	Arizona	11/29/2017	Y	N	0
Big tank	2	31.87108	−109.403	Arizona	11/29/2017	Y	N	0
Big tank	3	31.8709	−109.403	Arizona	11/29/2017	Y	Y	5.6
Big tank	4	31.87096	−109.403	Arizona	11/29/2017	Y	N	0
Big tank	5	31.87086	−109.403	Arizona	11/29/2017	Y	Y	36.9
Big tank Site 6	09	31.87075	−109.402	Arizona	12/13/2017	Y	N	0
Big tank Site 6	10	31.87058	−109.403	Arizona	12/13/2017	Y	N	0
UNFH Yaqui catfish tank	1A	29.186111	−99.833055	Texas	3/30/2018	Y	Y	3863.1
UNFH Yaqui catfish tank	1B	29.186111	−99.833055	Texas	3/3/2018	Y	Y	1289.5
UNFH Yaqui catfish tank	2A	29.186111	−99.833055	Texas	3/30/2018	Y	Y	1156.6
UNFH Yaqui catfish tank	2B	29.186111	−99.833055	Texas	3/3/2018	Y	Y	57648.4
UNFH Yaqui catfish tank	3A	29.186111	−99.833055	Texas	3/30/2018	Y	Y	1808.1
UNFH Yaqui catfish tank	3B	29.186111	−99.833055	Texas	3/3/2018	Y	Y	402294.4
UNFH Yaqui catfish tank	4A	29.186111	−99.833055	Texas	3/30/2018	Y	Y	3248.8
UNFH Yaqui catfish tank	4B	29.186111	−99.833055	Texas	3/30/2018	Y	Y	23148.1
UNFH Yaqui catfish tank	1	29.186111	−99.833055	Texas	3/30/2018	Y	Y	2464.4
UNFH Yaqui catfish tank	2	29.186111	−99.833055	Texas	3/30/2018	Y	Y	888.1
UNFH Yaqui catfish tank	3	29.186111	−99.833055	Texas	3/30/2018	Y	Y	884.9
UNFH Yaqui catfish tank	4	29.186111	−99.833055	Texas	3/30/2018	Y	Y	1715.8
UNFH Yaqui catfish tank	5	29.186111	−99.833055	Texas	3/30/2018	Y	Y	18508.9
UNFH Yaqui catfish tank	6	29.186111	−99.833055	Texas	3/30/2018	Y	Y	2576.3
UNFH Yaqui catfish tank	7	29.186111	−99.833055	Texas	3/30/2018	Y	Y	3064.3
UNFH Yaqui catfish tank	8	29.186111	−99.833055	Texas	3/30/2018	Y	Y	4263.9
UNFH Yaqui catfish tank	9	29.186111	−99.833055	Texas	3/30/2018	Y	Y	2131.6
UNFH Yaqui catfish tank	10	29.186111	−99.833055	Texas	3/30/2018	Y	Y	883.57
UNFH Yaqui catfish tank	11	29.186111	−99.833055	Texas	3/30/2018	Y	Y	1476.6
UNFH Yaqui catfish tank	12	29.186111	−99.833055	Texas	3/30/2018	Y	Y	4135.5
UNFH Yaqui catfish tank	13	29.186111	−99.833055	Texas	3/30/2018	Y	Y	2103.5

needed to obtain a sample, DNA from half of each filter was extracted. The eDNA extracts were then separately analyzed with each assay using a QuantStudio 3 Real-Time PCR System in triplicate 15 µl reactions comprising 7.5 µl Environmental Master Mix 2.0, forward primer (300 nM for Yaqui catfish; 900 nM for channel catfish), reverse primer (900 nM for Yaqui catfish; 900 nM for channel catfish), 250 nM probe, 4 µl eDNA extract (~0.4 ng), TaqMan Exogenous IPC, which included 1.5 µl of 10× IPC assay and 0.30 µl of 50× IPC DNA, and PCR-grade water for the remaining volume. Using the IPC to screen for qPCR inhibitors, reactions were considered inhibited if the there was a > 1 C_t shift in the IPC relative to the no-template control. If a reaction was inhibited, the sample was treated with an inhibitor removal kit (Zymo Research) and reanalyzed in triplicate. All qPCR experiments included a qPCR-positive control containing 0.4 ng tissue-derived DNA from the target species and a negative control consisting of PCR-grade water in place of template. Experiments were set up in a room physically isolated from other rooms and inside a

Table 2. Collection information and detection results for *in vivo* testing of the channel catfish assay. Samples were collected from 3 waterbodies in the southwestern USA known to be occupied by channel catfish. Y: yes; N: no. Dates are given as mo/d/yr

Waterbody	Site no.	Latitude	Longitude	State	Collection date (mo/d/yr)	Channel catfish expected	Channel catfish DNA detected	Mean DNA copies per liter
Byron Pond 16	1	36.839444	−98.181388	Oklahoma	8/8/2019	Y	Y	6027.71
Byron Pond 16	2	36.839444	−98.181388	Oklahoma	8/8/2019	Y	Y	3712.093
Byron Pond 09	3	36.829444	−98.18111	Oklahoma	8/8/2019	Y	Y	8597.301
Byron Pond 09	4	36.840000	−98.18110	Oklahoma	8/8/2019	Y	Y	1578.236
Boomer Lake	1	36.155833	−97.060555	Oklahoma	8/12/2019	Y	Y	32.71224
Bubbling Ponds Hatchery	02	34.76474	−111.89451	Arizona	3/11/2016	Y	Y	–
Red Ives Creek	103-1	47.06161	−115.27692	Montana	7/7/2015	N	N	–
Upper Willow Creek Tributary	875-1	46.51384	−113.51090	Montana	6/27/2016	N	N	–
Dahlman Creek	867-1	48.00491	−116.22800	Idaho	7/2/2018	N	N	–
Dahlman Creek	867-2	48.01077	−116.22447	Idaho	7/2/2018	N	N	–
Swamp Creek	1207-1	47.96591	−115.58031	Montana	7/18/2018	N	N	–
Swamp Creek	1207-2	47.97144	−115.57082	Montana	7/18/2018	N	N	–
Pack River	721-1	48.52973	−116.59233	Idaho	7/14/2018	N	N	–
North Fork Indian Creek	421-4	48.64770	−116.68695	Idaho	8/28/2018	N	N	–

