



Fish community composition detected using traditional fishing and eDNA in Dianchi Lake, Southwest China

Kai Zhang · Zhiqiang Xia · Zijin Hong · Zihao Fu ·
Qiuhan Li · Xuexiu Chang · Guangjie Chen ·
Hugh J. MacIsaac

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Abstract Dianchi Lake is a eutrophic lake in Yunnan, China with many endangered or extinct native fish species. We sought to explore the fish community using traditional netting and trapping methods and, for the critically endangered golden-line barbel *Sinocyclocheilus grahami*, environmental DNA (eDNA) to detect its presence and distribution in the lake. Using traditional netting and trapping methods, we found that the fish community has been almost completely converted to non-native species (~97% of abundance). Loss of native fishes was serious, with ~77% of historical species not detected. We designed sensitive species-specific primers for the mitochondrial COI gene specific to *S. grahami* and sampled eDNA throughout the lake. While only two

individuals of *S. grahami* were caught using traditional methods, eDNA analysis revealed its presence across the central and southern regions of the lake. While it is unclear if the fish detected represent a recovering population or recently stocked individuals, occurrence records for the fish were inversely related to regions of the lake that suffered serious cyanobacteria blooms. This study highlights the sensitivity and utility of eDNA for non-destructively detecting presence of an endangered fish species.

Keywords Endangered fish · Aquatic invasive species · eDNA · Conservation · Plateau lakes · Quantitative real-time PCR

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K. Zhang · Z. Xia · X. Chang · H. J. MacIsaac (✉)
Great Lakes Institute for Environmental Research,
University of Windsor, Windsor, ON N9B 3P4, Canada
e-mail: hughm@uwindsor.ca

Z. Xia
College of Quality and Safety Engineering, China Jiliang
University, Hangzhou 210018, Zhejiang, China

Z. Hong · Z. Fu · H. J. MacIsaac
School of Ecology and Environmental Science, Yunnan
University, Kunming 650091, China

Q. Li
Faculty of Environmental Science and Engineering,
Kunming University of Science and Technology,
Kunming 650500, China

X. Chang
Yunnan Collaborative Innovation Center for Plateau Lake
Ecology and Environmental Health, College of Agronomy
and Life Sciences, Kunming University, Kunming 650214,
China

G. Chen
Yunnan Key Laboratory of Plateau Geographical Processes
and Environment Change, Faculty of Geography, Yunnan
Normal University, Kunming 650500, China

Introduction

Management of rare species (e.g., newly introduced nonindigenous species or endangered species) is an important aspect of biodiversity conservation (Lynch et al., 2016). Endangered fish protection is a conservation goal worldwide, but lack of effective and standardized detection methods inhibits management. Traditional fish collection methods include netting, line and hook, trapping, or electrofishing, though their capture probabilities can be low (Dejean et al., 2011), thus these methods are more suitable for species detection when abundance is moderate to high (i.e., species is not endangered) (McDonald, 2004). These methods are less conducive to detecting rare fishes (Piggott et al., 2020) as they are prone to type II errors (i.e., false negatives; Gu & Swihart, 2004; Zhan & MacIsaac, 2015). In addition to the difference in detection sensitivity, traditional fish survey methods have multiple deficiencies. For example, netting and traps may be biased towards specific species or sizes of fish or particular behaviour types, and electroshocking and gillnetting can harm or kill individuals of both target and non-target species (Lintermans, 2015). Therefore, these methods have well-defined limitations and disadvantages when used to detect endangered fish (Costello et al., 2016).

Over the past 15 years, technology has developed permitting detection of species' DNA in the environment. This technique is referred to as eDNA-based techniques (Hebert et al., 2003; Taberlet et al., 2012; Lacoursière-Roussel & Deiner, 2021). eDNA is shed by organisms as they carry out everyday activities, allowing researchers to collect and analyze for its presence from environmental samples. Such conveniences in eDNA sampling make it non-destructive to organisms (Piggott et al., 2020). eDNA has progressed as a high-speed, high-sensitivity, and high-accuracy technology (Harper et al., 2019) and is now widely used for species detection and community structure profiling in freshwater ecosystems (Bohmann et al., 2014; Sepulveda et al., 2020; Xia et al., 2021; Wang et al., 2023). eDNA-based techniques can have greatly improved sensitivity (i.e., reduced type II errors) of rare species detection (Wang et al., 2023). For example, eDNA was deemed to be ~44% more effective than other methods in marine species detection (Boussarie et al., 2018). Method selection is an important component that needs to be considered

to improve detection sensitivity (Xia et al., 2021). Real-time quantitative PCR (hereafter qPCR) has higher sensitivity and has largely displaced end-point conventional PCR (cPCR) and is now widely used to detect the presence and distribution of single species in the environment (e.g., Piggott et al., 2020; Xia et al., 2021). Droplet digital PCR (ddPCR) is the newest approach, has very high sensitivity potential, and can overcome PCR inhibitors (Harper et al., 2019), although it is not commonly used yet (Xia et al., 2021).

Yunnan Province, China is a global biodiversity hotspot that accounts for a large fraction of China's total biodiversity (Yang et al., 2004). Plateau lakes in this province belong to different watersheds, leading to a high level of geographical isolation and high native fish biodiversity (Chen, 2013). However, over the past few decades multiple stressors including overfishing, river damming, habitat loss, metal contamination, and especially species introduction and cultural eutrophication have had large impacts on the biodiversity of these lakes (Wang et al., 2021). Fish species have been heavily impacted, particularly in Dianchi Lake, the largest and most seriously impacted lake in this plateau region. In Dianchi, ~90% of endemic fish species are now considered extinct (Wang et al., 2013a), including the golden-line barbel (*Sinocyclocheilus grahami*) (Chen & Yang, 2008). *S. grahami* grows slowly and was once common and commercially harvested (Pan et al., 2009). It usually lives in slow-moving waters, preferring habitats with pebblestone and large aquatic plants. The spawning period extends from January to April, and eggs are laid on hard surfaces in flowing waters (Pan et al., 2009). The species has suffered from an array of problems that have caused its population size to plummet (Tang et al., 2019). Currently, the species is classified as being critically endangered (Chen et al., 2008). Extensive effort has been made to breed and restock the species with annual stocking of captive-bred fish, though no natural breeding has been reported in the lake in recent years (Zhang et al., 2021).

