

Marine Ecology Enhancement Fund (MEEF)

MEEF2024012A Completion Report

1. Funded Project Information

Funding Scheme:	Marine Ecology Enhancement Fund (MEEF)		
Project Number:	MEEF2024012A		
Project Title:	Exploring the mystery of Chinese Bahaba in Hong Kong western waters using revolutionary eDNA techniques		
Name of Organization:	Lingnan University		
Reporting Period:	From:	01/7/2025	To: 31/3/2026
Date of Report Submission:	30 June 2026		
Project Leader:	Dr. Chi-Ho IP, Assistant Professor		
Signature:			
Official Chop of Organization, Science Unit, Lingnan University:			

I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorization has been obtained in respect of information owned by third parties.

Any opinions, findings, conclusions or recommendations expressed in this report do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

(i) Executive Summary (1-2 pages);

Project Background and Objectives

The Chinese Bahaba (*Bahaba taipingensis*) is a critically endangered marine species and a First Class Protected species in China. Therefore, comprehensive and effective species-specific monitoring is imperative. Environmental DNA (eDNA) approaches are emerging as valuable tools for biodiversity monitoring and conservation; however, there has been a lack of eDNA-based detection methods specifically for the Chinese Bahaba.

In this project, we are developing target-specific quantitative PCR (qPCR) eDNA assays as a rapid and non-invasive method to uncover the hidden populations of the Chinese Bahaba in Hong Kong's western waters. By incorporating Phase 1—targeted qPCR assays (MEEF2024012) and Phase 2—eDNA metabarcoding approaches (MEEF2024012A), this study aims to provide essential baseline information to better understand the status of the Chinese Bahaba and to support future conservation strategies in western Hong Kong waters.

Key Activities and Progress

During the previous **Phase 1** period from November 1, 2024, to June 30, 2025, several key activities were successfully executed to advance the project. These included recruiting a Research Assistant, setting up the wet lab, developing and validating species-specific primers for the Chinese Bahaba, and conducting field monitoring from January to June 2025, along with qPCR analyses of all samples. During **Phase 2** (July 2025–March 2026), we successfully completed the final field monitoring and qPCR analyses in September 2025. We then performed eDNA metabarcoding of 552 eDNA samples [spanning Phases 1 and 2; 23 sites x (3 replicates/site + 1 control/site) x 6 surveys] and constructed 96 Illumina libraries (1,104 PCR products) using 12S-V5 and MiFish-U universal fish primers. The pooled PCR products were subsequently sent to Novogene (China) for Illumina sequencing on the NovaSeq X Plus platform with the paired-end 150-bp (PE150) mode. The raw Illumina sequencing data was then retrieved and analyzed using our bioinformatics pipeline for sequence data processing, including quality control, trimming, denoising, merging, and taxonomic annotation.

Highlights of key results

- Completed six rounds of eDNA sampling across 23 sites in western Hong Kong waters in 2025, generating one of the most comprehensive fish eDNA datasets for the region;
- Achieved the first successful field detection of Chinese Bahaba eDNA in Tai O and in/near three marine parks in Hong Kong waters (September 2025) using the developed species-specific qPCR assay, providing important baseline information on its occurrence;
- Generated approximately 304 Gb of Illumina sequencing data using dual-primer (12S-V5 and MiFish-U) eDNA metabarcoding, detecting 266 vertebrate taxa, including the Chinese

White Dolphin, the Finless Porpoise, and nine IUCN threatened fish species (six Vulnerable and three Endangered: *Anguilla japonica*, *Colia mystus*, and *Sillago parvisquamis*)

- Demonstrated significant spatial and seasonal variation in fish community composition, identifying distinct ecological assemblages among sampling locations and between wet and dry seasons
- Identified temperature, dissolved oxygen, salinity and pH as important environmental gradients influencing fish community structure, with Chinese Bahaba detections occurring under relatively warmer environmental conditions
- Successfully integrated species-specific qPCR detections with community-level eDNA metabarcoding, enabling investigation of the ecological context surrounding Chinese Bahaba occurrence
- Applied Weighted Gene Co-occurrence Network Analysis (WGCNA) to identify recurrent fish assemblages, revealing red and yellow modules as the communities most strongly associated with Chinese Bahaba detections
- Identified several potential indicator species and co-occurring fish assemblages associated with Chinese Bahaba, including *Ilisha melastoma*, *Acentrogobius pflaumii*, *Thryssa dussumieri*, *Bathygobius hongkongensis*, and *Ambassis buruensis*, which may serve as ecological indicators of habitats favorable to Chinese Bahaba

Overall Summary

This project successfully developed and applied an integrated eDNA monitoring framework (species-specific qPCR + dual-primer metabarcoding) for the critically endangered Chinese Bahaba in Hong Kong's western waters. The first confirmed field detections of Bahaba eDNA, alongside insights into associated fish communities, environmental drivers, and potential indicator species, provide essential baseline data for conservation. These outcomes establish reliable molecular tools and ecological context that will support future monitoring, habitat protection, and evidence-based management strategies for this protected species in Hong Kong and the Pearl River Estuary region.

(ii) Project title and brief description of the Project;

Title: Exploring the mystery of Chinese Bahaba in Hong Kong western waters using revolutionary eDNA techniques

Description of the Project

Chinese Bahaba (*Bahaba taipingensis*) is a critically endangered fish species, recognized as a Class I protected wild animal in China. Hong Kong western waters are one of the major estuarine habitats and spawning aggregations for Chinese Bahaba, unfortunately, their population has experienced a dramatic decline since the 1960s due to overfishing and pollution, leading them to nearly local extinction. To gain crucial insight into the current status and conservation of this threatened species, it is imperative to have comprehensive and species-specific monitoring. However, conventional surveys such as bottom trawling and gill netting are costly and destructive to the remaining wild population. In this project, we propose using the environmental DNA (eDNA) approach as a rapid and non-invasive method to uncover the hidden population of Chinese Bahaba in Hong Kong's western waters (**Figure 1**). Through intensive eDNA sampling in the western waters, we will utilize a targeted quantitative PCR (qPCR) to detect Chinese Bahaba eDNA and trace its potential distribution, seasonality, and habitat preferences of Chinese Bahaba. Furthermore, by incorporating eDNA metabarcoding approach, we will gain a comprehensive understanding of the overall biodiversity of the ecosystem, which will provide insights into the species composition, interactions, and potential threats within the habitats associated with the Chinese Bahaba. Overall, this study will provide essential baseline information to better understand the status of Chinese Bahaba and support future conservation strategies in western Hong Kong waters.

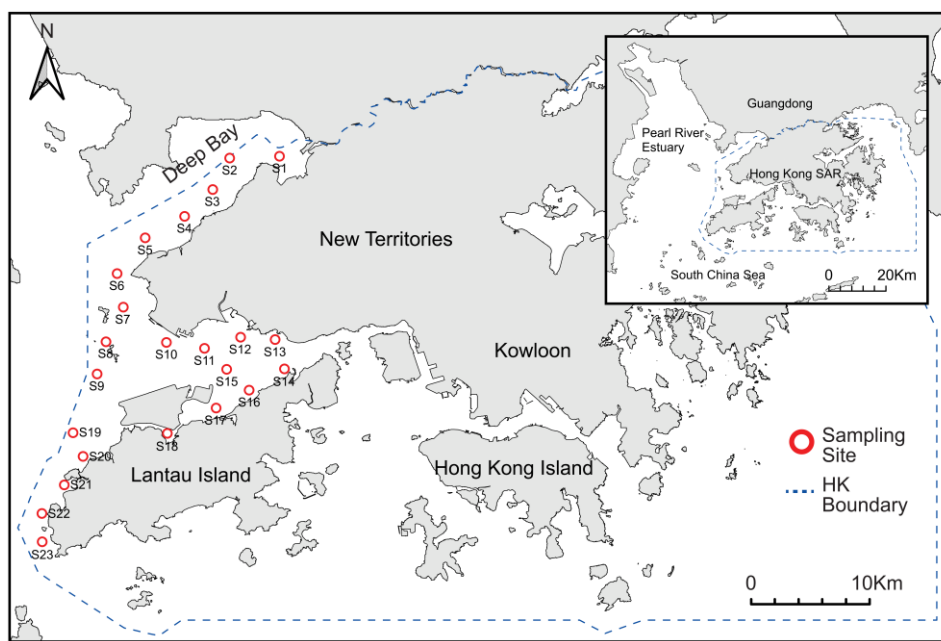


Figure 1. Site map for eDNA survey in western Hong Kong waters ($n=23$).

(iii) Completed activities against the proposed Work Schedule;

No.	Activity / Task for the Project	Date (Start & End)	Actual date (Start & End)	Progress	Details of the Activity
1	Task 1 – Water sampling and qPCR	Jul 2025 to Oct 2025	Jul 2025 to Oct 2025	Completed	Conducting the wet season field trip to collect water samples in western waters, completing eDNA experiments and qPCR analysis for Task 1.
2	Task 2-eDNA metabarcoding	Jul 2025 to Jan 2026	Jul 2025 to Mar 2026	Completed	Conducting eDNA metabarcoding and integrating the data of Chinese Bahaba to investigate spatial-temporal distribution and species interaction for Task 2.
3	Writing Phase 2 progress report	Nov 2025	Nov 2025	Completed	Submit progress report for phase 2 project period Jul 2025 to Nov 2025.
4	Writing Phase 2 completion report	Feb 2026 to Mar 2026	Mar 2026 to June 2026	Completed	Paper and completion report writing.

During the reporting period from July 2025 to March 2026, several key activities were successfully carried out to advance the project. The final field sampling was conducted in September 2025 (Round 6), marking the end of the wet season. All eDNA samples were extracted, PCR, gel electrophoresis, purification, and PCR product pooling was completed, pooled into 96 Illumina libraries and sent to Novogene (China) for Illumina sequencing. The resulting 304Gb of raw sequencing data was received in November 2025, the resulting data prepared and ran through our bioinformatics pipeline for analysis, the Phase 2 paper and final report writing shortly followed marking a significant step forward in our ongoing research efforts.

Summary of Key Outcomes

- Completed six rounds of seasonal eDNA surveys across western Hong Kong waters, generating one of the largest contemporary fish eDNA datasets for the region.
- Produce an assessment of fish biodiversity and environmental conditions in western Hong Kong waters, including the detection of threatened and conservation-important species.
- Identify ecological assemblages, environmental gradients, and potential indicator species associated with Chinese Bahaba occurrence
- Demonstrate the potential of integrated eDNA approaches as an effective, non-invasive tool for monitoring rare and threatened marine fishes, supporting future biodiversity conservation and fisheries management.

(iv) Results/ descriptions on the completed activities with appropriate analysis, with the support of photos, videos, social media platforms, etc., if any;

During the reporting period from July 1, 2025, to March 31, 2026, the following key activities were conducted:

- (July 2025 – October 2025): Final field sampling of Round 6 samples in September. Completion of all eDNA eDNA extractions, gel electrophoresis, qPCR analysis of Sep round, gel purification, and PCR product pooling which was then sent for Sequencing.
- (November 2025): Received raw Illumina sequencing data back from Novogene (China), and the beginning of bioinformatics and data analysis. Submitted progress report of phase 2 project period.
- (December 2025 – March 2026): Conducting eDNA metabarcoding and integrating the data of Chinese Bahaba to investigate spatial-temporal distribution and species interaction for Task 2. Paper and completion report writing for Phase 2.