Table 3. Mean (SD) and range of environmental conditions in sampled study sites within the Yaqui River basin, Mexico, measured in the field or from geospatial data

Covariate	Modeled	Unit	Mean (SD)	Range
Depth	Detection	cm	0.27 (0.21)	0.04–1.20
Secchi tube depth	Detection	cm	0.84 (0.42)	0.06–1.20
Substrate	Detection	mm	42.56 (60.65)	2.00–180.00
Temperature	Detection	°C	23.88 (4.00)	15.3–34.3
Flow	Detection	$m\ s^{-1}$	0.17 (0.22)	0.00–1.00
Canopy cover	Detection	%	22.45 (29.25)	0.00–100
Class (0 = reservoir, 1 = riffle, 2 = run, 3 = pool)	Detection/occupancy	Categorical	1.21 (0.78)	0.00–3.00
Large woody debris (1 = present, 0 = absent)	Detection/occupancy	Categorical	0.41 (0.49)	0.00–1.00
Elevation (above mean sea level)	Occupancy	m	708.91 (451.36)	95.05–1646.73
Slope	Occupancy	Δ elevation	1.22 (1.03)	0.16–4.82
Stream order (Strahler)	Occupancy	Rank integer	3.83 (1.60)	1.00–6.00
Area of catchment	Occupancy	km^2	28.88 (21.17)	0.73–98.81
Total upstream area	Occupancy	km^2	15667.45 (23729.58)	18.00–65236.30
Annual average actual evapotranspiration	Occupancy	mm	407.44 (104.76)	238.00–655.00
Annual average potential evapotranspiration	Occupancy	mm	1810.12 (150.10)	1443.00–2018.00
Annual average natural discharge	Occupancy	$m^3\ s^{-1}$	17.92 (28.08)	0.02–78.81
Vegetation class (2 = tropical deciduous forest/woodland, 8 = mixed forest, 10 = grassland steppe, 11 = dense shrubland, 12 = open shrubland)	Occupancy	Categorical	6.6 (4.33)	2.00–12.00
Forest cover extent in reach catchment	Occupancy	%	87.91 (21.02)	5.00–100.00
Land cover class (2 = tree cover, broadleaved, deciduous, closed, 4 = tree cover, needle-leaved, evergreen, 12 = shrub cover, closed-open, deciduous [with or without sparse tree layer])	Occupancy	Categorical	3.31 (1.81)	2.00–12.00
Mean annual precipitation averaged across the reach catchment	Occupancy	mm	501.44 (120.83)	306.00–812.00
Predicted probability that a river ceases flowing for at least 1 day per year	Occupancy	%	77.56 (12.05)	56.61–97.67
Predicted probability that a river ceases to flow for at least 1 month (30 d) per year	Occupancy	%	71.05 (13.27)	46.23–92.52

hood in which all consumables, pipettes, and tube racks were previously exposed to UV light for at least 1 h. A sample was considered positive for the presence of the target species if at least 1 of the 3 qPCR reactions amplified DNA of that species.

Site-level covariates included presence of channel catfish mitochondrial DNA, also determined from eDNA sampling, and several landscape-scale parameters associated with stream segments from the global prevalence of intermittent rivers and ephemeral streams (GIRES) database (Messager et al. 2021), as well as 100 m digital elevation models from the Instituto Nacional de Estadística y Geografía (INEGI; http://en.www.inegi.org.mx/default.html). Associations between site locations and landscape data were made in ArcGIS 10.6 software by proximity and manual inspection to ensure accuracy. In 2 instances, 2 sites each were associated with the same GIRES stream segment and 1 instance could be not be associated with a GIRES segment. In those cases, we assigned associated landscape variables from the stream segments obtained from INEGI.

2.4. Occupancy modeling

The eDNA sampling design requires that T samples are collected from R sites. Repeated sampling at a site yields a detection history, e.g. 0,0,0,1,1 for a site sampled 5 times with detection in the fourth and fifth samples. Thus, the detection history partitions the zero sites (e.g. 0,0,0,0,0) into those where the mitochondrial DNA of a species occurs but was not detected from those where it simply does not occur (MacKenzie et al. 2003). For a model in which there are 2 interacting species, the data are the number of detections for each of T samples at R sites for which a subordinate or dominant species is detected (MacKenzie et al. 2004, Richmond et al. 2010, Rota et al. 2016). In the co-occurrence modeling framework, we classified mitochondrial eDNA detections from native Yaqui catfish as subordinate to those of non-native channel catfish, where the occurrence of mitochondrial eDNA from the subordinate species depends on the occurrence of the mitochondrial eDNA from the dominant species. However, mitochondrial eDNA of the dominant species is independent of mitochondrial eDNA of the subordinate species. Therefore, we consider the observed elements of mitochondrial eDNA detection histories of binary observations, y, from each $t = 1,...,T$ samples within each set of $i = 1,...I$ sites. The observed data, y_{it}, can be denoted by the matrix of mitochondrial eDNA histories from the survey as $Y = \{y_{it}: i = 1,...I; t = 1,...,T\}$ and conditional on a state process z_{it}, where the observation model is $y_{it} | z_{it}, p_{it} \sim \text{Bernoulli}(z_{it} p_{it})$. The state process is the result of a Bernoulli trial indicating the latent occupancy state of Yaqui catfish or channel catfish with $z = 1$ indicating presence and $z = 0$ indicating absence. The detection probability p_{it} is conditional on $z = 1$. Here, the $\mathbf{Y}$ matrix of binary observations (y_{it}^A, y_{it}^B) and state variables (z^A, z^B) for species A = Yaqui catfish and B = channel catfish. Therefore, $\psi^B = \Pr(z^B = 1)$ is the probability of occurrence of channel catfish; $\psi^{A|B} = \Pr(z^A = 1 | z^B = 1)$ is the conditional probability of occurrence of Yaqui catfish given that channel catfish is also present; and $\psi^{A|\bar{B}} = \Pr(z^A = 1 | z^B = 0)$ is the conditional probability of occurrence of Yaqui catfish given that channel catfish is absent. Using these parameters, the joint probability of the occupancy of Yaqui catfish and channel catfish can be estimated following these Bernoulli processes (Waddle et al. 2010):