The goals of this study were to: (1) characterize the fish community structure and distribution, including *S. grahami*, of Dianchi Lake using multiple survey methods; and (2) detect *S. grahami* using eDNA and assess its distribution relative to key stressors in the system.

Methods

Fish and water sample collection

Dianchi Lake (24.40–25.02°N, 102.37–102.48°E) is a large, shallow lake (1887 m a.s.l.; mean water depth 4.4 m, maximum water depth 11.0 m) with a surface area of ~300 km² (Fan et al., 2017). Sampling sites were established at eight existing national water quality-monitoring sites (each site was marked with a buoy). We also included eight other sites which coincided with the existing Kunming Institute of Zoology fish survey sites in the lake. To increase sample representativeness, we added eight water-sampling sites to the existing ones, resulting in a total of 16 sites (Fig. 1, Table 1).

Many fishes in Dianchi Lake are small-bodied species, including many benthic species (Chen, 2013). Gillnets and fish traps are common fishing methods used throughout the lake. In this study, fishes were collected from eight sites (Fig. 1) in January 2018 by local anglers and trappers. Specifically, three different mesh size gillnets (10, 6, and 1.5 cm, respectively; 2 m in height and 16 m in length) and fish traps (mesh size 0.5 cm with a frame of 35×25 cm and 10 m long) were used. Gillnets and fish traps were set to the lake bottom at each site. Nets were set at dusk and retrieved the next morning, with each deployment lasting for ~12 h. Fishes captured were identified to species level following Cheng (1987), counted, and intact individuals were preserved in 95% ethanol, placed on ice, and transported to the laboratory within 12 h of collection.

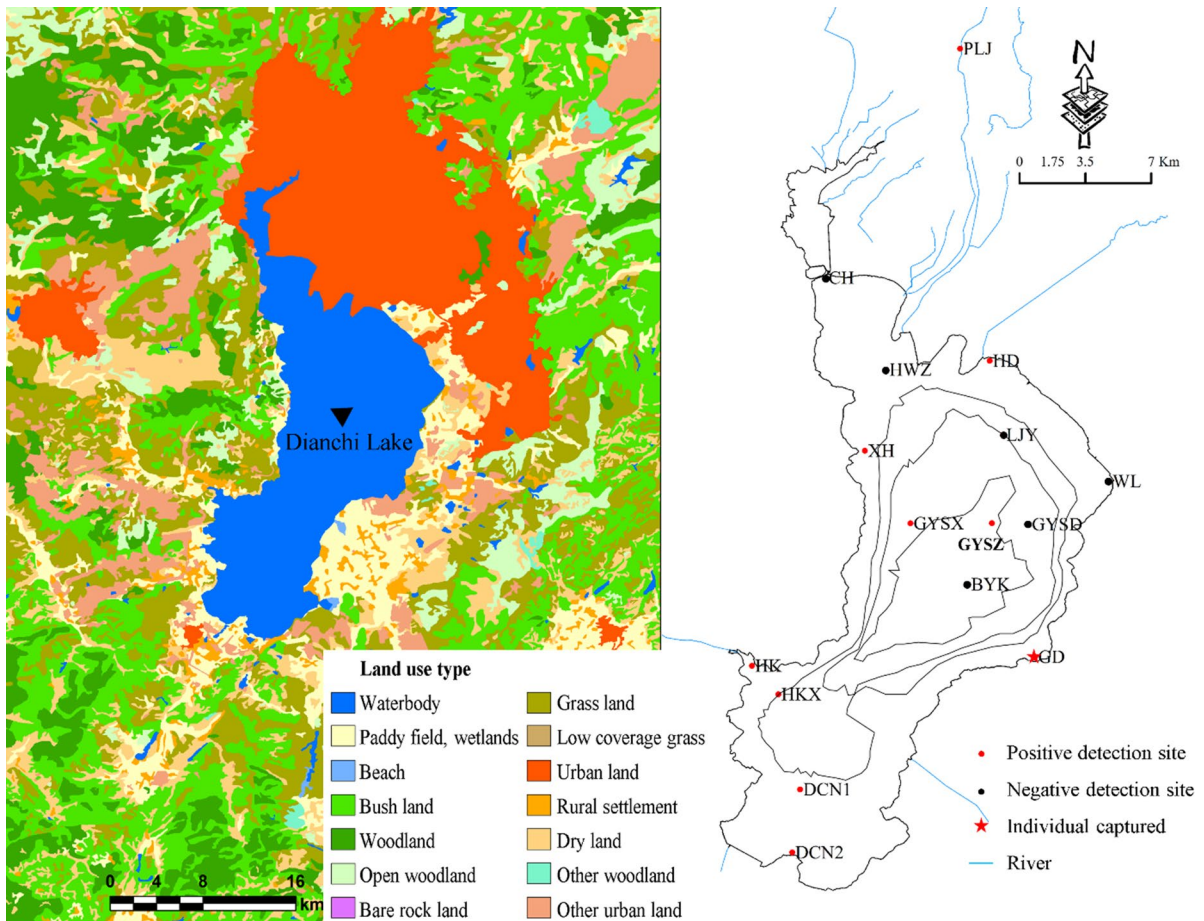


Fig. 1 Location, land use and sampling sites in this study (red dots in the right panel) in Dianchi Lake, China. PLJ is a river control site and a historical natural distribution area of *Sinocyclocheilus grahami*