Task 1. To conduct eDNA surveys and qPCR analysis to monitor Chinese Bahaba in western Hong Kong waters

Methodology

Primer development and *in silico* testing

Compared with nuclear DNA, mitochondrial DNA (mtDNA) is generally a more suitable molecular marker for eDNA assays due to its substantially higher cellular copy number (Rees et al., 2014). Based on previous eDNA studies involving fish taxa or marine environment (Baker et al., 2018; Tsuji et al., 2018; Zhang et al., 2020), we selected three mtDNA regions —12S-Ribosomal RNA (12S), NADH dehydrogenase 5 (ND5), and Control Region (D-loop) for species-specific primer and probe development. Complete mitochondrial genome sequences of *B. taipingensis* were retrieved from NCBI GenBank. Additionally, based on studies about phylogenetic analysis of *B. taipingensis* (Zhao et al., 2015), mitochondrial genomes from ten phylogenetically related species in family Sciaenidae (*Collichthys lucidus*, *C. niveatus*, *Larimichthys crocea*, *L. polyactis*, *Parapristipoma trilineatum*, *Dendrophysa russelii*, *Pennahia argentata*, *Miichthys miiuy*, *Nibea albiflora*, and *N. coibor*), all of which potentially overlap with *B. taipingensis* in geographic distribution, were obtained for comparison. All sequence data used for primer development of *B. taipingensis* are listed in [Table 1](#).

Table 1. Reference sequence information of Chinese Bahaba specific primer development.

Marker gene regions	Species	Accession number	Nucleotide differences from <i>Bahaba. taipingensis</i> NC_018347.1 (Nt)			Nucleotide differences from <i>Bahaba. taipingensis</i> NC_018347.1 (%)		
			Forward	Reverse	Probe	Forward	Reverse	Probe
12S	<i>Collichthys lucidus</i>	NC_014350. 1	2	5	0	8.0	20.8	0.0
	<i>Collichthys niveatus</i>	HM219223.1	3	5	0	12.0	20.8	0.0
	<i>Dendrophysa russelii</i>	NC_017606. 1	4	8	2	16.0	33.3	9.5
	<i>Larimichthys crocea</i>	NC_011710. 1	3	5	0	12.0	20.8	0.0
	<i>Larimichthys polyactis</i>	NC_013754. 1	3	5	0	12.0	20.8	0.0
	<i>Miichthys miiuy</i>	NC_014351. 1	3	5	2	12.0	20.8	9.5
	<i>Nibea albiflora</i>	NC_015205. 1	2	7	1	8.0	29.2	4.8
	<i>Nibea coibor</i>	NC_025307. 1	2	7	2	8.0	29.2	9.5
	<i>Parapristipoma trilineatum</i>	NC_009857. 1	10	10	0	40.0	41.7	0.0
	<i>Pennahia argentata</i>	NC_015202. 1	2	7	2	8.0	29.2	9.5
ND5	<i>Collichthys lucidus</i>	NC_014350. 1	5	5	5	20.8	20.8	25.0
	<i>Collichthys niveatus</i>	HM219223.1	4	4	4	16.7	16.7	20.0
	<i>Dendrophysa russelii</i>	NC_017606. 1	4	6	5	16.7	25.0	25.0
	<i>Larimichthys crocea</i>	NC_011710. 1	5	5	4	20.8	20.8	20.0
	<i>Larimichthys polyactis</i>	NC_013754. 1	4	4	4	16.7	16.7	20.0
	<i>Miichthys miiuy</i>	NC_014351. 1	3	4	2	12.5	16.7	10.0
	<i>Nibea albiflora</i>	NC_015205. 1	5	5	8	20.8	20.8	40.0
	<i>Nibea coibor</i>	NC_025307. 1	5	5	5	20.8	20.8	25.0
	<i>Parapristipoma trilineatum</i>	NC_009857. 1	5	4	10	20.8	16.7	50.0
	<i>Pennahia argentata</i>	NC_015202. 1	13	11	8	54.2	45.8	40.0
DLP	<i>Collichthys lucidus</i>	NC_014350. 1	15	17	19	62.5	70.8	70.4
	<i>Collichthys niveatus</i>	HM219223.1	14	18	15	58.3	75.0	55.6
	<i>Dendrophysa russelii</i>	NC_017606. 1	15	7	14	62.5	29.2	51.9
	<i>Larimichthys crocea</i>	NC_011710. 1	15	18	17	62.5	75.0	63.0
	<i>Larimichthys polyactis</i>	NC_013754. 1	14	18	16	58.3	75.0	59.3
	<i>Miichthys miiuy</i>	NC_014351. 1	6	3	10	25.0	12.5	37.0
	<i>Nibea albiflora</i>	NC_015205. 1	8	3	6	33.3	12.5	22.2
	<i>Nibea coibor</i>	NC_025307. 1	8	10	14	33.3	41.7	51.9
	<i>Parapristipoma trilineatum</i>	NC_009857. 1	11	17	14	45.8	70.8	51.9
	<i>Pennahia argentata</i>	NC_015202. 1	5	4	12	20.8	16.7	44.4

Multiple sequence alignments were conducted in Geneious Prime v2025.0.2 (Biomatters, New Zealand) to identify highly variable interspecific regions within each target locus. Primer and minor-groove binding (MGB) probe design, along with preliminary screening, were performed using Primer3.0 (Untergasser et al. 2012) under criteria specifying optimal melting temperature of 60 °C, GC content around 40-60 %, and minimal self-complementarity. The primer-probe sets for each target region were further in-silico-evaluated using NCBI Primer-BLAST to confirm amplification specificity and eliminate potential off-target matches (2 or less mismatches to the primer). Details of the three selected primer-probe sets are provided in [Table 2](#).

Table 2. Details of the new primer-probe sets targeting the mitochondrial genome of Chinese Bahaba.

Name	Sequence (5'–3')	Annealing Temperature (°C)	Amplicon size (bp)
Bhb12S_F752	TGTTGAAATACACGTCTGAAGGAGG	60	120
Bhb12S_R871	CTGAGTTCAATTAGTTCGGGGAGA		
Bhb12S_MGB797	TAGAGTGTCCCGCTGAAATTG		
BhbND5_F1571	TACTACCTCACCATTCTCCAACA	60	114
BhbND5_R1684	AATCGACTATTTGGGTGGCAATTG		
BhbND5_MGB1610	CAGCCATTATTCACCGCAGC		
BhbDLP_F85	TGTCACACATTCATACACCACCAT	60	120
BhbDLP_R204	CTTATGGACGGAAGTGTAGGAGC		
BhbDLP_MGB145	CCAACAATCCTTTATATAATTGCAGGC		

Specificity and sensitivity testing

We evaluated the specificity of the designed *B. taipingensis* primers by conducting PCR using *B. taipingensis* tissue DNA (fin clips) as positive control and six closely related sciaenid species: *L. crocea*, *C. lucidus*, *Johnius. belangerii*, *Pennahia. argentata*, *N. albiflora*, and *N. squamosa*. For *B. taipingensis*, DNA extraction and PCR analysis were conducted in co-author Dr. Wang's eDNA Lab at South China Normal University, using tissue collection from the Chinese Bahaba breeding farm in Huizhou, Guangdong province, China. Tissues of the other commercial species were obtained from Hong Kong's fish market. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions, and subsequently, PCR reactions were performed in a total volume of 25 µL, consisting of 1 µL each of forward and reverse primers (10 µM), 12.5 µL of Taq PCR Master Mix (Qiagen, Germany), 9.5 µL of nuclease-free water, and 1 µL of DNA template. The thermal cycling program included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 8 min. PCR products were visualized on 2 % agarose gel electrophoresis to identify the successful and specific amplicon of *B. taipingensis*.

We also evaluated primer performance using positive eDNA samples collected from two sources: (1) an aquaculture tank containing *B. taipingensis* and (2) environmental samples from survey sites spiked with *B. taipingensis* tissue DNA through a ten-fold serial dilution (10^{-1} to 10^{-4} ng/µL) within a natural eDNA background. Real-Time PCR (qPCR) was performed with on a

StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Reaction setup was conducted in a pre-PCR laboratory dedicated to preventing amplicon contamination. Each 20- μ L reaction consisted of 10 μ L TaqMan™ Environmental Master Mix 2.0 (Applied Biosystems, USA), 0.5 μ L of each forward and reverse primer (10 μ M), 0.5 μ L of probe (10 μ M), 6.5 μ L nuclease-free water, and 2 μ L of eDNA template. Thermal cycling conditions began with 50 °C for 2 min, 95 °C for 2 min, followed by 45 cycles of denaturation at 95 °C for 15 s and 60 °C for 1 min.

All positive PCR and qPCR products from above testing were purified and submitted to BGI Health (HK) Company Limited (Hong Kong, China) for Sanger sequencing. Resulting sequences were inspected in Geneious and compared against the NCBI GenBank database (Aug. 2025 version) using BLAST to confirm species identity, with *B. taipingensis* identified as the best match based on similarity > 98% and E-value threshold of 1e-30. The limit of detection (LOD) and limit of quantification (LOQ) for each primer pair were determined from standard curves generated from qPCR assays (consistent setting with positive eDNA testing qPCR) of a serial dilution of synthetic target DNA (Taihe Biotechnology, China), ranging from 2×10^1 to 2×10^7 copies per reaction. LOD and LOQ were calculated using curve-fitting modeling method in R script customized by (Klymus et al., 2020), with 95% detection probability for LOD and 35% Coefficient of Variation for LOQ; detailed criteria were described by Klymus et al. (2020); the quantification cycle (Cq) threshold was defined for each primer set based on the highest Cq value observed among positive reactions in the LOD/LOQ tests.

Field survey, eDNA Extraction and qPCR analysis

To investigate the migration of *B. taipingensis* across Hong Kong waters, we conducted six surveys during the migration season (March–June) and additional surveys before (January) and after (September) the migration period in 2025. Water samples were collected from 23 evenly distributed sites in the western Hong Kong waters, covering Mai Po Nature Reserve and three marine parks: Sha Chau and Lung Kwu Chau Marine Park, The Brothers Marine Park, and Southwest Lantau Marine Park (**Figure 2**). At each site, three 1-L surface seawater replicates and one 1-L negative control (sterile distilled water) were collected. Each seawater replicates were collected twice (500 mL per collection) using plastic water sampler, transferred into sterile sampling bags, and immediately stored on ice until filtration. **A total of 552 water samples were collected in this study (23 sites x (3 replicates/site + 1 control/site) x 6 surveys).** All sampling equipment was sterilized before each survey by soaking in 1% bleach for 15 min, rinsing with sterile distilled water, and placing under UV light for 30 min; water sampler was sprayed with 1% bleach and cleaned with distilled water between sites as well during each survey. Following transportation to the laboratory, each sample was filtered through 47-mm mixed cellulose ester (MCE) filter with a pore size of 0.45 μ m (Millipore, USA) inside a dedicated UV-sterilized eDNA hood within 6 hours. Filters were transferred to sterile Falcon petri dishes and immediately stored at -80 °C until DNA extraction.