$$z_i^B \mid \psi_i^B \sim \text{Bernoulli}\left(\psi_i^B\right) \quad (1)$$

$$z_i^A \mid z_i^B, \psi_i^{A|B}, \psi_i^{A|\bar{B}} \sim \text{Bernoulli}\left(z_i^B \psi_i^{A|B} + \psi_i^{A|\bar{B}}\left(1 - z_i^B\right)\right) \quad (2)$$

These equations represent occupancy of Yaqui catfish depending on occupancy of channel catfish, which is based on 2 probabilities: (1) the probability that Yaqui catfish is present based on the presence of channel catfish $\psi^{A|B}$, (2) the probability that Yaqui catfish is present based on the absence of channel catfish $\psi^{A|\bar{B}} = \Pr(1 - z_i^B)$. Using these parameters, each element of the encounter history of Yaqui catfish (A) is modeled as:

$$y_{it}^A \mid z_i^B, \psi_i^{A|B}, \psi_i^{A|\bar{B}}, p_{it}^A \sim \mathit{Bernoulli}\left(p_i^A \left\{z_i^B \psi_i^{A|B} + \psi_i^{A|\bar{B}}\left(1 - z_i^B\right)\right\}\right) \quad (3)$$

We modeled the detection probability of each species as a logit function of both sample- and site-level covariates on detection probabilities for each species and represented generally as:

$$\text{logit}\left(p_{it}\right) = \alpha_0 + \sum_{v=1}^{w} a_v x_{v,i} \quad (4)$$

We also incorporated potential covariate effects in the occupancy model using a logit-link function specified as:

$$\text{logit}\left(\psi_i\right) = \beta_0 + \sum_{v=1}^{w} \beta_v x_{v,i} + \gamma_i + \varepsilon_i \quad (5)$$

where x_v are predictors $v = 1,2 ... w$ measured at site i. The α's and β's are the intercept and slope parameter estimates and ε is the independent error term. The

effects of categorical variables (e.g. large wood debris [lwd] and land cover [land]) were modeled as logit-scale parameters, e.g.:

$$\text{logit}\left(\alpha_{it}^{B}\right) = \alpha_{1}^{B}\text{lwd}_{it} + \alpha_{2}^{B}\left(1-\text{lwd}_{it}\right) \quad (6)$$

$$\text{logit}\left(\psi_{i}^{B}\right) = \beta_{1}^{B}\text{land}_{i} + \beta_{2}^{B}\left(1-\text{land}_{i}\right) + \gamma_{i} + \varepsilon_{i} \quad (7)$$

where lwd_{it} indicates the presence of woody debris (1 = large woody debris, 0 = no large woody debris) and land_{it} indicates the habitat (1 = cropland, 0 = natural vegetation). The parameters of each categorical variable can be thought of as the logit-scale probabilities of occurrence of channel catfish or Yaqui catfish in habitats with and without large woody debris and habitat types. The γ term is a latent spatial random error term that accounts for spatial autocorrelation, where γ and θ take the form of a conditional autoregressive model and follow by defining the distribution conditionally as:

$$\gamma_{i} \mid \gamma_{i-1}, \phi, \sigma_{\gamma}^{2} \sim N\left(\phi \sum_{i' \neq i} \frac{w_{ii'}}{w_{i.}} \gamma_{i'}, \frac{\sigma_{\gamma}^{2}}{w_{i.}}\right) \quad (8)$$

where σ_{γ}^{2} is the variance parameter for the spatial random error term. The parameter ϕ is interpreted as a measure of the strength of the spatial correlation, with $\phi = 0$ implying independent and higher values of $|\phi|$ leading to greater positive or negative correlation. Here, the weights w reflect the different distances between sites, where $w_{i.} = \sum_{i'=1}^{L} w_{ii'}$, the row totals of the neighbors' weights for a site. If the weights are large (perhaps due to spatial distance between 2 sites), its conditional variance will be larger than if w_i is small. Thus, the neighborhood structure also affects the precision with which site occupancy is estimated. The probability density of γ is given by:

$$\wp\left(\gamma \mid \phi, \sigma_{\gamma}^{2}\right) \propto \exp\left\{-\frac{1}{2\sigma_{\gamma}^{2}} \phi' \mathbf{M}^{-1}\left(\mathbf{I} - \phi \mathbf{C}\right)\phi\right\} \quad (9)$$

where $\mathbf{M}$ is a diagonal matrix with the ith diagonal element $1/w_i$, and $\mathbf{C} = \mathbf{MW}$ is the scaled weight matrix (Banerjee et al. 2004). The equation specifies the kernel of a multivariate normal distribution with mean vector 0 and covariance matrix $\sum_{\gamma} \sigma_{\gamma}^{2}\left(I - \phi \mathbf{C}\right)^{-1}\mathbf{M}$ (Tognelli & Kelt 2004).