Table 1 Sample collection information. Refer to Fig. 1 for site locations

Site	GYSX	DCN1	GYSZ	HKX	HD	GD	DCN2	HK	PLJ ^a	XH	HWZ	LJY	GYSZ	BYK	CH	WL
Date (MM/DD)	01/24	01/24	01/24	01/24	01/20	01/12	01/14	01/25	01/08	01/20	01/24	01/24	01/24	01/24	01/11	01/21
Water Samples collected	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Fish Samples collection	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes

^aSite PLJ is a control river site

One 1-L water sample for chemical parameters (HDPE bottles) was collected from the subsurface (~20 cm) at each site. Samples were placed on ice in a cooler and transported to the laboratory within 12 h of collection. Control bottles filled with double-distilled water (ddH₂O) from the lab were bought to the field, remained open, and then returned to the lab until being analyzed the same way as field samples. To avoid cross contamination, gloves were used during sample collection, and all bottles were cleaned using ddH₂O and rinsed three times with sample water before being filled with the sample. Water samples were stored in different coolers and separated from fish samples throughout the entire sampling process.

Dissolved oxygen concentration (DO), water temperature (site WT) and oxidation–reduction potential (ORP) of the 15 samples were measured on site (site CH was not measured due to equipment failure). Measurements were done on sites using YSI 556 handheld multiparameter instrument (YSI Incorporated, Yellow Springs, OH, USA).

Water chemistry analyses

Total nitrogen (TN) was measured by taking 10 ml of the well-mixed water sample, and 5 ml of alkaline potassium persulfate was added to a 25 ml colorimetric tube and placed in an autoclave for digestion for 30 min at 120 °C. Two blank controls with ultra-pure water (Mili-Q, conductivity of <0.056 μS cm⁻¹ and <50 ppb of Total Organic Carbon (TOC)) was included in analysis. After being cooled to room temperature, 1 ml of hydrochloric acid solution was added, and ultra-pure water was added to make up to 25 ml. Samples were then analyzed using an automatic discrete analyzer cleverchem 380 (DeChem-Tech, Hamburg, Germany).

Total phosphorus (TP) was measured by taking 25 ml of well-mixed water sample in a 50-ml colorimetric tube, then 4-ml potassium persulfate solution was added, following which samples were shaken and covered with a stopper. All samples were placed in an autoclave at 120 °C at a pressure of 1.1–1.4 kg/cm² for 30 min. After cooling to room temperature, the samples were diluted to 50-ml with pure water. Two blank controls using ultra-pure water were included in analysis.

eDNA samples collection and handling

We collected water samples in January 2019 from the lake surface at all 16 sampling sites (Fig. 1). All sampling bottles (300-ml HDPE bottle) were cleaned with 20% bleach and washed three times with sample water before collection (Huggett et al., 2013). We collected four replicate samples per site, and gloves were used and changed between sites. An additional bottle filled with ddH₂O that served as a blank control was exposed to air, sealed, and stored with water samples.

Owing to a large amount of particulate matter in the water samples, we conducted a pilot extraction experiment comparing a pre-filtering and non-pre-filtered protocol. Specifically, water samples were pre-filtered using a 45- μ m metal mesh before being filtered using an acetate filter membrane (pore size: 0.45 μ m; diameter: 50 mm, Tianjin Keyilong Lab Equipment Co., Ltd., Tianjin, China). Metal mesh was cleaned with 20% bleach and washed three times with ddH₂O before each use. A NanoDrop test (Desjardins & Conklin, 2010) demonstrated that the pre-filtered samples led to higher DNA quantity than the unfiltered samples. As a result, all eDNA water samples were pre-filtered in our formal analysis. All water samples were filtered within 24 h of collection, and filters were stored at -20°C until DNA extraction.

Half of each filter was used for extraction following the PCI extraction method by Balasingham et al. (2017), and the remaining half was stored for future use. Tissue DNA (i.e., total genomic DNA) of the target species and other captured fish species was extracted from fin samples using same method. For the in vitro specificity test to determine a set of primers will amplify only one group of sequences (the target species) but no others (the non-targeted species). Blank (negative) controls and tissue samples (positive controls) were used for each batch of extractions. All tissue samples were extracted in a different room from environmental samples, and decontamination and cleaning were performed between extractions. Negative controls were used in all steps of processing environmental samples. We assessed extracted DNA quality and estimated concentration (ng/ μ l) using a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific).

Primer design and sensitivity testing

No species-specific genetic markers for *S. grahami* were available in existing studies; thus, we designed species-specific primers using the mitochondrial COI and D-loop regions based on the complete mitochondrial genome (NCBI reference sequence: NC_013189.1). We used the Oligo 7 and Primer Premier 5 to design primers. Initially, a total of 104 primer pairs were designed and then tested online for non-specific amplification using the Primer-blast (NCBI) tool (i.e., in silico specificity check). Specifically, primers were sorted in descending order of the scores detected by Primer Premier 5, and then the Primer-BLAST online tool (NCBI) was used to blast with the non-redundant (NR) nucleotide database and fish database (tax id 7898) one by one.

Fourteen primer pairs that passed the online specificity check were synthesized by Sangon Biotech (Shanghai, China), following which an optimal annealing temperature check was performed. We conducted an in vitro specificity test for non-specific amplification for each primer pair against all sympatric fish species with available tissue samples that were either collected during this study or bought from local markets. Tissue DNA was diluted to 1 ng/ μ l before being subjected to conventional PCR amplification, with the product being checked on 2% agarose gel. Three positive controls were included using the total genomic DNA (i.e., tissue DNA) of the target species.