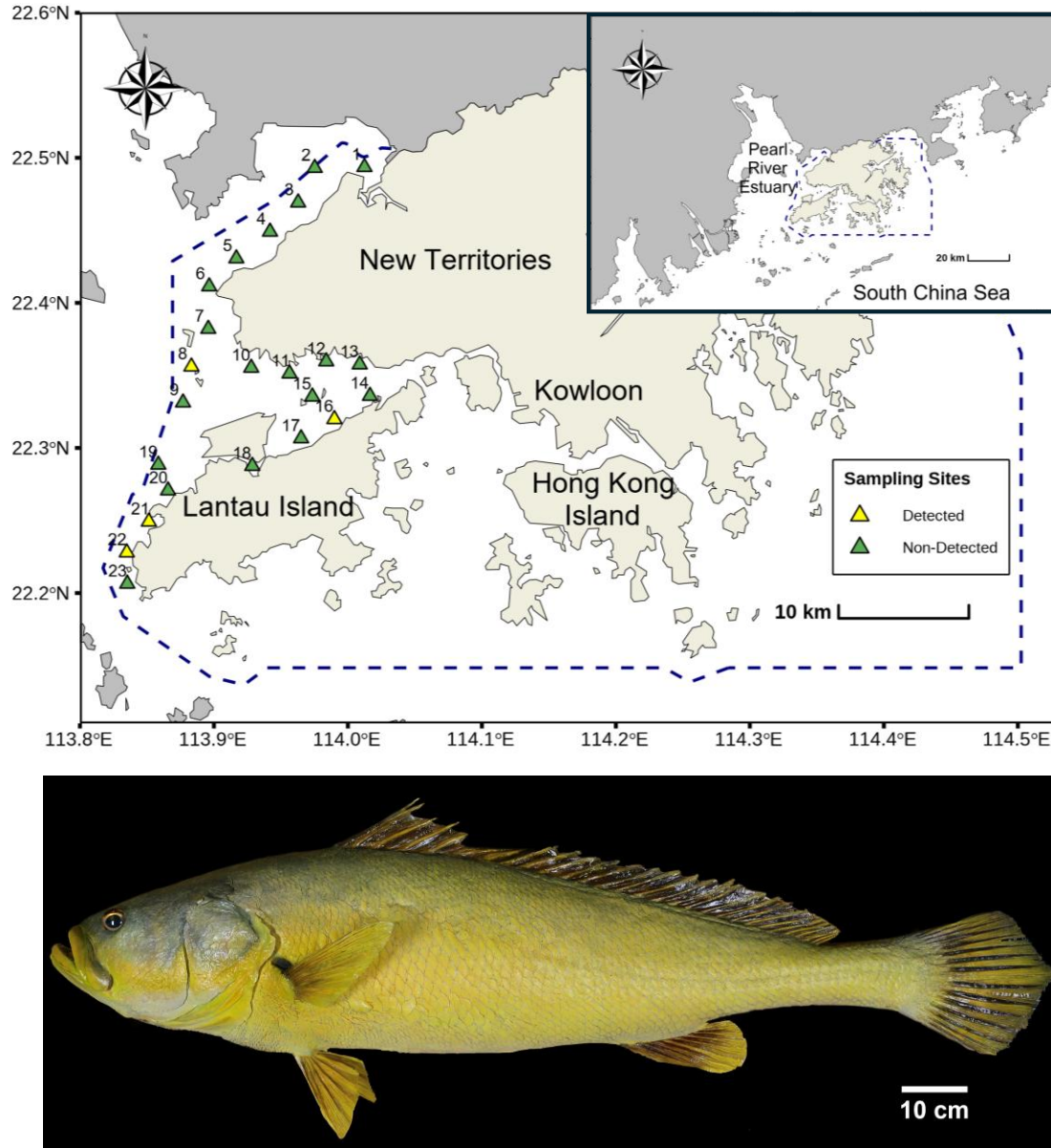


Figure 2. Map of western Hong Kong showing the 23 sampling sites used in this study (green, upper panel). Four sites yielded Chinese bahaba eDNA detections (yellow): Sha Chau and Lung Kwu Chau Marine Park (S8), The Brothers Marine Park (S16), Tai O (S21), and Southwest Lantau Marine Park (S22). Lower panel: photograph of an adult Chinese bahaba specimen (total length 152 cm) from the Bahaba Nature Reserve, Dongguan.

eDNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's protocol and the extracted DNA quantity was examined using Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). Apart from site blank controls, each run included two blanks (nuclease-free water) as negative controls and two reactions containing *B. taipingensis*

tissue DNA as positive controls. A site was considered positive if at least one replicate showed a clear amplification curve and a Cq value below the predefined threshold. For positive reactions, only quantitative results calculated by a standard curve with $R^2 > 0.98$ and an amplification efficiency of 90–110% were accepted. Positive qPCR products were purified and submitted for Sanger sequencing, and the resulting sequences were identified using BLAST against the GenBank database.

Results and discussion

Primer specificity and sensitivity

Three selected primer-probe sets (namely: Bhb_12S, Bhb_ND5 and Bhb_DLP) demonstrated high specificity to *B. taipingensis*. Both in-silico and in-vitro tests confirmed the specificity of primers, with no off-target amplification detected in Primer-BLAST and testing PCR results (Figure 3). All three primers also exhibited high sensitivity, as confirmed by qPCR results from positive eDNA samples. The LOD at 95% confidence was determined to be 701.65 copies per reaction for Bhb_12S and Bhb_ND5, and 83.69 copies per reaction for Bhb_DLP. The LOQ was similarly established at 701.65 copies per reaction for Bhb_12S and Bhb_ND5, and 153 copies per reaction for Bhb_DLP. Based on the testing qPCR results, the detection thresholds (Cq) were set at 41.96 for Bhb_12S, 40.38 for Bhb_ND5, and 42.06 for Bhb_DLP, ensuring reliable detection of *B. taipingensis* eDNA. Although all primer pairs showed good performance, Bhb_12S was selected for field screening due to its superior performance in LOD/LOQ tests for trace eDNA (<100 copies per reaction) and a lower likelihood of generating by-products (Figure 4; Table 3)

Table 3. LOQ & LOQ test results for Chinese Bahaba qPCR assay. CT = Cycle Threshold

Primer Pair	synDNA quantity (copies)	Detect probability (%)	CT Mean (n = 12)	CT SD (n = 12)
Bhb12S	10 ¹	75	38.65	1.71
	10 ²	100	33.97	0.25
	10 ³	100	30.46	0.17
	10 ⁴	100	26.29	0.49
	10 ⁵	100	23.04	0.21
	10 ⁶	100	19.42	0.13
	10 ⁷	100	15.87	0.14
BhbND5	10 ¹	75	39.11	0.73
	10 ²	100	35.33	0.41
	10 ³	100	31.66	0.14
	10 ⁴	100	27.75	0.35
	10 ⁵	100	24.52	0.15
	10 ⁶	100	20.86	0.15
	10 ⁷	100	17.17	0.15
BhbDLP	10 ¹	67	36.83	2.34
	10 ²	100	33.36	0.42
	10 ³	100	28.88	0.36
	10 ⁴	100	25.53	0.32
	10 ⁵	100	21.96	0.28
	10 ⁶	100	18.34	0.24
	10 ⁷	100	14.61	0.27

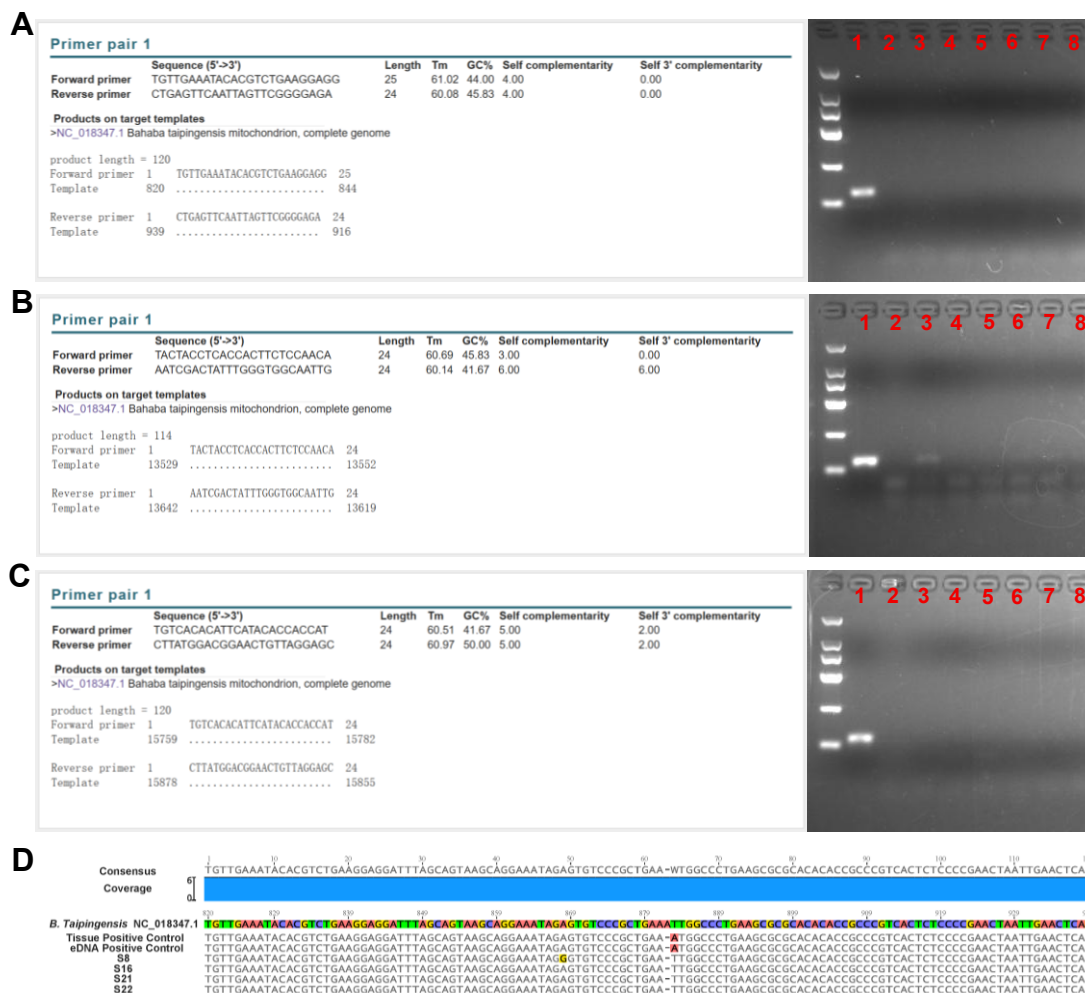


Figure 3. *In silico* and *in vitro* specificity testing of the three species-specific qPCR assays for *Bahaba taipingensis*. *In-silico* and *in-vitro* test result of three selected primers. (A) Bbh12S; (B) BbhND5; (C) BbhDLP. Template used for each *in-vitro* PCR test (from #1 to #8 in gel electrophoresis): *B. taipingensis* tissue, Nuclease-free water, *Larimichthys crocea* tissue, *Collichthys lucidus* tissue, *Johnius belangerii* tissue, *Pennahia argentata* tissue, *Nibea albiflora* tissue, and *N. squamosa* tissue. (D) Multiple alignment result of positive controls and positive samples (S8, S16, S21, S22 during Sep 2026 survey) with reference sequence of *B. taipingensis* from NCBI (Accession number: NC_018347.1).