Covariates were standardized with a mean of 0 and SD of 1, and only predictor variables that had a correlation coefficient (r) < 0.60 were used in the same model to reduce multicollinearity (Dormann et al. 2013). Additionally, if a variable was redundant in its hypothesis description with another variable, then only 1 of the 2 variables was considered in the model set. We evaluated the strength of evidence for covariate effects by estimating posterior model probabilities using an inclusion parameter to identify the most probable predictors (Kuo & Mallick 1998, Congdon 2005). The inclusion parameters were specified as latent binary variables (Bernoulli) using a balanced prior probability for x_v, such that the prior probability that variable v was included in a species-specific model was 0.50. Therefore, when the posterior probability of inclusion for each variable was $x_v = 0$, variable v had zero effect. If $x_v = 1$, this corresponds to the variable v having a linear effect and indicating a high degree of support for the covariate being in the 'best' model. We considered variables having ($\Pr(x_v > 0.65)$) to be highly supported and considered those in the final models, where the best overall model was based on the Bayesian information criterion, which is used to approximate the posterior model probability (Thomson et al. 2010). We fit the models using Markov chain Monte Carlo implemented in WinBUGS version 1.4 using R2WinBUGS (Sturtz et al. 2005) in R (R Core Team 2021, Lunn et al. 2000). Models were fit using 100 000 iterations with a burn-in of the first 15 000 iterations and thin of 1.

3. RESULTS

3.1. Mitochondrial eDNA assays

The Yaqui catfish assay detected DNA from all *Ictalurus pricei* tissue samples and did not detect DNA from within the no-template controls. BLAST searches did not return any North American fishes outside of Ictaluridae with significant homology to the assay primers or probes. eDNAssay analysis indicated that no other fish species previously observed in the Yaqui basin had an amplification assignment probability greater than 0.5 (Table S1). None of the DNA from any non-target species we tested was detected *in vitro* (Table S4). The standard curve demonstrated a reaction efficiency of 99.046% ($r^2 = 0.993$, y-intercept = 38.357, slope = −3.345) and a limit of detection (the lowest concentration with >95% amplification success; Bustin et al. 2009) of 2 copies per reaction; DNA was detected in all 6 replicates at this concentration. We did not detect Yaqui catfish DNA in any eDNA samples taken where the species was expected to be absent. Further, we detected Yaqui catfish DNA in all waterbodies where the species was known to be present, but not all samples from all occupied waterbodies detected Yaqui catfish (Table 1). Although the missed detections are due to undetermined reasons, it is possible that the degree of

pond mixing, number of fish present, the volume of water, and additional unmeasured variables all influenced the probability of Yaqui catfish detection. The channel catfish assay detected DNA from all *I. punctatus* tissue samples and did not detect DNA within no-template controls. BLAST searches did not return any North American fish species outside of Ictaluridae with significant homology to the assay primers or probes. eDNAssay analysis did not indicate any other Yaqui basin fish species with assignment probabilities greater than 0.3 (Table S3). Regardless, we tested the assay *in vitro* against DNA from the 3 ictalurids known from the Yaqui basin (Table S5). No DNA from any non-target species we tested was detected with the assay (Table S5). We observed uniformly low eDNAssay assignment probabilities produced with the channel catfish assay across the ictalurids we were able to examine *in silico*, regardless of how closely related the species was (Supplement 2). This is suggestive that the channel catfish assay could be specific across more of Ictaluridae than we were able to investigate. Additional testing which bears out this finding could allow for eDNA sampling using the assay over a larger geographic area. The channel catfish standard curve demonstrated a reaction efficiency of 91.82% (r^2 = 0.997, *y*-intercept = 38.357, slope = −3.535) and a limit of detection of 10 copies per reaction; DNA was detected in all 6 replicates at this concentration and in 4 replicates at the 2 copy level. Channel catfish DNA was detected in all environmental samples from waters that were known to contain channel catfish individuals (Table 2).

3.2. Species occurrences and occupancy

Stream characteristics varied among the 35 sites and among samples (Fig. 1, Table 3). Habitat conditions were typically comprised of shallow water and riffle habitats, low flow with low-moderate canopy cover, large woody debris present, and highly variable substrate size. In general, the sites were located in medium-sized streams, within a forested landscape, and many streams had a high probability of flow ceasing at some point in the year. We observed 69 Yaqui catfish mitochondrial eDNA detections at 22 of the 35 sites, and 59 channel catfish mitochondrial eDNA detections at 20 of the 35 sites (Table 4; Supplement 3). Within sites, 83% of positive detections occurred in all PCR replicate samples; 98% of positive detections were a result of positive detections in ⩾50% of PCR replicate samples. Only 1 site had a positive detection based on less than 50% of PCR replicate samples being positive for the target mitochondrial eDNA. Yaqui catfish and channel catfish eDNA were present at 17 of the same sites, distributed throughout the sampled area, often in main river channel habitats. Otherwise, 5 sites contained solely Yaqui catfish mitochondrial eDNA, 3 only channel catfish mitochondrial eDNA, and 10 sites did not contain mitochondrial eDNA from either species. The 5 sites that contained only Yaqui catfish eDNA were distributed among 4 sub-basins: Rio Chico (2 sites), Rio Sahuaripa, Rio Bacanora, and Rio Moctezuma; mostly in upper portions of the drainages. Two sites that contained only Yaqui catfish mitochondrial eDNA were reservoirs (Presa Las Calabazas and Presa Mojonera).