To determine the limit of detection (LoD; the lowest concentration of analyte in the test sample that can be reliably detected but not necessarily quantitated) and limit of quantification (LoQ; the lowest amount of analyte in a sample that can be suitably quantified) of our assays (Klymus et al., 2020), we performed a series of amplification using serial dilutions of total genomic DNA following the method by Yun et al. (2006). Specifically, the LoD was determined based on 3 \times serial dilutions (Agersnap et al., 2017), and one successful amplification of five technical replicates was considered a positive detection (Goldberg et al., 2016). Likely, the LoQ of our assay was determined but with 10 \times dilutions and three technical replicates per concentration. Primer pairs that passed the specificity check were tested by serial dilution. To quantify target DNA concentration in water samples, we constructed a standard curve for each batch of amplifications using triplicate 10 \times serial dilutions.

Inhibitors are widely present in eDNA samples, and we conducted a gradient dilution experiment to reduce amplification inhibition for water samples (Huggett et al., 2013) before we formally amplified our eDNA samples. Specifically, we prepared three replicates of 2×, 4×, 8×, and 16× diluted eDNA extracts and tested them in a 20-cycle conventional PCR, and the quality and brightness of each amplification product were examined by gel electrophoresis to determine the optimal dilution factor (Schabacker et al., 2020). 8× dilution led to the best results and then used in the formal analysis.

Quantitative PCR and Sanger sequencing

Primers that passed the Specificity or sensitivity tests were used for qPCR examination of water samples. We used PowerUp SYBR Green master mix (Thermo Fisher Scientific, MA, USA) for all qPCR reactions. Each 20 µl PCR cocktail contained 10 µl master mix, 0.6 µl forward and reverse primer, respectively (concentration: 2 µM), 2 µl diluted (8× diluted) eDNA sample (i.e., templates), and 6.8 µl ultrapure water. No-template (negative) controls and standard curves (also serving as positive controls) using total genomic DNA were also included in triplicate for each qPCR in a 96-well plate. A 2 min 50 °C heat start was set at the beginning of each run following the prescribed instructions of the master mix (PowerUp SYBR Green master mix user guide). Thermal cycler conditions were set to an initial 95 °C denaturation for 3 min, followed by 50 cycles of 95 °C denaturation for 30 s, then annealing at 58 °C for 30 s and 70 °C for 60 s, with a default melting curve stage performed at the end. All qPCR data were analyzed on the QuantStudio 7 Flex real-time PCR System (Thermo Fisher Scientific, MA, USA). qPCR products were sent to Sangon Biotech (Shanghai, China) for Sanger sequencing to confirm species identity of the amplified product. We then used Basic Local Alignment Search Tool (BLAST) to align resulting sequences against GenBank nucleotide database sequences for *S. grahami* to verify amplification of the target species (Table S4). Samples with a similarity higher than 97% were considered to be *S. grahami*.

Three water sample replicates and three technical (i.e., intra-sample) replicates provided a total of nine replicates for each sampling site. Concentrations of target DNA in positive amplifications were calculated

according to the standard curve. Some sites that had a higher cycle number than the LoQ were also count as positive for further analysis and comparison.

Statistical analysis

To calculate the actual DNA concentration, we used linear regression to determine the best fit for empirical data. Spatial variation in fish composition was analyzed and visualized using principal component analysis (PCA) on fish absolute abundance data across the six sampling sites (site PLJ was a river control, and site CH's data was incomplete). Data were Hellinger-transformed to reduce weights of variables with low fish counts and zeros. The PCA based on the abundance of the 13 fish species across the six sites was used to visualize the spatial characteristics of the fish community. Spatial variation in target species DNA detections and other fish abundances were analyzed and visualized using PCA. Relationships between fish abundance (i.e., fish catch) and environmental factors were analyzed by calculating the Kendall correlation coefficient (Myers et al., 2013). A binary logistic regression model was developed to relate detection probability (i.e., yes or no) to environmental factors. We performed all statistical analyses using R (version 4.0.3), with package “ggplot2” used for plotting (Wickham, 2016).

Results

Physical and chemical parameters

Total phosphorus (TP) ranged from 0.105 mg/l (site XH) to 0.384 mg/l (site GYSX), with an average of 0.272 ± 0.096 (mean \pm SD) mg/l. The northeast part of the lake, closest to the major city of Kunming, had the highest TP concentration (Fig. 1). On the other hand, TP concentration was comparatively low along the west bank and in the downstream, southwest area (Table 2). Total nitrogen (TN) ranged from 1.568 mg/l (site DCN1) to 3.058 mg/l (site PLJ) and averaged at 2.116 ± 0.375 mg/l across the lake (Table 2). TN concentration was generally higher in the east and middle sections of the lake than other areas (Fig. 1).