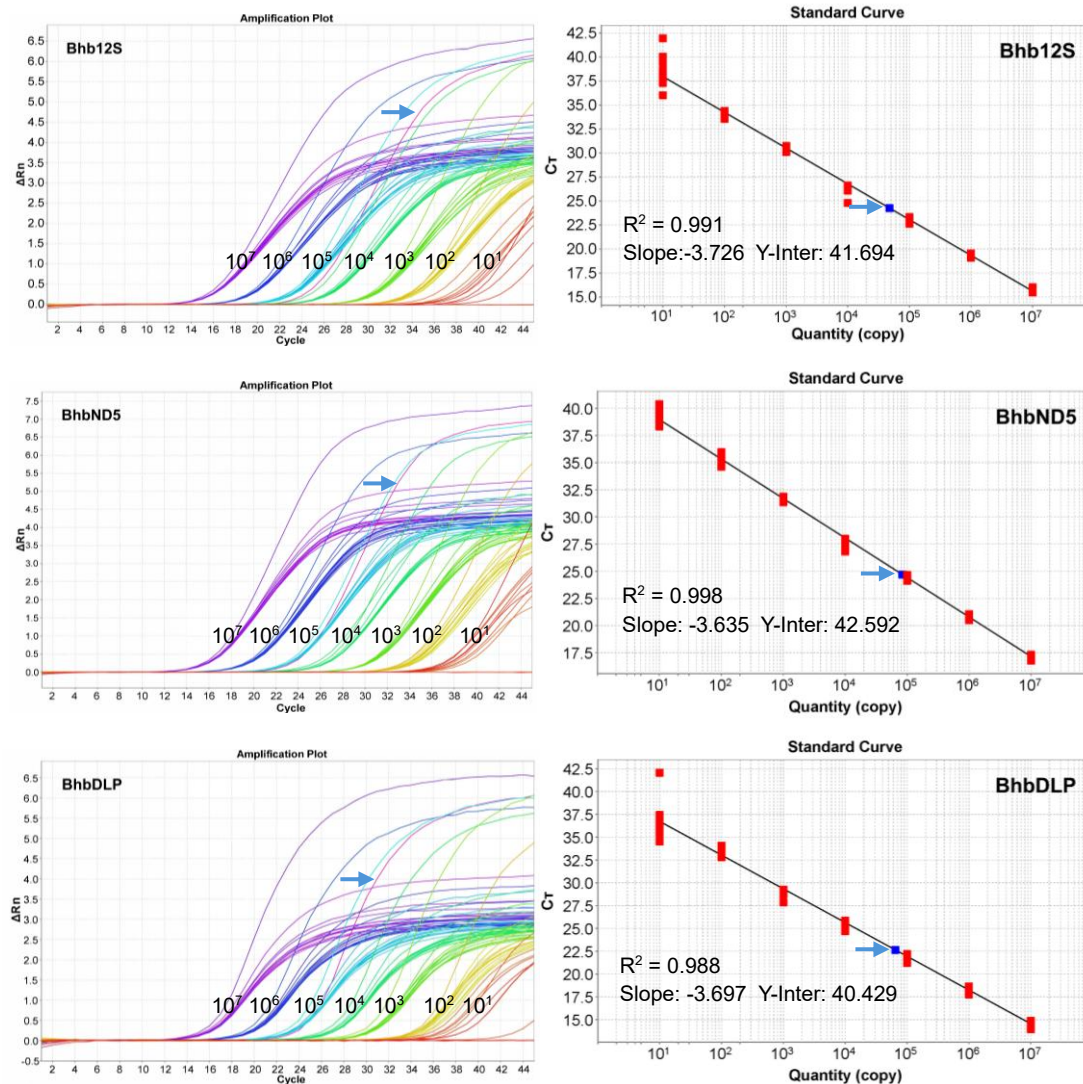


Figure 4. Limit of detection & limit of quantification test results of Three developed primer pairs. Left: amplification plot of the synthetic DNA serial dilution, ranging from 10^7 to 10^1 copies per reaction; right: standard curve of each test, with the generated linear regression; blue arrows indicate result of qPCR positive control.

Detection of Chinese Bahaba eDNA in study area

To investigate whether *B. taipingensis* migrates across Hong Kong waters, we collected 414 seawater samples from 23 sites with control waters across western Hong Kong waters in 2025. During the migration period in 2025, *B. taipingensis* eDNA was not detected; however, interestingly, positive signals were identified at four out of the 23 sites (S8, S16, S21, and S22; **Figures 2 & 4**) during the September 2025 survey. **The number of positive qPCR replicates is 5 replicates (Site 8 with 1/3 reps, Site 16 with 1/3 reps, Site 21 with 2/3 reps, Site 22 with 1/3 reps) with the C_q thresholds of 41.96 (based on LOD test; Table 4).** The concentration of *B. taipingensis* eDNA in positive reactions ranged from 0.71 to 2.85 copies, with an average of 2.05

copies (**Table 4 & Figure 5**), with no amplification in the corresponding site blank and qPCR negative controls. **Apart from passing the C_q threshold and showing negative results in the field blanks, we also performed gel recovery and Sanger sequencing of the positive samples to further confirm the species identity of the qPCR products. All S8, S16, S21, and S22 sequences matched *B. taipingensis*, indicating the presence of eDNA signals at these sites (Figure 3D).**

Table 4. Summary of qPCR results for positive field samples, detailing the number of replicates, average CT values, and average copy numbers.

Sample	No. Positive Reps	CT value	No. of average copy
R6-S8	1/3	39.94	2.04
R6-S16	1/3	39.99	1.38
R6-S21	2/3	38.93 (average)	2.79 (average)
R6-S22	1/3	40.97	0.72

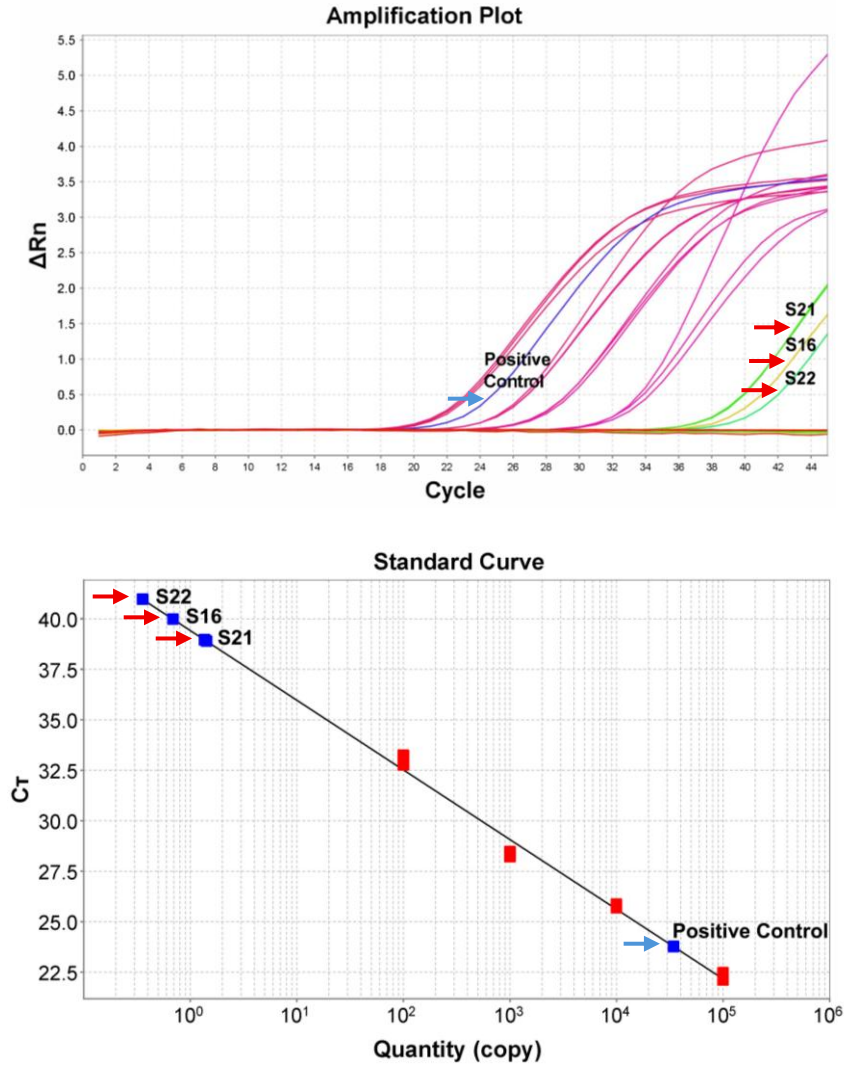


Figure 5. qPCR results showing positive sample signals from the Round 6 (September 2025) survey (indicated by red arrows). Blue arrows denote the qPCR positive control.

The development of species-specific eDNA assays represents an important advancement for monitoring critically endangered species such as the Chinese Bahaba, particularly when conventional survey methods are ineffective because of the species' rarity and cryptic behaviour. Here, we present the first validated qPCR assays targeting the mitochondrial 12S, ND5, and D-loop regions of *B. taipingensis*, filling a critical methodological gap in the conservation toolkit for this IUCN Critically Endangered fish.

Developing species-specific primers requires careful selection of target loci and reference sequences from phylogenetically related species to maximize specificity and minimize off-target amplification (Tsuji et al., 2017; Hong et al., 2025; Osathanunkul & Suwannapoom, 2025). By comparing mitochondrial sequences from *B. taipingensis* and ten co-occurring Sciaenidae species, we identified primer-probe sets with no significant matches to non-target taxa. Validation using

both tissue-derived DNA and positive eDNA samples confirmed high specificity and sensitivity, while tests against closely related Sciaenids showed no cross-amplification. These assays satisfy level 4 of the eDNA validation framework proposed by Thalinger et al. (2021), incorporating in silico, in vitro, and preliminary field testing.

Application of the Bhb_12S assay resulted in the first successful detection of *B. taipingensis* eDNA from natural seawater samples in Hong Kong. Positive detections were recorded at four sites in western Hong Kong waters during September 2025, shortly after the release of approximately 1,300 individuals into the Pearl River Estuary in August 2025 (China Report 2025). Tai O (S21), a historically important fishing ground for the species (Sadovy & Cheung, 2003), exhibited the highest detection frequency and lowest Cq values. Although all detections occurred at very low concentrations (0.71–2.85 copies per reaction), low-copy detections are common when monitoring rare species and should not be dismissed, as they may represent genuine biological signals (Kralik & Ricchi, 2017; Klymus et al., 2020). The absence of amplification in negative controls and confirmation of amplicon identity through Sanger sequencing strongly supports the authenticity of these detections.

Given the severe population decline of *B. taipingensis* due to overfishing and habitat degradation, these detections provide novel evidence of potential habitat use in Hong Kong waters and have direct implications for conservation planning, including marine protected area designation and evaluation of restocking programs (Robinson et al., 2019; Hong et al., 2025). Species-specific eDNA monitoring has similarly proven effective for assessing restocking and conservation outcomes in other endangered species (Osathanunkul & Suwannapoom, 2023).

The extreme rarity of *B. taipingensis* makes conventional fisheries-dependent surveys largely impractical. In contrast, eDNA monitoring provides a highly sensitive, non-invasive alternative capable of generating spatially extensive distribution data with relatively limited effort. Repeated eDNA surveys also offer a practical framework for long-term monitoring of population persistence, responses to conservation interventions, and temporal shifts in distribution. Similar approaches have proven valuable for monitoring other threatened aquatic taxa, including cetaceans in the Pearl River Estuary and Yangtze sturgeon (Yu et al., 2021; Ushio et al., 2025).

Despite its advantages, eDNA has inherent limitations. Detection probabilities are influenced by DNA shedding, degradation, and transport processes, and low-copy detections remain vulnerable to false negatives (Harrison et al., 2019; Ficetola et al., 2016; Sahu et al., 2025). Consequently, multi-replicate sampling and integration with complementary datasets remain essential. Future studies should quantify detection probabilities at known occupied and unoccupied sites to achieve full level 5 operational validation (Thalinger et al., 2021).

Overall, this study provides the first validated species-specific qPCR assays for detecting *B. taipingensis* eDNA and demonstrates their applicability under natural field conditions. Integration of these assays with broader eDNA metabarcoding approaches could substantially improve biodiversity monitoring in data-limited coastal systems such as the Pearl River Estuary and enhance our understanding of habitat use and species associations to better inform future conservation management (Zou et al., 2020; Ip et al., 2024; Chen et al., 2025).