Table 4. Summary of occupancy of Yaqui catfish and channel catfish at 35 sites in the Rio Yaqui basin, Mexico, as determined by mitochondrial environmental DNA (mt eDNA) analysis of catfish collected in 2019

Yaqui catfish mt eDNA detected	Channel catfish mt eDNA detected: No	Yes	Total
No	10	3	13
Yes	5	17	22
Total	15	20	35

Posterior probabilities for inclusion parameters of sample- and site-specific environmental correlates confirmed that detection probability and occupancy were affected by habitat factors describing land cover, forest extent, and water (Table 5). Site detection probabilities differed between species with occupancy status of channel catfish mitochondrial eDNA being the only covariate affecting detection of Yaqui catfish eDNA (Fig. 2). Although site detection probabilities were high (mean > 0.6) in all cases, detection of Yaqui catfish mitochondrial eDNA was near 1 when channel catfish mitochondrial eDNA was present. Detection of channel catfish mitochondrial eDNA was related to variable water temperatures (negative), elevation (negative), and substrate size (positive), but was still generally high (>0.5) in all instances (Fig. 3). Covariates affecting occupancy probability of Yaqui catfish eDNA were average natural stream discharge (positive), percent forest (positive), and probability of the stream reach drying (negative; Fig. 4). The 3 sites that contained only channel catfish eDNA were within 2 sub-basins: Rio Moctezuma and Rio Agua Prieta (2 sites) and included river sites (n =1) and reservoir sites (n = 2). Channel catfish mitochondrial eDNA was more likely to occupy sites in watersheds where the major-

Table 5. Estimated detection probability and parameters for mitochondrial environmental DNA (eDNA) Yaqui catfish *Ictalurus pricei* and channel catfish *I. punctatus*. Pr: inclusion probability

Parameter	Mean slope (SD)	90% CI	Pr
Yaqui catfish			
Occupancy			
Annual average natural discharge ($m^3 s^{-1}$)	2.33 (1.10)	0.59, 4.28	0.72
Percent forest extent	2.37 (1.20)	0.51, 4.45	0.88
Probability of a reach drying at least once in a year	−1.51 (1.15)	−3.48, 0.32	0.74
Channel catfish present	0.75 (0.19)	0.36, 0.97	0.65
Channel catfish absent	0.36 (0.23)	0.06, 0.79	0.51
Detection			
Channel catfish present	0.98 (0.02)	0.95, 0.99	1.00
Channel catfish absent	0.66 (0.13)	0.43, 0.85	1.00
Channel catfish			
Occupancy			
Land cover categories	2.52 (1.04)	0.91, 4.29	0.97
Total upstream area (km^2)	2.04 (0.89)	0.72, 3.62	0.93
Probability of a reach drying at least once in a year	−2.29 (0.89)	−3.80, −0.88	0.93
Detection			
Water temperature (°C)	−0.47 (0.39)	−1.09, 0.18	0.68
Elevation (m)	−1.52 (0.56)	−2.48, −0.66	0.85
Modal substrate size	0.67 (0.50)	−0.15, 1.52	0.65

ity of land cover was conifer trees and shrub types, with larger amounts of upstream area above the stream segment and lower probabilities of stream drying compared to areas with different land cover types (Fig. 5).

4. DISCUSSION

Our development and application of new eDNA assays successfully detected the mitochondrial DNA of these species in both sympatry and allopatry with high levels of sensitivity and specificity. Given the expansive range of channel catfish, we recommend future practitioners validate the assay with their population(s) of interest prior to conducting eDNA sampling. As is common for eDNA approaches, we used the maternally inherited DNA from the mitochondria, and when both species were discovered in a single sample, we interpreted the findings as either both species being present or a hybrid combination of channel and Yaqui catfish being present (Bylemans et al. 2018, Evans & Lamberti 2018). Additionally, we acknowledge that detection of DNA from a single species could represent a hybrid individual at the nuclear genome level because of the maternal inheritance of mitochondrial DNA, but we have no assessment regarding the degree of this potential. Given local evidence of hybridization (Gutiérrez-Barragán et al. 2021), the presence of mitochondrial DNA of both species at a site certainly implies a high probability of hybrids. However, the true extent of hybridization at the basin scale and beyond is unknown,

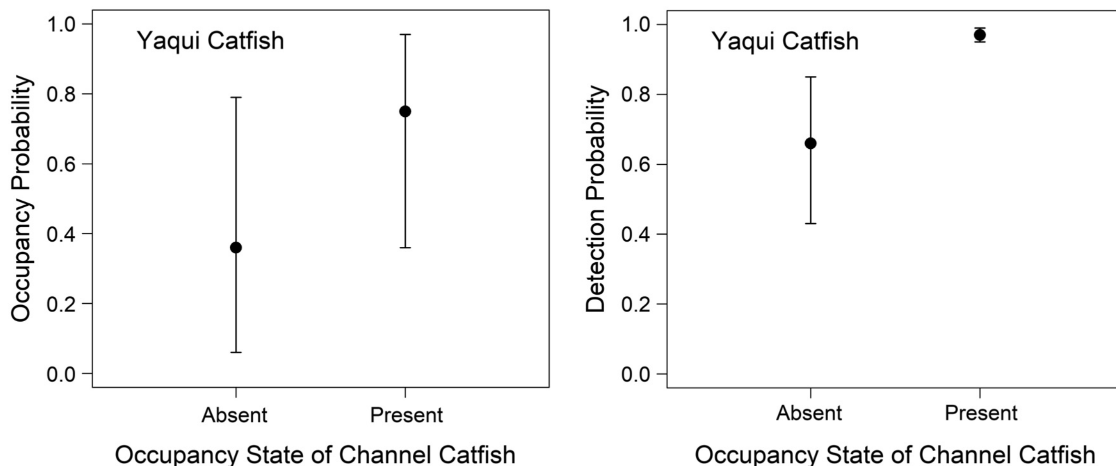

Fig. 2. Estimates of probability of occupancy and detection of mitochondrial environmental DNA (eDNA) from Yaqui catfish *Ictalurus pricei* in the presence and absence of mitochondrial eDNA from channel catfish *I. punctatus*. Error bars indicate 90% credibility intervals