Average DO was 8.97 ± 1.02 (\pm SD) mg/l and ranged from 7.30 mg/l (site PLJ) to 11.45 mg/l (site

Table 2 Physical and chemical parameters at different sampling sites in Dianchi Lake

Sampling site	GYSX	DCNI	GYSZ	HKX	HD	GD	DCN2	HK	PLJ	XH	HWZ	LJY	GYSZ	BYK	WL	CH
TP (mg/l)	0.384	0.232	0.348	0.34	0.256	0.224	0.148	0.288	0.348	0.105	0.376	0.372	0.284	0.412	0.172	0.204
TN (mg/l)	1.805	1.568	1.968	1.888	2.683	1.928	2.106	1.926	3.058	2.255	2.478	2.089	1.959	2.448	1.768	1.752
DO (mg/l)	7.7	8.5	8.4	8.9	9.7	8.4	8.6	8.9	7.3	9.5	11.4	10.2	8.8	9.6	8.6	NA
WT (°C)	12.9	13.6	14.2	12.2	14.9	14.4	13.3	12.2	13.9	14.7	15.6	14.5	14.6	12.9	15.5	NA
ORP (mV)	168.8	174.8	167.7	121.3	198.4	131.3	176.0	121.3	188.0	174.8	180.9	167.6	171.4	168.8	164.0	NA

Sampling sites correspond to Fig. 1

TP total phosphorus, TN total nitrogen, DO dissolved oxygen, WT water temperature, ORP oxidation–reduction potential, NA not available due to equipment damage

HWZ) (Table 2). Water temperature was highly variable among sites, ranging from 12.2 °C (site HK and HKX) to 15.6 °C (site HWZ), with an average of 14.0 ± 1.1 °C (Table 2). ORP ranged from 198.4 mV (site HD) to 121.3 mV (site HK and HKX), with an average of 165 ± 22 mV (Table 2).

Fish community distribution

A total of 478 individual fish were collected from eight sites (Table 3). Introduced species numerically dominated the fish community, with a total of 463 individuals (97%) belonging to 11 non-native species and only 15 individuals belonging to three native species (Table 3). Four non-native species were identified as numerically dominant (> 7%): *Neosalanx taihuensis* (~36%), *Rhinogobius giurinus* (~19%), *Cultrichthys erythropterus* (~13%), and *Pseudorasbora parva* (~9%).

The distribution of native species was very limited. *S. grahami* was only found at one site (GD) in the lake (1 of 7 sites) and the site PLJ in Panlong River (Fig. 1), while *Misgurnus anguillicaudatus* was distributed near the estuary (2 of 7 sites). *Carassius auratus* was distributed at site CH (Caohai area, 1 of 7 sites) (Fig. 1). On the contrary, most non-native species were widely distributed throughout the lake, especially the numerically dominant ones. *Neosalanx taihuensis* (7 of 7 sites), *Cultrichthys erythropterus* (6 of 7 sites), and *Pseudorasbora parva* (7 of 7 sites) were all widely distributed and dominant in the whole lake, while *R. giurinus* was distributed in all areas except the densely populated southeast coast of the lake (4 of 7 sites).

The first two principal components accounted for 38.1% and 26.5% of the total variation in fish abundance (Fig. 2, Table S2, Supplementary information). *S. grahami*, *Rhinogobius cliffordpopei*, *Cyprinus carpio* and *Hemiramphus sajori* had a similar contribution rate to axis one (~19.5%), with the distribution of the four species concentrated at site GD (an artificial wetland). Non-native *R. giurinus*, *Micropercops swinhonis*, *Hypomesus olidus*, *Abbottina rivularis* and *P. parva* had the highest contributions to the second axis (> 10% each).

Rhinogobius giurinus, *P. parva*, *M. anguillicaudatus*, *Hemiculter leucisculus* and *C. erythropterus* were numerically dominant at sites DCN2, XH, and HK in west and south parts of the lake (Fig. 2). *M.*

Table 3 Fish collected from Dianchi Lake by fish traps and nets

Species name	Sample site										
	Non-native Species	DCN2	GD	HD	CH	WL	PLJ	XH	HK	Total	
<i>Sinocyclocheilus graham</i> (Regan, 1904)		0	2	0	0	0	3	0	0	5	
<i>Misgurnus anguillicaudatus</i> (Cantor, 1842)		2	0	0	0	0	0	0	2	4	
<i>Carassius auratus</i> (Linnaeus, 1758)		0	0	0	6	0	0	0	0	6	
<i>Rhinogobius cliffordpopei</i> (Nichols, 1925)	+	0	2	0	0	0	1	0	0	3	
<i>Rhinogobius giurinus</i> (Rutter, 1897)	+	25	14	0	0	0	2	35	14	90	
<i>Neosalanx taihuensis</i> (Chen, 1956)	+	21	30	20	2	30	0	40	26	169	
<i>Cultrichthys erythropterus</i> (Basilewsky, 1855)	+	17	6	8	0	15	0	15	2	63	
<i>Hemiculter leucisculus</i> (Basilewsky, 1855)	+	17	2	4	0	0	0	2	0	25	
<i>Micropercops swinhonis</i> (Günther, 1873)	+	3	3	0	0	0	0	0	0	6	
<i>Cyprinus carpio</i> (Linnaeus, 1758)	+	0	5	0	4	0	0	0	0	9	
<i>Hypomesus olidus</i> (Pallas, 1814)	+	0	1	10	0	0	0	1	0	12	
<i>Hemiramphus sajori</i> (Temminck & Schlegel, 1846)	+	0	25	4	0	0	0	0	0	29	
<i>Abbottina rivularis</i> (Basilewsky, 1855)	+	0	0	8	5	0	0	0	0	13	
<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)	+	10	2	4	11	0	2	7	8	44	
Total		95	92	58	28	45	8	100	52	478	

swinhonis occurred mainly in the southern lake basin, and *N. taihuensis* did not show strong spatial distribution patterning (Fig. 2). *C. carpio*, *S. grahami*, *R. cliffordpopei* and *H. sajori* were more concentrated at site GD, while *A. rivularis* and *H. olidus* were most abundant at sites GD and WL in the northeast part of the lake (Fig. 2).

A Kendall correlation coefficient matrix was produced to visualize interactions between the 13 fish species and five environmental parameters across the six sites (Fig. S1). *S. grahami* was positively correlated with the non-native species *R. cliffordpopei* and native *C. carpio*. *P. parva* and *M. anguillicaudatus* were positively correlated, as were *H. olidus* and *H. sajori*. *M. swinhonis* was negatively correlated with *N. taihuensis* and with DO concentration. The distribution and abundance of *M. anguillicaudatus* were negatively correlated with water temperature. *P. parva* was negatively correlated with the redox potential.