Task 2. To incorporate eDNA metabarcoding to investigate the whole community associated with the Chinese Bahaba Methods

Methodology

eDNA Metabarcoding Preparation and Illumina Sequencing

We adopted multiple primer sets for species detection, 12S-V5 (Riaz et al., 2011) and MiFish-U (Miya et al., 2015) primer assays for the mitochondrial 12S RNA gene were used for marine vertebrate detection. The forward and reverse primers were indexed with a unique 8-bp index sequence allowing the cost-effective sequence of multiple amplicons in the same library. PCR amplification was carried out in 25µl reactions using 1µl of template eDNA, 12.5µl of KOD One PCR master mix (Toyobo, Japan), 1µl of each forward and reverse primers, and 9.5µl of nuclease-free water. The thermal cycling conditions that were used is as follows: an initial denaturing step of 98°C for 1min, followed by 40 cycles of denaturation at 98°C for 10s, annealing at T_m (60°C for 12S-V5, 59°C for MiFish-U) for 5s, extension at 68°C for 5s, and then a final extension at 68°C for 5min. The quality of the PCR product was checked using a 2% agarose gel and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, USA). DNA concentration of the PCR product was normalized, pooled, and submitted to Novogene (China) for quality control, library preparation with the DNA PCR-Free Library Prep kit (Illumina, USA), and sequencing on the Illumina NovaSeq X Plus platform with the paired-end 150-bp (PE150) mode.

Bioinformatics and Taxonomic Annotation

A total 304Gb of raw Illumina sequencing data was obtained (155Gb from 12S-V5, and 149Gb from MiFish-U) and analyzed using our bioinformatics pipeline using the QIIME2 2024.10.1 pipeline (Bolyen et al., 2019). Demultiplexing of the raw reads was done followed by the primer removal from the forward and reverse reads using q2-cutadapt. Further quality control was done on the trimmed reads by denoising the paired-end reads, merging, and removing chimeric sequences, and identifying the Amplicon Sequence Variants (ASVs) using the q2-dada2 and the unoise3 algorithm from the Usearch pipeline. The sequences were compared against the GenBank database using BLASTn 2.16.0 with “perc_identity” of 90% and “evalue” of 1e-30. The resulting ASVs with <10 reads were discarded to account for false positive reads, and the ASV reads present in our negative controls were addressed using the subtraction method from (He et al., 2023).

Finally, the taxonomic occurrence of each taxa was checked against FishBase (<https://www.fishbase.se>), SeaLifeBase (<https://www.sealifebase.ca>), the Hong Kong Biodiversity Information Hub (<https://bih.gov.hk>), and the Hong Kong Register of Marine Species (<https://www.marinespecies.org/hkrms/>). The conservation status of detected species was evaluated using the IUCN Red List (<https://www.iucnredlist.org>).

To investigate the ecological associations of Chinese Bahaba, species-specific qPCR detections were integrated with fish community data from eDNA metabarcoding. Chinese Bahaba detections obtained from qPCR were incorporated as an additional species within the presence/absence community matrix where applicable, enabling environmental ordination and co-occurrence network analyses to examine the environmental conditions and fish assemblages associated with Bahaba occurrence.

Statistics and Diversity Analysis

Diversity and multivariate analyses were conducted using the R package *vegan* and the alpha diversity was calculated using observed species richness and the Shannon index (H') from presence-absence matrices via the “diversity” function. The normality and homogeneity of the Shannon diversity values were assessed for all sites using the Shapiro-Wilk test ($\alpha = 0.05$) and the Levene’s test to determine the appropriate use of parametric/non-parametric tests. Effects of sampling round and sites on alpha diversity were evaluated using the Kruskal Wallis test followed by post hoc Dunn’s test for pairwise comparisons. We analyzed the spatial-temporal differences in species composition among our sites, habitats, and sampling round (seasonality) using a Principal coordinate analysis (PCoA) based on the presence/absence Jaccard coefficient and Analysis of Similarities (ANOSIM) with the “anosim” function using presence/absence data. Pairwise community dissimilarities among samples were calculated using the Jaccard dissimilarity index and hierarchical clustering was subsequently performed using Ward’s minimum variance method to identify groups of samples with similar species compositions. To visualize patterns of community similarity, a two-dimensional non-metric multidimensional scaling (NMDS) ordination was performed using the same Jaccard distance matrix. NMDS was implemented using the “metaMDS” function in the *vegan* package. The optimal number of ecological clusters was determined through silhouette analysis, where the average silhouette widths were calculated across a range of candidate cluster numbers. The cluster number producing the highest average silhouette width was selected as the optimal partition of the data. Similarity of percentages (SIMPER) analysis was conducted to determine which species are driving the differences in fish assemblages within each sampling site/zone and round. Finally, to determine the optimal number of biological replicates per sample for vertebrate eDNA detection, species accumulation curves were constructed using the *iNEXT* package based on the species presence/absence detection from both 12S-V5 and MiFishU assays.

To identify environmental and biological associations of Chinese Bahaba, presence–absence matrices from both metabarcoding assays were merged into a single community matrix, and replicate-level qPCR detections of Chinese Bahaba were incorporated as an additional species occurrence record. Environmental variables were filtered for multicollinearity using variance inflation factor (VIF) analysis, and highly correlated variables ($VIF > 10$) were iteratively removed prior to ordination using the “vif” function from the *car* package. Redundancy analysis (RDA) was performed using Hellinger-transformed community data and the retained environmental variables. Site scores from the first two constrained axes were extracted and used to visualize environmentally structured patterns in fish community composition. To examine patterns of fish diversity across environmental conditions, species richness values were merged with sample-level environmental measurements, including water temperature, dissolved oxygen, conductivity, and pH. Relationships between species richness and environmental gradients were visualized using scatterplots in *ggplot2*, to assess whether Bahaba-positive samples occupied distinct regions of environmental space. A locally weighted scatterplot smoothing (LOESS) regression was fitted to the data, with 95% confidence intervals displayed as shaded regions to highlight potential non-linear relationships between environmental variables and species richness.

Weighted Gene Co-occurrence Network Analysis (WGCNA) was adapted to identify groups of co-occurring fish species and investigate their relationships with Chinese Bahaba detections and environmental gradients using the *WGCNA* package. Read count data generated from the 12S-V5 metabarcoding assay were Hellinger transformed using the “decostand” function in the *vegan* package prior to network analysis. Hierarchical clustering based on Euclidean distances was performed to identify potential sample outliers. The scale-free topology criterion was then assessed using the “pickSoftThreshold” function across candidate soft-thresholding powers ranging from 1 to 20. A soft-thresholding power of $\beta = 2$ was selected because it provided the highest scale-free topology fit ($R^2 = 0.72$) while maintaining adequate network connectivity. Species co-occurrence modules were identified using the “blockwiseModules” function. Network construction used a signed adjacency network with an unsigned topological overlap matrix (TOM). The modules were detected using dynamic tree cutting with a minimum module size of five species, a merge cut height of 0.30, and a “deepSplit” parameter of four. Each module was assigned a unique color identifier representing groups of species that exhibited similar occurrence patterns across sampling locations and time periods. Module eigengenes, representing the first principal component of each module, were calculated and correlated with environmental variables, including temperature, dissolved oxygen, salinity, pH, sampling round, sampling site, Round 6 occurrence, and Chinese Bahaba detections. Pearson correlation coefficients and associated p-values were visualized using module-trait heatmaps. Species importance within modules was then evaluated using intramodular connectivity (kWithin), module membership (kME), and species significance for Chinese Bahaba detections. A driver score was calculated as the product of kWithin and species significance, enabling identification of highly connected taxa whose occurrence patterns most closely tracked Chinese Bahaba detections. Correlation networks were exported to Cytoscape for visualization of module structure and putative ecological assemblages.

Results and Discussion

A total of 96 Illumina libraries from two primer assays (total of 1,104 PCR products) yielded 1.38 billion clean reads (12S-V5: 1,265,350,826; MiFish-U: 114,696,116) after quality filtering and demultiplexing. Mean read counts per replicate were 1.91 ± 0.103 million SEM (12S-V5) and 0.207 ± 0.023 million SEM. After denoising, 14,636 ASVs remained for 12S-V5, and 1,268 ASVs for MiFish-U. Taxonomic annotation further assigned 11,812 12S-V5 and 1,188 MiFish-U ASVs to marine vertebrates. To evaluate whether sequencing depth was sufficient to capture the full diversity of each target community, rarefaction curves were generated for observed ASVs as a function of sequencing depth (Figure 6). For the 12S-V5 assay, the rarefaction curve approached asymptotes across all samples at ~5 million reads per replicate, respectively.

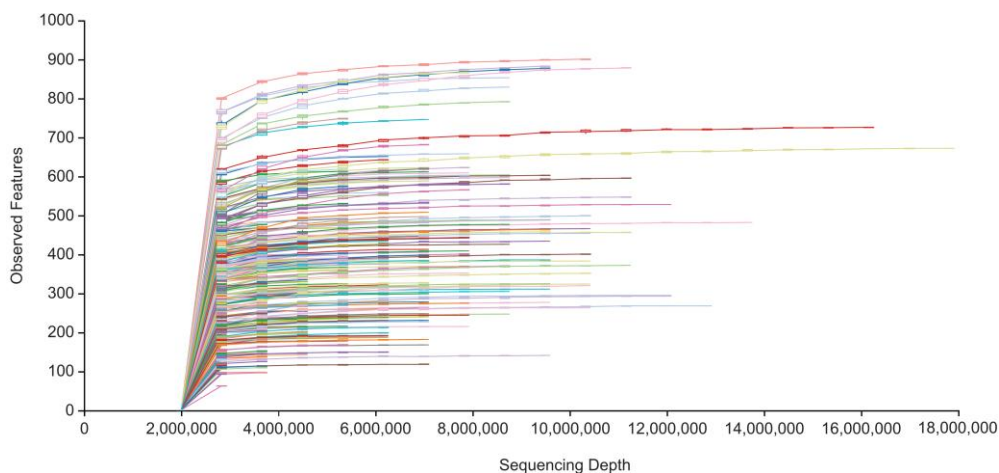


Figure 6. Alpha rarefaction of minimum and maximum sequencing depth between samples generated by the Qiime2 pipeline and visualized using the online Qiime2View website.

Fish community composition reveals high biodiversity and strong spatial-temporal turnover

A total of 266 taxa were detected from the eDNA metabarcoding survey, of which, 44 fish were identified to the family level, 55 taxa to the genus level (54 fish and one elasmobranch), and 167 to the species level, comprising of 167 vertebrate fish, two marine mammals (Chinese White Dolphin *Sousa chinensis* and Finless Porpoise *Neophocaena phocaenoides*) and one elasmobranch (*Telatrygon zugei*) (Table 5). Overall, 9 species detected were listed under the IUCN Red List of Threatened species (6 Vulnerable and 3 Endangered: *Anguilla japonica*, *Colia mystus*, and *Sillago parvisquamis*). Species rarefaction based on replicate-level sampling effort indicates a relatively low replication size can capture a majority of the local fish eDNA community. Two 1-L replicates were able to capture 33 species (90% coverage) and 35 species captured at three replicates (95% coverage) (Figure 7).

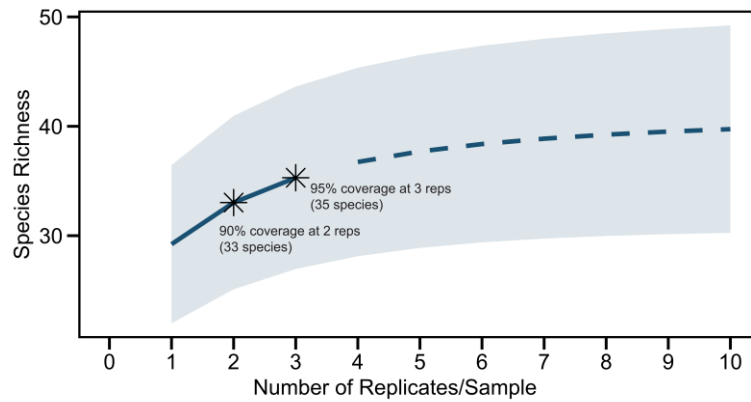


Figure 7. Optimization of eDNA replicate effort for vertebrate detection. Species accumulation analysis shows that 90% and 95% of vertebrate diversity is captured within two and three replicates. The curve illustrates the proportion of total observed species detected as a function of increased sampling replicates per water sample. Solid lines represent interpolated (observed) species richness while dotted lines indicate extrapolated species richness, the shaded area represents the standard deviation of species captured between replicates across all sites.