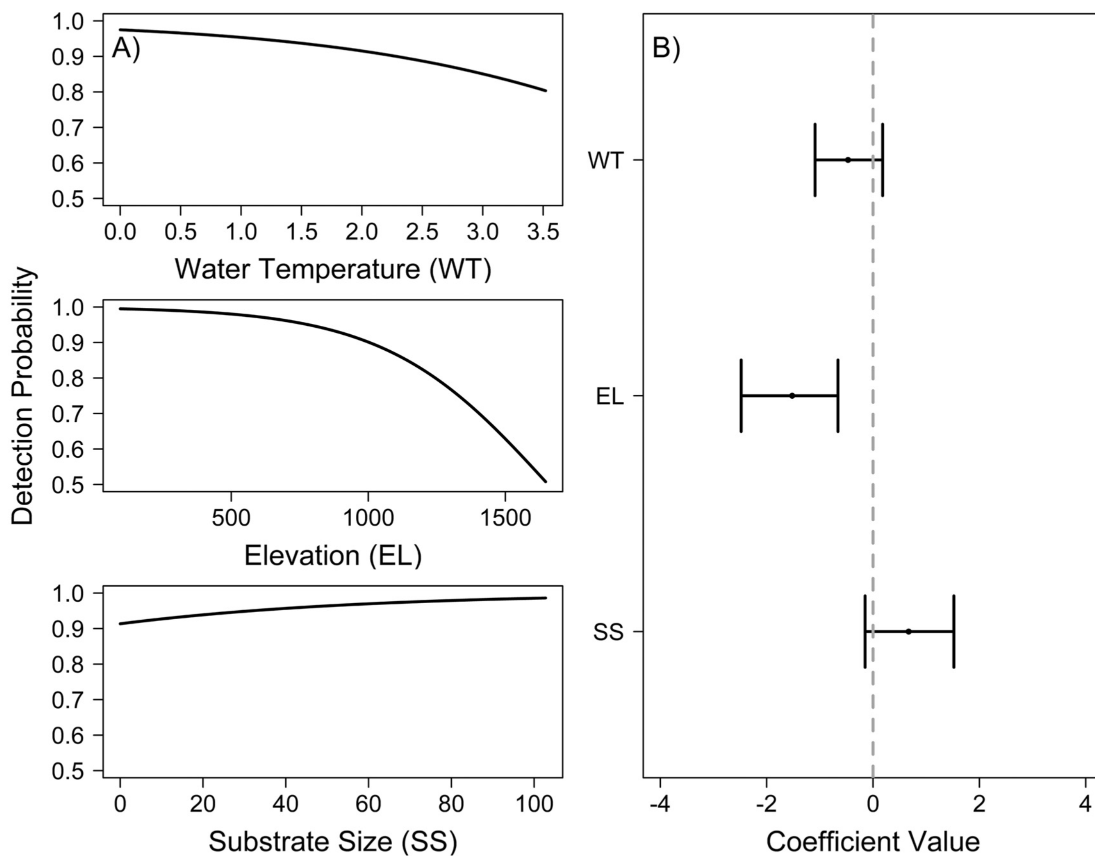

Fig. 3. Channel catfish *Ictalurus punctatus* mitochondrial environmental DNA (eDNA) (A) detection probabilities and (B) parameter estimates in relation to water temperature (°C), elevation (m), and modal substrate size (mm). Parameter estimates with 90% credibility intervals that intersect the reference line at 0 are not strongly supported

making hybridization outcomes and hybrid zones unclear. This information is essential for understanding the extent and locations of putative pure Yaqui catfish, channel catfish, and their hybrids. For example, at a local scale, Gutiérrez-Barragán et al. (2021) studied hybridization in the Arroyo Cajon Bonito, a small stream in northwest Mexico, and found evidence of putative pure Yaqui catfish, channel catfish, and their hybrids (with hybrids identified on the maternal side for channel catfish and Yaqui catfish) using nuclear genes. Our mitochondrial markers detected both channel catfish and Yaqui catfish mitochondrial eDNA at this site (Arroyo Cajon Bonito), which would infer the potential for hybridization because of co-occurrence. Our findings confirm that mitochondrial DNA from Yaqui catfish and channel catfish are frequently found together throughout the basin, particularly in mainstem habitats, suggesting that hybridization is high in the basin. However, the degree of hybridization remains unknown and would require additional physical sampling of individual fish to determine.

Five locations were identified as containing mitochondrial eDNA signatures of solely Yaqui catfish, indicating the potential existence of refuges for this imperiled species. Potentially, eDNA signatures of Yaqui catfish mitochondrial eDNA could represent a hybrid with Yaqui catfish maternal genes. Because of relatively high detection rates of mitochondrial eDNA from both species, our results indicate the presence of Yaqui catfish mitochondrial eDNA and the absence of channel catfish mitochondrial eDNA at these locations, but physical sampling of fish from these locations would be required to verify this assumption. Two locations where only Yaqui catfish mitochondrial eDNA was found occurred in reservoirs, ranging in size from 40 to 81 ha, where the dams may have created a barrier to invasion by channel catfish (Jackson and Pringle 2010). Additional sampling in these 2 locations and upstream would help verify if they represent situations free of channel catfish presence. If so, further investigation could identify the state of the population (e.g. abundance, size structure) and whether the reservoirs provide habitat that supports