Real-time qPCR for environmental samples

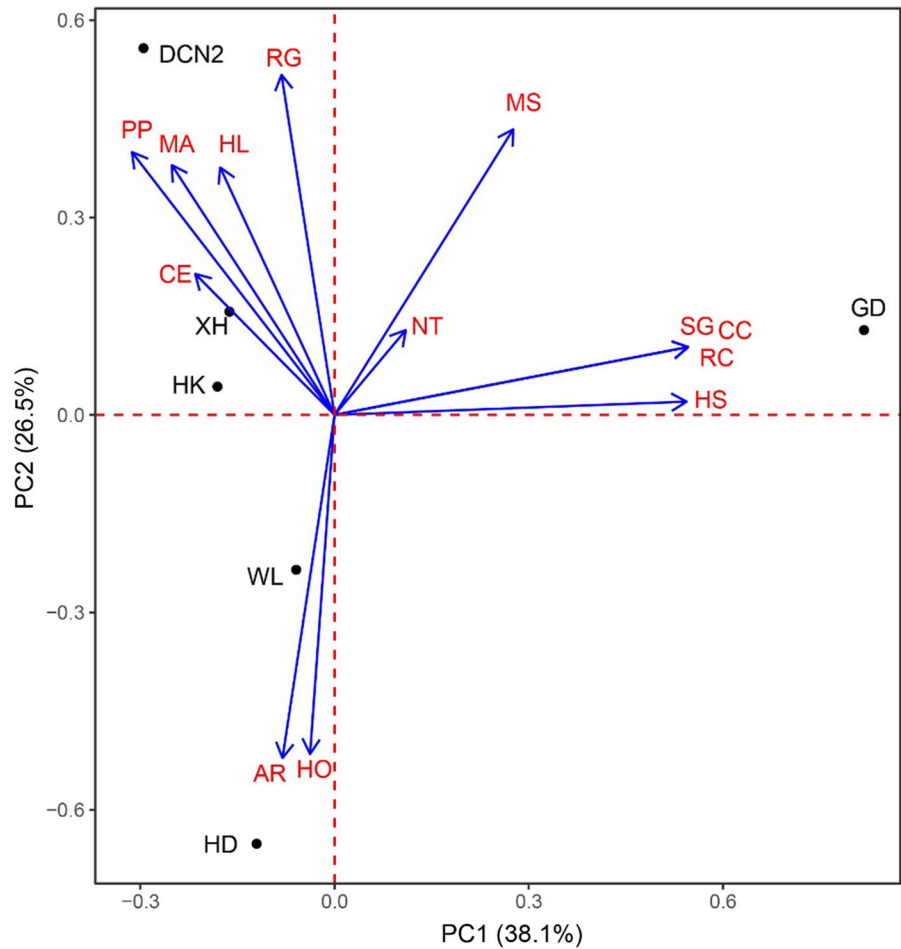
We selected the species-specific primer set SGCOI-1f (5'-CTACACTGATTCCCTACTAACC-3') and SGCOI-1r (5'-TCCGATGGATAACACCGTG-3') to amplify the eDNA samples of *S. grahami*. It does

not amplify any non-target fish in-silico or in-vitro, the length of other possible amplified sequences is very different from the length of the DNA sequence of the target species, which is easy to distinguish. We observed that the LoD of this primer set was excellent (1×10^{-8} ng/ μ l) (Fig. S2). We determined that the LoQ was 38 and 42 cycles using two standard curves using the same 96-well-plate as per environmental water samples (Figs. S3 A and B), equivalent to 1×10^{-4} ng/ μ l, and an amplification efficiency between 94 and 93%.

Dilution prior to qPCR amplification can minimize inhibitory effects and reduce errors between samples (McKee et al., 2015). The sampling blanks yielded two (of 3) positive results, but their melting temperature was 73.05 °C and 72.18 °C versus the 82.18 °C by the positive controls, suggesting that no contamination occurred during the sampling process. Sanger sequencing determined that PCR products were specific for *S. grahami*, with 98%+ similarity to the reference sequences (75,366) (Table S3).

Ten of the 16 sampling sites (62.5%) tested positive for *S. grahami* DNA, mainly in the southwest, west, and deep area of the northern basin (GD, DCN1, and GYSX; Fig. 1). Since some positive samples had CT values higher than the LoQ, we used the

Fig. 2 Principal component analysis (PCA) on the abundances of 13 fish species (blue arrows) across six sampling sites (black dots) in Dianchi Lake. Note that the arrows for SG, CC and RC are completely overlapped. Refer to Table 3 for abbreviations for species names



occurrence data (i.e., positive/negative detection) for subsequent analysis.

We combined DNA detection data for *S. grahami* with other fish individual abundance measures across the six sampling sites and visualized it using principal component analysis (Fig. 3). PC1 and PC2 accounted for 31% and 27% of the total variation, respectively (Fig. 3; Table S3). Fish species with the highest contribution to the PC1 were: *H. sajori* (21%), *R. clifordpopei* (20%) and *C. carpio* (20%). *M. swinhonis* (18%), *R. giurinus* (15%) and *A. rivularis* (13%) had the highest contribution to PC2. DNA detection showed much wide distribution (9 in 15 sites) of *S. grahami*, while the traditional method only caught two individuals at site GD.