Table 5. Presence/absence table of eDNA-detected species in each sampling round and site.

Taxa	Species	Region	Environment	IUC N	Round						Site																						
					1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Elasmobranchi i	<i>Telatrygon zugei</i>	HK/SC S	Demersal	VU	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	x	✓	x	x	x	x	x	x	x	x	
Marine Mammal	<i>Sousa chinensis</i>	HK	Pelagic	VU	✓	✓	✓	✓	✓	✓	x	x	x	x	x	x	✓	✓	✓	x	x	x	✓	✓	✓	x	x	x	✓	✓	✓	✓	
Marine Mammal	<i>Neophocaena phocaenoides</i>	HK	Pelagic	VU	✓	x	x	✓	x	x	✓	✓	✓	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	✓	✓	✓	✓	✓	
Neopterygii	<i>Tylosurus crocodilus</i>	HK/SC S	Reef-associated		x	x	✓	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓		
Neopterygii	<i>Arius manillensis</i>	SCS	Benthopelagic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Plotosus lineatus</i>	HK/SC S	Reef-associated		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Coptodon zillii</i>	HK	Benthopelagic		✓	✓	x	x	x	x	✓	✓	✓	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Neopterygii	<i>Oreochromis aureus</i>	HK	Benthopelagic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	✓	✓	✓	x	x	x	x	x	x	x	x	x	✓	✓	
Neopterygii	<i>Oreochromis niloticus</i>	HK	Benthopelagic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	✓	x	x	x	x	x	x	x	✓	x	x	✓	✓	x	
Neopterygii	<i>Carassius auratus</i>	SCS	Benthopelagic		✓	x	✓	x	x	x	✓	✓	✓	x	x	x	x	✓	✓	✓	x	✓	x	x	x	x	x	x	x	x	x	x	
Neopterygii	<i>Hypophthalmichthys molitrix</i>	SCS	Benthopelagic		✓	x	✓	✓	✓	x	✓	✓	✓	✓	✓	x	x	x	x	x	x	x	✓	✓	x	x	x	x	x	x	x	x	
Neopterygii	<i>Lateolabrax japonicus</i>	HK/SC S	Reef-associated		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Entomacrodus stellifer</i>	HK	Reef-associated		x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	
Neopterygii	<i>Omobranchus fasciolatoceps</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓	x	x	x	✓	✓	✓	✓	✓	x	✓	✓	✓	
Neopterygii	<i>Omobranchus punctatus</i>	HK/SC S	Benthopelagic		✓	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Parablennius yatabei</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	x	✓	x	x	✓	✓	x	x	x	✓	✓	✓		
Neopterygii	<i>Scartella emarginata</i>	SCS	Demersal		✓	x	✓	x	✓	✓	x	✓	x	x	x	x	x	x	x	x	x	✓	x	✓	x	x	x	x	x	x	✓	✓	
Neopterygii	<i>Enneapterygius etheostoma</i>	HK/SC S	Demersal		✓	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x	x	✓	x	x	x	x	x	
Neopterygii	<i>Alepes vari</i>	HK/SC S	Pelagic-neritic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Caranx ignobilis</i>	HK/SC S	Reef-associated		✓	x	x	x	x	x	✓	x	x	x	✓	✓	✓	✓	✓	✓	x	x	x	✓	✓	✓	x	x	x	x	x	x	
Neopterygii	<i>Decapterus maruadsi</i>	HK/SC S	Reef-associated		x	✓	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	✓	x	✓	✓	✓	✓	
Neopterygii	<i>Megalaspis cordyla</i>	HK/SC S	Reef-associated		✓	x	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	
Neopterygii	<i>Pelates quadrilineatus</i>	HK/SC S	Reef-associated		x	x	x	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	x	x	x	x	x	
Neopterygii	<i>Rhynchopelates oxyrhynchus</i>	HK/SC S	Benthopelagic		✓	x	✓	x	x	x	x	x	x	✓	x	✓	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	x	x	
Neopterygii	<i>Terapon jarbua</i>	HK/SC S	Demersal		x	x	✓	✓	✓	✓	x	x	x	✓	✓	✓	x	x	x	x	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x
Neopterygii	<i>Drepane punctata</i>	HK/SC S	Reef-associated		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Ambassis buruensis</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Scatophagus argus</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Nibea mitsukurii</i>	SCS	Benthopelagic		✓	✓	✓	✓	✓	✓	x	x	x	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Pennahia argentata</i>	HK/SC S	Benthopelagic		x	✓	✓	✓	x	✓	✓	✓	✓	x	x	✓	x	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	x	✓	✓	
Neopterygii	<i>Siganus canaliculatus</i>	HK/SC S	Reef-associated		✓	✓	✓	✓	✓	✓	x	x	x	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	x	
Neopterygii	<i>Siganus doliatus</i>	SCS	Reef-associated		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Sillago aeolus</i>	HK/SC S	Demersal		✓	x	✓	✓	✓	✓	x	x	x	x	x	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	x	x	
Neopterygii	<i>Sillago parvisquamis</i>	SCS	Demersal	EN	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Sillago sihama</i>	HK/SC S	Reef-associated		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Sphyrna pinguis</i>	HK/SC S	Pelagic-neritic		x	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	
Neopterygii	<i>Gerres decacanthus</i>	SCS	Benthopelagic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Neopterygii	<i>Butis butis</i>	SCS	Demersal		x	✓	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	x	x	

Neopterygii	<i>Pampus punctatissimus</i>	SCS	Benthopelagic		✓	x	✓	✓	x	✓	x	x	x	x	x	x	x	x	✓	x	✓	✓	✓	x	x	x	x	x	x	x	x	✓
Neopterygii	<i>Nemipterus bathybius</i>	HK/SC S	Demersal		x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x
Neopterygii	<i>Acanthopagrus pacificus</i>	HK/SC S	Pelagic-neritic		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Neopterygii	<i>Acanthopagrus schlegelii</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Neopterygii	<i>Upeneus tragula</i>	HK/SC S	Reef-associated		x	x	x	✓	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x
Neopterygii	<i>Takifugu alboplumbeus</i>	HK/SC S	Demersal		✓	x	✓	x	x	✓	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	✓	x	x	x	✓	✓	✓	✓	✓
Neopterygii	<i>Takifugu flavidus</i>	HK/SC S	Demersal		✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	x	x	✓	✓	x	✓	✓	✓	✓	✓
Neopterygii	<i>Elops machnata</i>	HK/SC S	Pelagic-neritic		✓	✓	✓	✓	✓	✓	x	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	x	✓	x	✓	✓	✓	x	✓

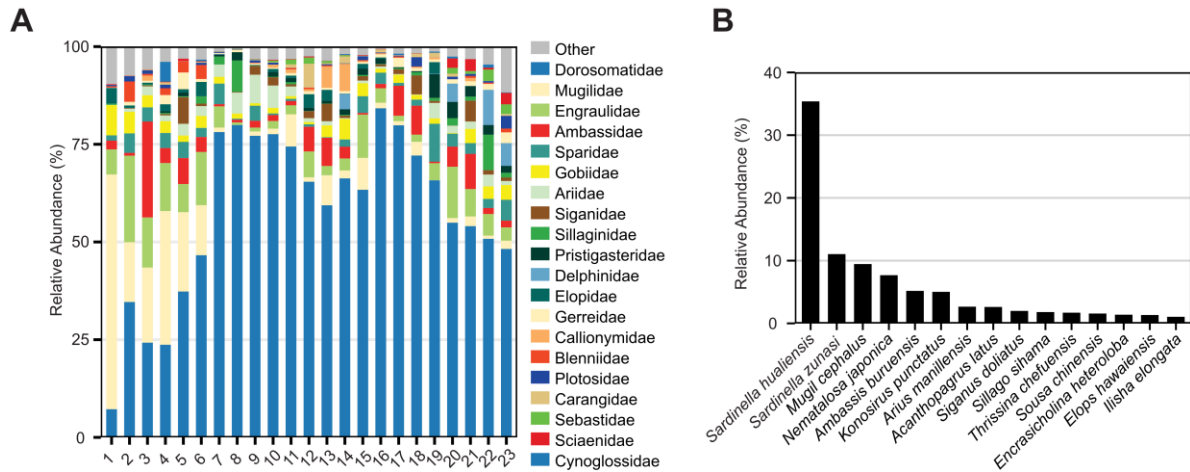


Figure 8. Stacked bar plot of the top 20 abundant families from each sampling site detected (A) and top 15 abundant species detected (B) based on the read counts from 12S-V5 metabarcoding assay.

The detected species primarily belonged to families Dorosomatidae, Mugilidae, Engraulidae, Ambassidae, and Sparidae with the fish top species based on read abundance (12S-V5) being *Sardinella hualiensis*, *Sardinella zunasi*, *Mugil cephalus*, *Nematalosa japonica*, and *Ambassis buruensis* which contributed to almost 70% of fish reads (Figure 8A and 8B). Numerous additional species contributed relatively small proportions of total read abundance, indicating that the fish assemblage consisted of a small number of highly abundant taxa together with a large component of less abundant species. Shannon diversity was strongly structured by sampling round (Kruskal-Wallis, $\chi^2(5) = 105.87$, $p < 0.001$), with the post-hoc Dunn's test revealing significant shifts in diversity between most sampling rounds. Spatially, species community structure also varied significantly (Kruskal-Wallis, $\chi^2(5) = 35.0$, $p < 0.05$). NMDS and PCoA ordination revealed strong temporal and spatial separation in community structure (Figure 9C). Separation was the most pronounced between sampling rounds, with wet and dry-season sampling rounds clustering distinctly along the PCoA axis (Figure 10). Similarly, species community structure is well-separated from Deep Bay and Lantau Sites, clusters of Deep Bay sites can be seen on the right-side of the first PCoA axis with Deep Bay sites clustering distinctly from those in North Airport, East-South Airport, and West Lantau, ANOSIM confirmed highly significant differences between sampling rounds ($R = 0.304$, $p < 0.001$) and sampling sites ($R = 0.295$, $p < 0.001$) (Table 6). SIMPER analysis reveals that similar species contributed to the variation in sampling sites/zones and sampling rounds. The differences in fish assemblages were mainly driven by the highly abundant Dorosomatidae species *Sardinella hualiensis*, *Sardinella zunasi*, and *Nematalosa japonica* (Table 7) with several overlapping species among sampling site/zones (Figure 9D). Collectively, these findings demonstrate that fish assemblages in western Hong Kong are characterized by spatial-

temporal turnover. The significant separation among sampling rounds and geographic regions indicates that fish communities are dynamic, emphasizing the importance of repeated seasonal sampling for accurately characterizing biodiversity and identifying suitable habitats for rare species such as Chinese Bahaba.