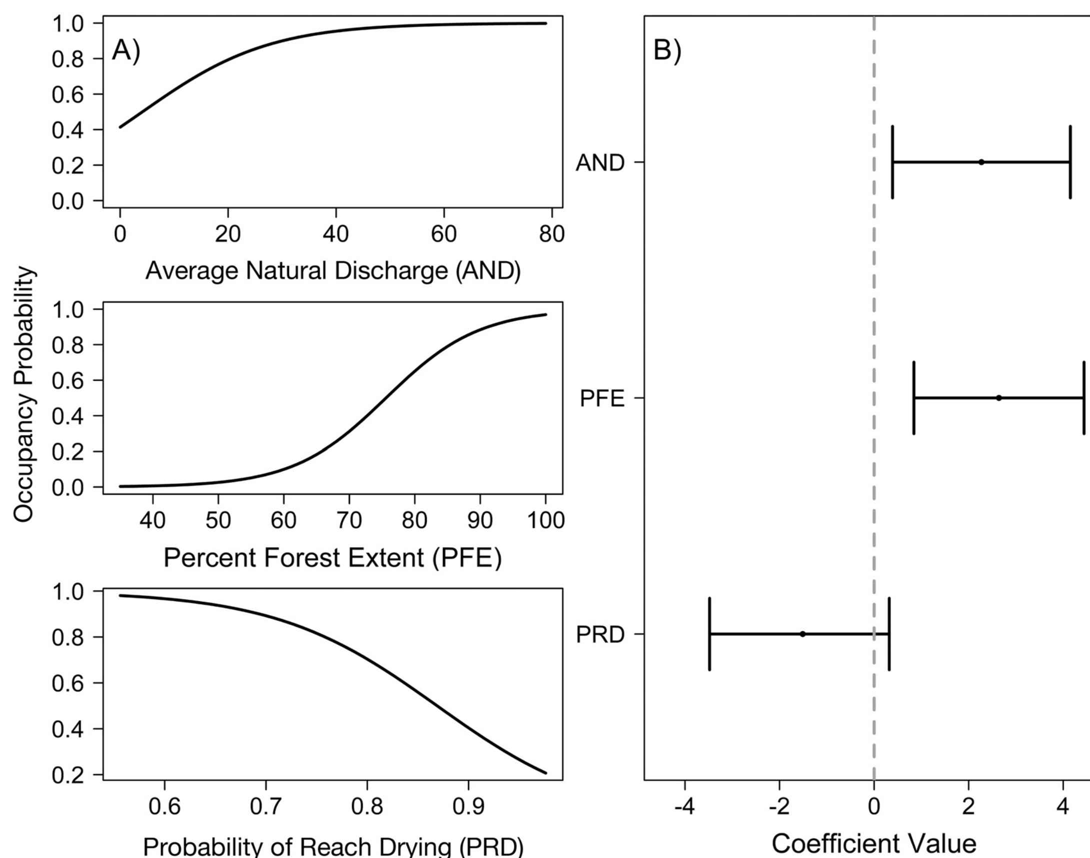

Fig. 4. Yaqui catfish *Ictalurus pricei* mitochondrial environmental DNA (eDNA) (A) occupancy probabilities and (B) parameter estimates in relation to annual average natural discharge ($m^3 s^{-1}$), percent forest extent, and probability of a reach drying at least once in a year. Parameter estimates with 90% credibility intervals that intersect the reference line at 0 are not strongly supported

natural reproduction. Small impoundments may not provide sufficient habitat for natural reproduction by Ictalurid catfishes. For example, Yaqui catfish stocked into ponds ⩽1.5 ha in size around San Bernardino National Wildlife Refuge in the late 1990s do not appear to have reproduced (Stewart et al. 2017). Similarly, lack of natural reproduction in small impoundments has been long noted for congeneric channel catfish (e.g. Marzolf 1957, Krummrich & Heidinger 1973), but Stewart & Long (2015) noted natural reproduction in a 223 ha impoundment in Oklahoma that had low perceived recruitment potential. In part, the recruitment potential of channel catfish has been limited by the scarcity of adequate spawning substrate in pebble, cobble, and boulder sizes (Hubert 1999). At the 81 ha Presa Calabazas, in the Rio Bacanora sub-basin, the modal substrate size we measured was in the pebble-cobble range (Udden 1914, Wentworth 1922, Blair & McPherson 1999), which is ideal for spawning by channel catfish, suggesting some potential for Yaqui catfish to persist in this system. Unfortunately, we did not measure substrate size at the 40 ha Presa Mojonera reservoir, where only Yaqui catfish mitochondrial eDNA was detected. Additional sampling of these locations would be useful to determine if they are suitable refuges for Yaqui catfish, and whether or not they are suitable for future stockings, such that management tools like genetic swamping can be implemented to ensure that the proportional makeup of pure Yaqui catfish in these areas remains high.

Historically, Yaqui catfish were distributed throughout the entire Yaqui River basin, where high temperatures and harsh desert environments are prevalent (Hendrickson et al. 1980). Many streams and rivers go completely dry during certain times of the year, making long-distance movements an important adaptation strategy to survive in these harsh conditions (Campoy-Favela et al. 1989, Hudson et al. 2005). Data on movement by Yaqui catfish is unknown, but congeneric channel catfish have exhibited long distance movement of up to 400 km (Hubert 1999), suggesting a similar potential for Yaqui catfish. With an east-to-west gradient in temperature, precipitation, elevation, and vegetation of the Yaqui River basin, the eastern