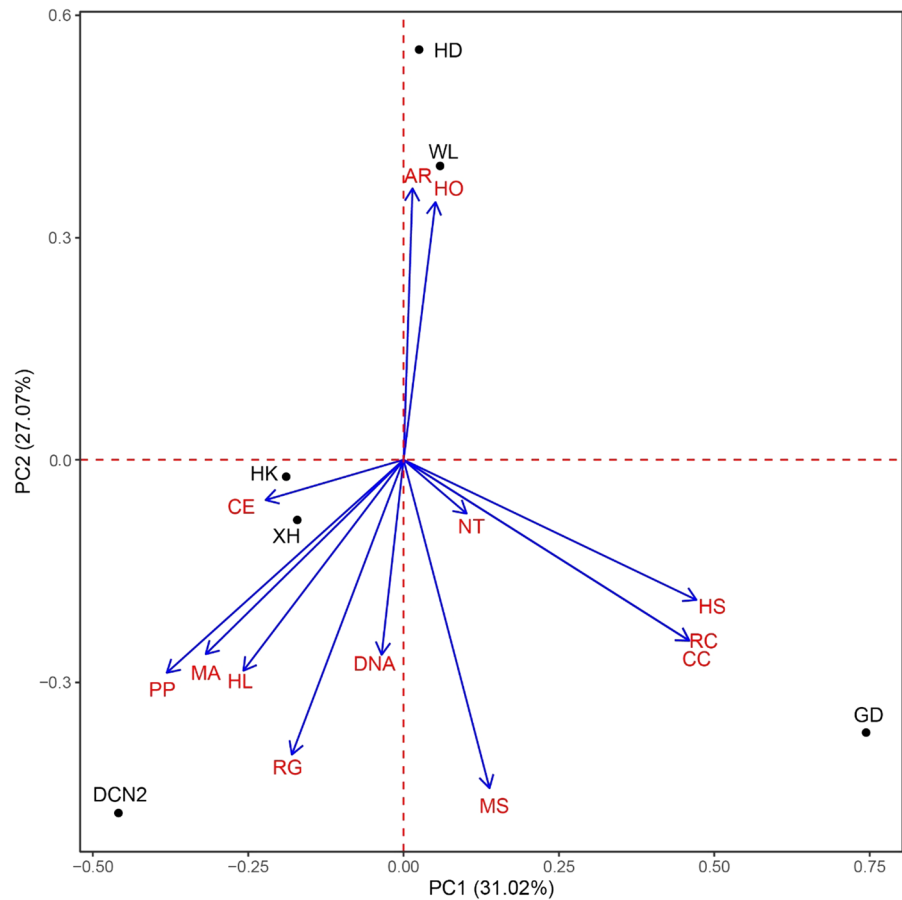
A binary logistic regression model of *S. grahami* DNA detection probability against environmental factors demonstrated that TP, DO and WT were all negatively correlated with detection success of

target DNA, though the model was non-significant ($P > 0.05$).

Discussion

Our study showed that non-native species dominated the fish community in Dianchi Lake (Table 2), with native species only accounting for <5% of the total abundance. Several non-native species with small body sizes and short life cycles were widely distributed throughout the lake as dominant species. These findings are consistent with previous fish surveys (Ye et al., 2015). While a total of 37 native fish species once occurred in Dianchi Lake, our study revealed the presence of only three, providing a snapshot on the apparent dramatic loss of native species (Yang et al., 2004; Luo et al., 2006). However, our results must be tempered by the fact

Fig. 3 Principal component analysis (PCA) on the DNA concentration of *Sinocyclocheilus grahami* (DNA) and other fish species across six sampling sites (black dots) in Dianchi Lake. Refer to Table 2 for abbreviations for environmental factors



that we sampled only once at a limited number of stations, using two collection techniques. Thus, our results based on netting and trapping may suffer from type II errors (i.e., species are present but are not detected) owing to relatively low sampling effort.

Water quality in Dianchi Lake has improved in recent years, especially regarding the input of treated sewage (He et al., 2020) and the construction of artificial wetlands around the lake (Niu et al., 2017). However, the large population size in Kunming (i.e., > 8 million) and the rapid development in this catchment still impose a significant impact on the lake (Yang et al., 2020). Recent studies have identified ongoing eutrophication and associated cyanobacteria blooms as serious problems (Zhao et al., 2021), while sediments remain an important source of pollutants (He et al., 2020; Qian et al., 2020). In addition, climate change has emerged as a new threat (Chen et al., 2020). These stressors, together with the introduction

of non-native fish, might have collectively contributed to the loss of endemic fish in Dianchi Lake.

According to the results of PCA analysis, endemic species were concentrated in the east-southeast section of the lake (Fig. 2). Individuals of the critically endangered species *S. grahami* were captured only at site GD. In addition, apart from the widely distributed and dominant species *N. taihuensis*, most non-native species had a higher density in the northeastern part of the lake where cyanobacteria blooms were more common (Zhao et al., 2021) (Fig. 2). Such discrepancies in spatial distribution of endemic and non-native fish species provide indirect evidence that harmful cyanobacterial blooms may affect native more than non-native fish species. In other words, degraded water quality may have imposed selective pressure on the fish community that favoured invaders over native species.

The native fish community (Table S1) of Dianchi Lake has been largely replaced by non-native and

commercial species (Table 3). Commercial species are very similar to those in other freshwater lakes in China (Zhao et al., 2016). This is a major component of biotic homogenization globally and Chinese systems in particular (Petsch, 2016; Kirk et al., 2020). We found only a small percentage of historically recorded species, suggesting loss of native diversity has likely been profound.

Results of the correlation analysis on abundance distribution showed that the only two species negatively correlated in distribution were *H. leucisculus* and *N. taihuensis* (Fig. S1). Previous work demonstrated that the distribution of the former species is strongly inhibited by the latter, especially with regard to reproduction due to resource competition and egg predation (Wang et al., 2013b).