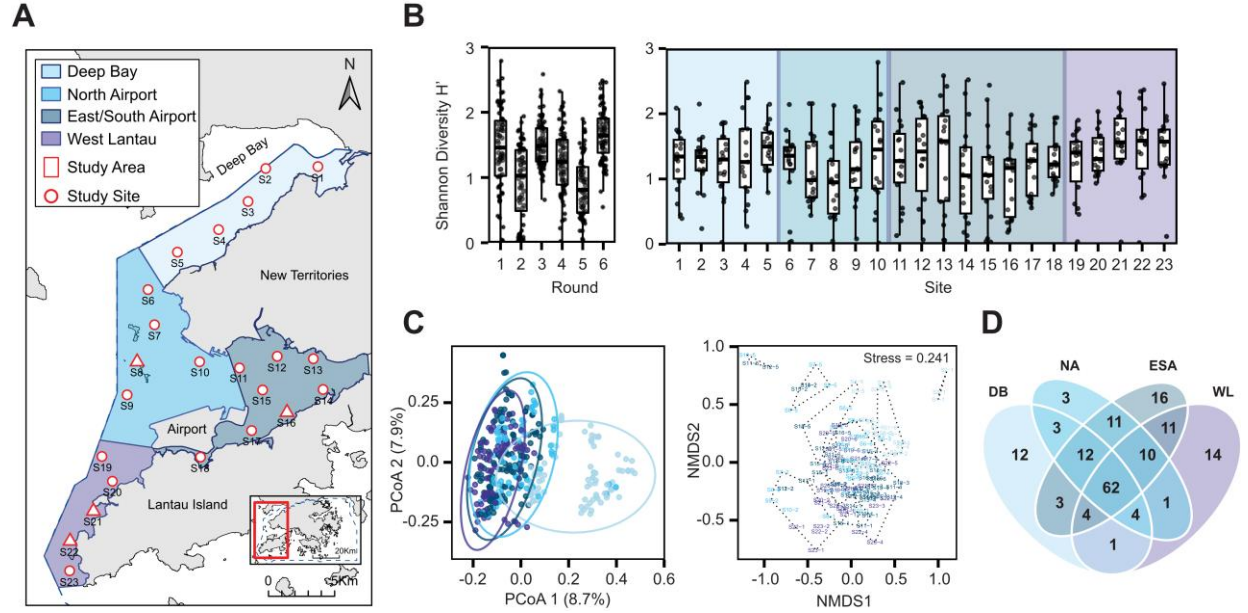


Figure 9. (A) Map of sampling sites and marine zones separating the sampling sites in the western waters of Hong Kong, the triangle points represent sites where Chinese Bahaba was detected. (B) Shannon diversity box plots created based on the sampling round and sites. (C) Principal Coordinate Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS) plots based on the presence/absence of species detected by metabarcoding, showing the spatial structure of species presence in relation to the colored marine zones. (D) Venn diagram of overlapping species detected in Deep Bay (DB), North Airport (NA), East/South Airport (ESA), and West Lantau sites (WL).

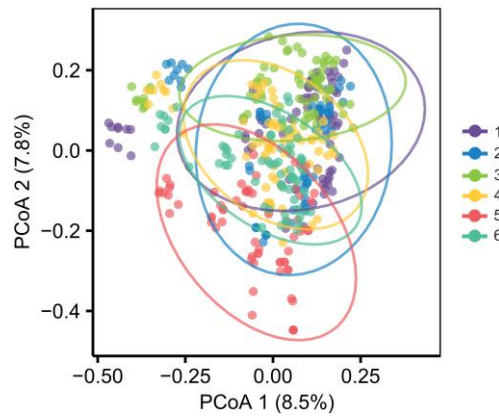


Figure 10. PCoA of fish assemblage based on the eDNA metabarcoding presence/absence from 12S-V5 and MiFish-U assays.

Table 6. Statistics of (A) comparison between Shannon diversity indices using Kruskal-Wallis test and (B) species composition between each round and site.

	Test	Variable	Statistic	p
A	Kruskal - Wallis	Round	$\chi^2(5) = 105.87$	<0.001
		Site	$\chi^2(22) = 35.0$	<0.05
B	ANOSIM	Round	$R = 0.304$	<0.001
		Site	$R = 0.295$	<0.001

Table 7. SIMPER analysis results of the fish assemblages based on the read count of 12S-V5 assay, the top five significant species contributing the most to the dissimilarity between each sampling site/zone and rounds.

Comparison	Species	Contribution_pct	Cumulative_pct
Deep Bay - EastSouth Airport	<i>Sardinella hualiensis</i>	26.96	32.12
	<i>Mugil cephalus</i>	14.14	48.97
	<i>Ambassis buruensis</i>	5.54	55.57
	<i>Scatophagus argus</i>	0.77	90.44
	<i>Oreochromis aureus</i>	0.64	91.20
Deep Bay - North Airport	<i>Mugil cephalus</i>	15.19	40.44
	<i>Konosirus punctatus</i>	6.36	59.71
	<i>Arius manillensis</i>	3.29	73.66
	<i>Omobranchus fasciolatoceps</i>	1.47	81.81
	<i>Thryssa hamiltonii</i>	0.92	86.68
Deep Bay - West Lantau	<i>Mugil cephalus</i>	12.92	29.86
	<i>Nematalosa japonica</i>	7.55	50.06
	<i>Thrissina chefuensis</i>	2.89	64.71
	<i>Sousa chinensis</i>	2.19	70.23
	<i>Ilisha elongata</i>	2.08	75.02
EastSouth Airport - West Lantau	<i>Nematalosa japonica</i>	7.39	49.52
	<i>Thrissina chefuensis</i>	2.61	66.25
	<i>Sousa chinensis</i>	2.12	68.90
	<i>Ilisha elongata</i>	1.73	78.06
	<i>Nibea mitsukurii</i>	0.95	87.33
North Airport - EastSouth Airport	<i>Sardinella hualiensis</i>	27.95	35.83
	<i>Thryssa dussumieri</i>	0.65	91.28
	<i>Drepane punctata</i>	0.44	94.41
North Airport - West Lantau	<i>Sardinella zunasi</i>	11.88	38.18
	<i>Nematalosa japonica</i>	8.15	48.29
	<i>Konosirus punctatus</i>	7.36	57.41
	<i>Arius manillensis</i>	3.19	61.36
	<i>Thrissina chefuensis</i>	2.70	64.71
R1 - R2	<i>Nematalosa japonica</i>	11.43	26.97
	<i>Konosirus punctatus</i>	9.35	37.97
	<i>Acanthopagrus latus</i>	3.89	56.33
	<i>Thrissina chefuensis</i>	3.34	60.25
	<i>Siganus doliatus</i>	3.06	63.85
R1 - R3	<i>Sardinella zunasi</i>	14.55	16.73
	<i>Konosirus punctatus</i>	9.79	39.75
	<i>Encrasicholina heteroloba</i>	4.20	59.99
	<i>Siganus doliatus</i>	3.30	63.79
R1 - R4	<i>Gerres decacanthus</i>	2.83	67.04
	<i>Sardinella hualiensis</i>	28.32	33.28
	<i>Konosirus punctatus</i>	7.62	42.24
	<i>Encrasicholina heteroloba</i>	3.26	65.73
	<i>Lateolabrax japonicus</i>	0.69	88.48
R1 - R5	<i>Neophocaena phocaenoides</i>	0.40	95.18
	<i>Sardinella hualiensis</i>	36.99	41.68
	<i>Acentrogobius viridipunctatus</i>	0.05	99.37
	<i>Sardinella aurita</i>	0.01	99.90
	<i>Sphyrna pinguis</i>	0.01	99.96
R1 - R6	<i>Lutjanus fulviflamma</i>	0.00	100.00
	<i>Konosirus punctatus</i>	9.30	28.41
	<i>Encrasicholina heteroloba</i>	3.73	51.72
	<i>Arius manillensis</i>	3.53	55.93
	<i>Siganus doliatus</i>	2.92	59.41
R2 - R3	<i>Ilisha melastoma</i>	2.39	65.53
	<i>Mugil cephalus</i>	13.73	14.95
	<i>Nematalosa japonica</i>	13.35	29.49
	<i>Sardinella zunasi</i>	11.22	41.71
	<i>Konosirus punctatus</i>	6.89	57.42

	<i>Acanthopagrus latus</i>	4.21	67.85
	<i>Nematalosa japonica</i>	10.76	38.13
R2 - R4	<i>Acanthopagrus latus</i>	3.62	71.94
	<i>Thrissina chefuensis</i>	3.22	75.52
	<i>Nibea mitsukurii</i>	1.06	87.33
	<i>Ostorhinchus kiensis</i>	0.06	99.35
	<i>Sardinella hualiensis</i>	32.34	35.94
	<i>Nematalosa japonica</i>	12.73	14.29
R2 - R6	<i>Mugil cephalus</i>	12.44	28.26
	<i>Ambassis buruensis</i>	6.75	48.63
	<i>Acanthopagrus latus</i>	4.15	60.78
	<i>Thrissina chefuensis</i>	3.57	64.79
	<i>Sardinella hualiensis</i>	33.48	44.27
R3 - R4	<i>Sardinella zunasi</i>	12.60	60.92
	<i>Ilisha elongata</i>	1.58	78.31
	<i>Glossogobius olivaceus</i>	0.45	91.65
	<i>Pennahia argentata</i>	0.18	96.38
	<i>Sardinella hualiensis</i>	41.96	50.40
R3 - R5	<i>Sardinella zunasi</i>	12.34	65.21
	<i>Tylosurus crocodilus</i>	0.27	95.74
	<i>Epinephelus lanceolatus</i>	0.11	97.91
	<i>Favonigobius gymnauchen</i>	0.09	98.48
	<i>Sardinella zunasi</i>	16.22	20.93
R3 - R6	<i>Arius manillensis</i>	3.28	60.32
	<i>Ilisha melastoma</i>	2.96	64.14
	<i>Plotosus lineatus</i>	2.25	67.04
	<i>Gerres decacanthus</i>	2.24	69.93
	<i>Sardinella hualiensis</i>	30.30	46.22
R4 - R5	<i>Oxyurichthys auchenolepis</i>	0.00	100.00
R4 - R6	<i>Sardinella hualiensis</i>	28.57	40.14
	<i>Ilisha melastoma</i>	2.18	71.15
	<i>Ilisha elongata</i>	2.00	73.96
	<i>Plotosus lineatus</i>	1.67	78.82
	<i>Thryssa dussumieri</i>	1.24	82.56
R5 - R6	<i>Sardinella hualiensis</i>	38.30	47.00
	<i>Sardinella zunasi</i>	11.47	61.07
	<i>Ilisha melastoma</i>	1.36	83.50
	<i>Tylosurus crocodilus</i>	0.26	96.05
	<i>Epinephelus lanceolatus</i>	0.09	98.11

Environmental gradients structure fish communities and are associated with Chinese Bahaba occurrence

There were four environmental variables remaining (Dissolved Oxygen, Salinity, Temperature, and pH) after VIF removal. Redundancy analysis (RDA) demonstrated that environmental gradients explained a small proportion of variation in fish community composition (**Figure 11A**). The first constrained axis accounted for 6.1% of constrained variation, whereas the second axis explained 4.5%. Environmental vectors indicated that increased dissolved oxygen, conductivity, salinity, and pH are the primary drivers of drivers between the community differences in Deep Bay sites versus the open waters of Lantau sites. Sampling sites where Chinese Bahaba was detected were associated with the positive end of dbRDA1 and were associated with relatively higher temperatures.