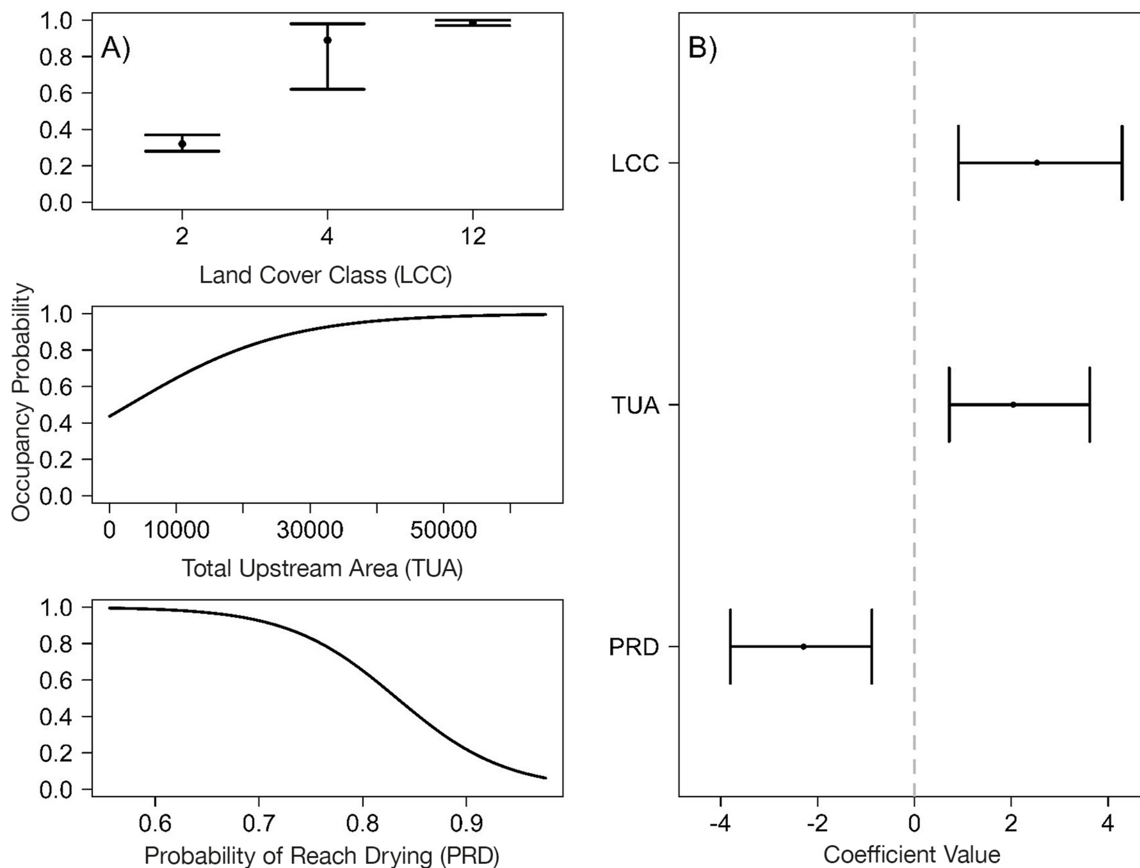

Fig. 5. Channel catfish *Ictalurus punctatus* mitochondrial environmental DNA (eDNA) (A) occupancy probabilities and (B) parameter estimates in relation to land cover (2 = deciduous forest, 4 = conifer forest, 12 = shrub), total upstream area (km^2), and probability of a reach drying at least once in a year. Parameter estimates with 90% credibility intervals that intersect the reference line at 0 are not strongly supported

part of the basin may serve as a refuge when most of the rivers of the western portion go dry (Brown 1982, Abarca et al. 1995, Munoz-Hernandez et al. 2011, Hudson et al. 2005), suggesting that connectivity may be important for Yaqui catfish. We were not able to sample in most of the eastern portion of the basin due to human safety concerns, but these environmental gradients may be important for securing sustainable Yaqui catfish populations by providing increased levels of natural discharge and lower probabilities of drying, which we found to be positively related to Yaqui catfish occupancy.

Beyond abiotic variables, the presence of channel catfish mitochondrial eDNA appears to be the most significant driver of Yaqui catfish mitochondrial eDNA occupancy in the Yaqui River basin, as determined through non-invasive sampling of eDNA. Non-native introductions can create a suite of challenges for native fish adapted to specific environments and conditions, whether it be from interspecific interactions (Gozlan et al. 2010, Britton et al. 2011) or hybridization (Gutiérrez-Barragán et al. 2021). Channel catfish were first stocked in the Yaqui River basin reservoirs for recreational fishing and as a food source (Ruíz-Campos et al. 2014), and have since expanded their distribution from reservoirs into mainstem rivers and even headwater streams. In addition to hybridization, the effects of channel catfish on Yaqui catfish likely include decreased growth rate and abundance (Cucherousset & Olden 2011), increased exposure to predation (Blanchet et al. 2008), and competitive exclusion (Fisk et al. 2007). The presence of non-native species and their hybrids will likely complicate conservation, requiring further steps to ensure species identity in areas of restoration or conservation. Studies focused on understanding the extent of hybridization and building effective genetic markers to distinguish hybrids from genetically pure fish are needed, involving crafting a robust sampling design (i.e. probabilistic sampling) starting from the genetic discovery of hybridization in the Arroyo Cajón Bonito in the headwaters of the Río Yaqui basin in Sonora (Gutiérrez-Barragán et al. 2021).

Moreover, and given that hybridization dynamics will vary substantially across locations, several replicate hybrid zones will need to be sampled to ensure that the molecular markers developed to identify the loci fixed for each species and hybrids are representative, while minimizing bias towards particular *Ictalurus* ancestries as a result of sampling practices (Mandeville et al. 2015, Kim et al. 2022, Rosenthal et al. 2022). Concurrently, using those tools with the appropriate sampling design to promptly develop a broodstock for a captive population is a logical next step for Yaqui catfish conservation. Additional research investigating interactions between Yaqui catfish and channel catfish, determining Yaqui catfish spawning habitats, and identifying related environmental cues for reproduction would aid in conservation planning efforts. Development of protocol elements to inform a responsible stock enhancement strategy to conserve Yaqui catfish also appears prudent to secure them in their native environment (Hendrickson & Varela-Romero 2002, Rosenfield et al. 2004, Hata et al. 2019, Montanari et al. 2016, Stewart & Long 2015).

The current number of Yaqui catfish in Mexico is unknown. As it will take time to plan, should conservation agencies be interested in developing conservation areas for Yaqui catfish or identify future release locations for hatchery-produced Yaqui catfish, it is critical to pinpoint areas with relatively 'pure' Yaqui catfish populations. Moreover, areas of refuge isolated from habitats with non-native species could further ensure long-term stability of such areas. This idea aligns with the recovery plan goals for San Bernardino and Leslie Canyon National Wildlife Refuges for native fishes of the Yaqui River basin: eradicate non-native species, protect critical habitat, and protect and conserve groundwater (USFWS 1995). We conclude that more surveys, including physical sampling and the use of mitochondrial and nuclear eDNA sampling, and genomics along with habitat modeling could better identify areas for future research and protection. Moreover, a parallel effort to capture individuals from the remaining Yaqui catfish population in Mexico to create a captive population could help secure the species in the interim. While scientists can play a critical role in informing conservation planning, a cooperative international effort involving governments and nongovernmental organizations would be necessary to develop and implement a long-term Yaqui catfish conservation plan.

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