The fish community in Dianchi Lake was almost entirely dominated by non-native species, with minor roles played by native species. DO content has played a role in limiting benthic fish such as *M. swinhonis* (Fig. S1). The distribution of *P. parva* and *M. anguillicaudatus* were negatively correlated with water temperature. In addition to the parameters measured in this study, disease (Minamoto et al., 2015), organic pollutants (Fan et al., 2017), and antibiotics (Wei et al., 2014) may also affect the distribution of fish in the lake. Habitat change followed by non-native species invasion was proposed as another main cause of fish community change in the lake (Wang et al., 2013a). At the same time, algal blooms associated with eutrophication and climate warming are also recurring problems (Ye et al., 2015; Zi et al., 2018).

Distribution of *Sinocyclocheilus grahami*

According to previous field surveys, the wild population of *S. grahami* disappeared from Dianchi Lake Basin more than 10 years ago (Chen & Yang, 2008). In our study, two individuals were caught at site GD using fish traps, but this location was restocked with artificially bred fry 2 months prior to our study. Thus, it was highly likely that the captured individuals were newly stocked to the lake. Furthermore, as eDNA is a more sensitive detection method than trapping, and *S. grahami* DNA was detected at 10 of the 16 sites, the species could have a much broader distribution. Target eDNA was mainly detected in the southern and western regions of the lake, as well as in the Panlong River (site PLJ) and its estuary

(site HD) (Fig. 1). This is likely a result of physical presence of target fish combined with DNA transported by advected water. Jerde (2019) suggested that eDNA results can be used as a basis for management and protection because it is able to provide signal of species presence much earlier than traditional methods. The capability of eDNA for early detection has demonstrated in many species, including fish (Jerde et al., 2019) and bivalves (e.g., Xia et al., 2023). On this basis, it is possible that *S. grahami* may be recovering at some sites with positive eDNA signals in the lake, though additional study is needed to validate our work with more surveys based on both eDNA and traditional methods.

eDNA detection of *S. grahami* was negatively correlated with two non-native fish (*A. rivularis* and *H. olidus*), both of which are omnivorous (Chen and Yang, 2008). It is unclear whether they compete with or prey on *S. grahami*. Likewise, a number of non-native goby species have been introduced to the lake, raising the possibility of reproduction failure in *S. grahami* associated with high predation on eggs, competition for resources or habitats cannot be excluded (Miano et al., 2019; Lutz et al., 2020).

The distribution of *S. grahami* may be affected by blooms of the cyanobacterium *Microcystis aeruginosa*, because it has a significant inhibitory effect on the heart and other organs of *S. grahami* (Zi et al., 2018). Algal blooms also affect embryonic development and can produce neuro-depression (Cai et al., 2022). The toxic *M. aeruginosa* is a dominant harmful cyanobacterium in Dianchi Lake, and mainly floats on the surface during the day (Ostrovsky et al., 2020). It might have accumulated in the central and northern parts of the lake as a result of southwest wind (Yu et al., 2020), inhibiting the growth of *S. grahami* (Zhang et al., 2020a). The distribution patterns of algae were consistent with the distribution of our eDNA signals, so it is possible that harmful cyanobacteria blooms could have contributed to the on-going impairment of *S. grahami* in Dianchi Lake. In addition, *S. grahami* is a cave fish that prefers lower water temperature and is sensitive to water quality (Yang et al., 2016). The northern and eastern lakeshores are densely populated, mostly urban areas, where human impacts are among the greatest (Zhang et al., 2020b); thus, it cannot be excluded that habitat changes also affected fish distribution in the lake.

False positives and false negatives

The primer design steps utilized in our study ensured high species-specificity and high sensitivity by carefully screening multiple candidates (Xia et al., 2018, 2021). In our study, false positives (type I error) could account for some or all of our eDNA hits in southern parts of the lake if the DNA was advected from areas where the fish do occur. Many of the rivers that enter Dianchi Lake are natural distribution areas of *S. grahami*, so it is possible that eDNA detected at some sites (e.g., HD) may have originated from these locations (Fig. 1).

It is possible that higher temperature or microbial activities in some parts of the lake may have elevated rates of DNA degradation, resulting in false negatives (Zulkefli et al., 2019). However, we sought to reduce DNA degradation by keeping samples in cool in the field and process them quickly in the lab. A third possible source of false negatives could relate to inhibition from presence of massive cyanobacteria blooms in certain parts of the lakes, which increased the difficulty of eDNA collection and extraction (Raemy and Ursenbacher, 2018). Our pre-filtration method reduced interference, thereby improving eDNA extraction rate and reducing the risk of this error (Turner et al., 2014). Although pre-filtration may cause eDNA loss, the method seemed to yield as much eDNA as possible while minimizing inhibitors (Hunter et al., 2019). Organic pollutants in Dianchi Lake may also inhibit PCR (Fan et al., 2017; Lance & Guan, 2020), though the dilution method used in our study appeared to effectively minimize these problems (Mckee et al., 2015).

Conclusions

We found that invasive and commercial species numerically dominate the fish community in Dianchi Lake, while native fish diversity and abundance were very low. One critically endangered species, *S. grahami*, was detected at very low abundance in the central southern part of the lake using traditional fish sampling, though eDNA signals indicated a much broader distribution. The eDNA distribution of *S. grahami* suggests an inverse occurrence versus that of harmful algal blooms in the lake. It is not clear whether eDNA detection patterns signal a recovering,

reproducing population or simply reflect recent stocking activities in the lake.

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Data availability The authors are willing to share the data supporting the findings of this study and can be contacted for data availability.

Declarations

Conflict of interest The authors declare that they have no conflict of interest related to this research.

Ethical approval The research involving human or animal subjects in this study complied with all relevant ethical guidelines and regulations.

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