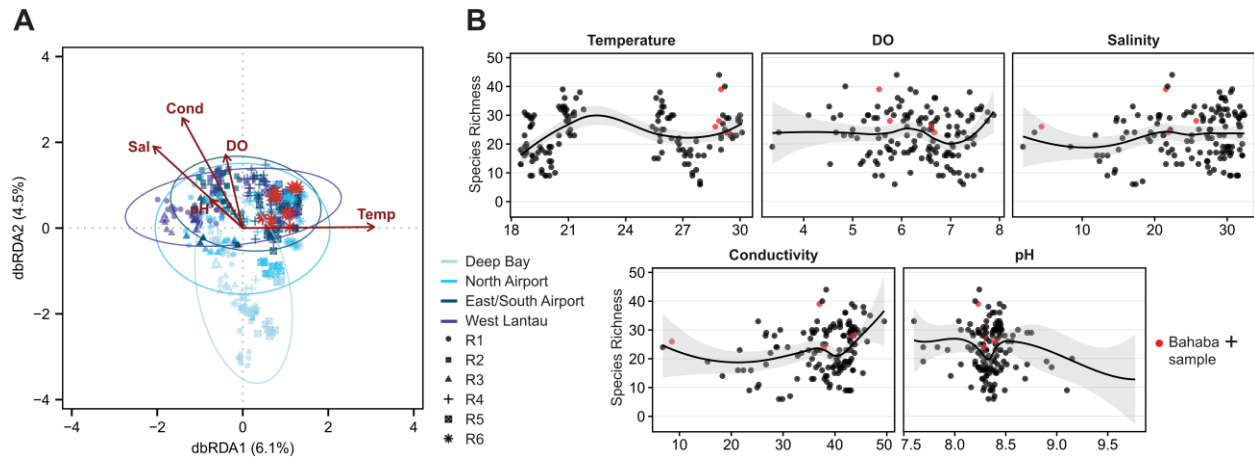


Figure 11. (A) Redundancy Analysis (RDA) ordination of site fish assemblages constrained by environmental variables after VIF removal: Temperature (Temp), Dissolved Oxygen (DO), Salinity (Sal), Conductivity (Cond), and pH. Points represent sampling sites colored according to ecological cluster identified by k-means clustering. Sites where Chinese Bahaba was detected are colored in red. The environmental vectors indicate the direction and relative strength of environmental gradients influencing the species community composition. (B) Relationships between species richness and environmental variables across all sampling rounds/sites. The black line represents locally weighted regression smoothers while red points indicate samples where Chinese Bahaba was detected.

Relationships between species richness and environmental variables were non-linear (**Figure 11B**). Species richness showed weak positive relationships with dissolved oxygen and salinity, whereas lower richness is observed at higher pH values. Apparent clusters can be seen at different temperature ranges which show the seasonal variation during sampling, with richness peaking $\sim 29^{\circ}\text{C}$. Samples where Chinese Bahaba was detected occurred across a range of environmental conditions but tended to be associated with moderate-higher species richness values, meaning their occurrence is tied to a broader ecological community. Although the measured environmental variables explained only a proportion of the overall variation in community composition, they revealed clear ecological gradients influencing fish assemblages across western Hong Kong. The association of Chinese Bahaba-positive samples with warmer conditions and relatively species-rich communities suggests that the species may occupy habitats supporting diverse fish assemblages rather than occurring independently of broader community structure. Chinese Bahaba uses estuarine and shallow coastal habitats, and adults aggregate in estuaries to spawn before moving to deeper waters afterward, which indicates that the species already shows seasonal movement between habitat zones (Wang et al., 2009). Its feeding habits also vary seasonally, with shrimp, crustaceans, and small fishes as major prey, so temporal shifts in estuarine food webs could plausibly influence when Bahaba is present in western Hong Kong (Wang et al., 2009). September (Round 6) detections under warmer conditions could therefore reflect an occupancy window related to post-spawning movement, foraging, or use of prey-rich estuarine habitat, rather than a

simple preference for high temperature alone. Species-specific presence data can be integrated with seasonal environmental measurements and assemblage composition to build more explicit habitat models, especially because sciaenid distributions in Chinese coastal waters are already known to respond strongly to dissolved oxygen, salinity, and sea surface temperature (Yang et al., 2023). Climate-related projections for Sciaenidae further indicate that suitable habitat can shift substantially over time and that some areas may become refuges while others contract (Yang et al., 2023). For a rare estuarine Sciaenid such as Chinese Bahaba, this means that repeated eDNA monitoring could be used not only to document occurrence, but also to detect long-term changes in the environmental niche space associated with occupancy. These findings indicate that environmental conditions alone are unlikely to determine Chinese Bahaba occurrence, and that habitat suitability is more likely governed by interactions between environmental gradients and the composition of local fish communities and that long term monitoring is required.

Weighted species co-occurrence network analysis identifies two ecologically distinct fish assemblages associated with Chinese Bahaba occurrence

Weighted Gene Co-occurrence Network Analysis (WGCNA) identified seven modules of co-occurring fish species, comprising the blue, brown, green, red, turquoise, yellow, and grey modules (**Figure 12A**). Scale-free topology analysis indicated that a soft-thresholding power of $\beta = 2$ provided the highest scale-free topology fit ($R^2 = 0.72$) while maintaining adequate network connectivity (**Figure 12B**). Module eigengene analyses revealed significant relationships between species co-occurrence modules and environmental variables (**Figure 12C**). Among all modules, the red and yellow modules exhibited the strongest positive associations with Chinese Bahaba detections. The red module showed positive correlations with Bahaba occurrence, sampling round, and Round 6 occurrence, while displaying negative slight relationships with dissolved oxygen and pH. Similarly, the yellow module demonstrated positive associations with Bahaba detections and Round 6 and weaker relationships with environmental variables. The turquoise module exhibited comparatively weak relationships with Bahaba detections but showed stronger correlations with environmental gradients, particularly dissolved oxygen and salinity, suggesting that this module primarily represented environmentally structured assemblages rather than species associated with Chinese Bahaba occurrence. Hierarchical clustering of module eigengenes demonstrated that modules differed substantially in their species occurrence patterns (**Figure 12D**). Modules with similar eigengene profiles clustered together, indicating partially shared ecological responses among groups of species. Mean module significance analyses further supported the importance of the red and yellow modules, which displayed the highest average species-level correlations with Chinese Bahaba detections (**Figure 12E**). The species co-occurrence network revealed clear modular organization of fish assemblages (**Figure 12F**). Several modules formed highly connected clusters containing numerous positive co-occurrence relationships, whereas other modules contained relatively few species and exhibited sparse connectivity. The network structure indicates that fish communities within western Hong Kong waters are composed of recurrent species

assemblages that have similar occurrence patterns across sampling locations and periods. Unlike traditional community analyses that primarily describe differences in species composition, WGCNA identified groups of species that consistently co-occurred across sampling locations and seasons, providing insight into the underlying ecological organization of the fish community. The associations between the red and yellow modules and Chinese Bahaba detections suggest that Bahaba occurrence is linked to distinct ecological assemblages rather than individual species alone.

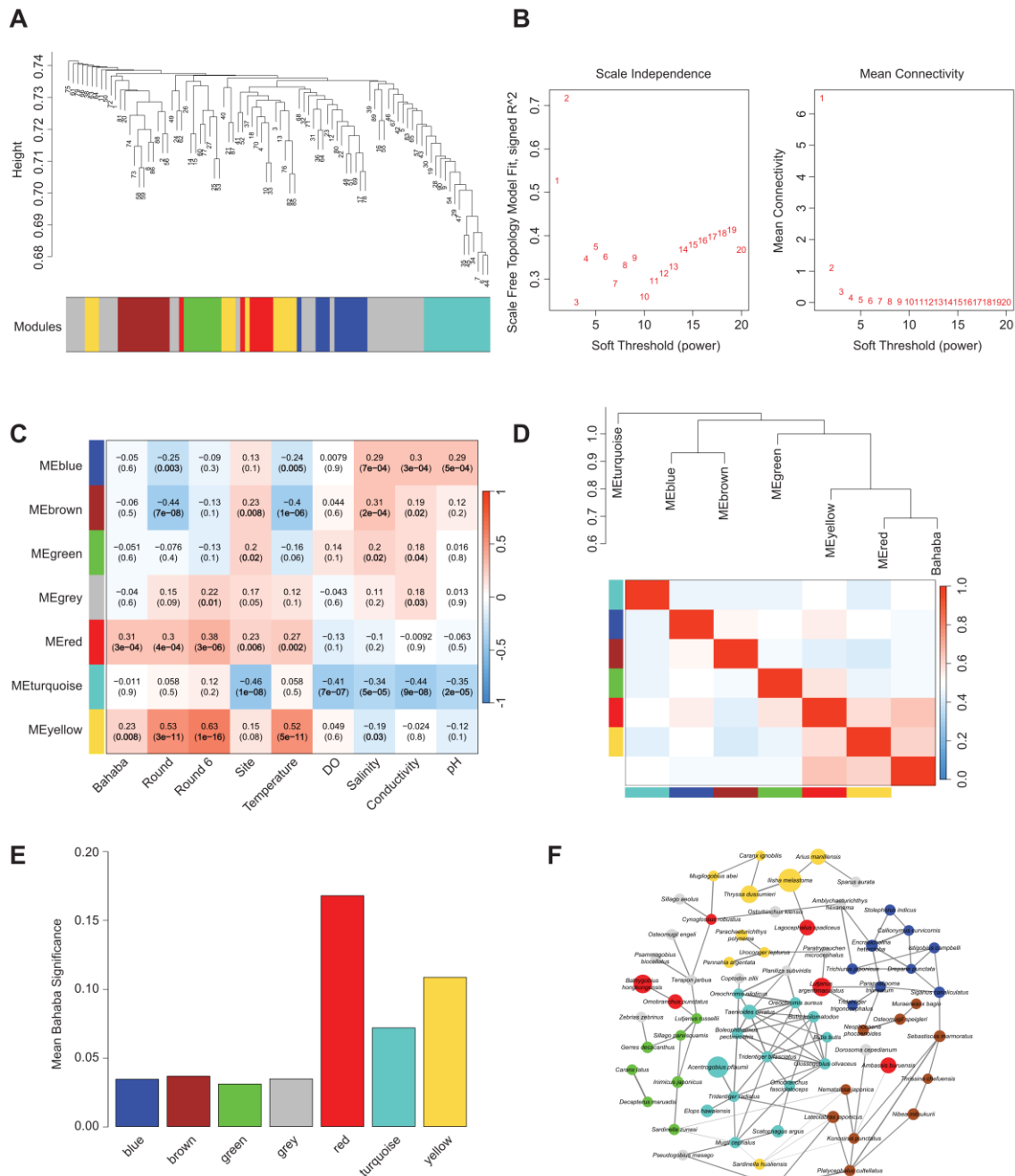


Figure 12. Weighted Gene Co-occurrence Network Analysis (WGCNA) of fish assemblages detected by 12S-V5 metabarcoding. **(A)** Hierarchical clustering of fish species based on topological overlap matrix (TOM) dissimilarities, with colored bars indicating module assignments identified by dynamic tree cutting. **(B)** Selection of the soft-thresholding power showing scale-free topology fit and mean network connectivity; a power of $\beta = 2$ was selected. **(C)** Module–trait relationships showing correlations between module eigengenes and Chinese Bahaba detections, sampling variables, and environmental parameters. Values represent Pearson correlation coefficients with associated p-values in parentheses. **(D)** Hierarchical clustering of module eigengenes illustrating similarities among co-occurrence modules. **(E)** Mean Bahaba association of module, calculated as the average absolute species-level correlation with Chinese Bahaba detections. **(F)** Species co-occurrence network showing fish species (nodes) and positive pairwise correlations (edges), with node colors representing WGCNA module assignments. The larger nodes indicate their correlation with Bahaba occurrence (higher correlation = larger node), edges represent positive pairwise Pearson correlations, with edge width proportional to correlation strength.

Because the red and yellow modules exhibited the strongest associations with Chinese Bahaba detections, these modules were examined in greater detail (**Figure 13A & 13B**). Relative abundance analyses demonstrated that the two modules differed substantially in taxonomic composition. The red module was strongly dominated by *Ambassis buruensis*, which accounted for approximately 80% of total module read abundance. Additional taxa, including *Plotosus lineatus* and *Omobranchus punctatus*, contributed considerably smaller proportions of total abundance. At the family level, Ambassidae and Gobiidae represented the dominant taxonomic groups within this module. In contrast, the yellow module was dominated by *Sardinella hualiensis*, which accounted for approximately 85% of total read abundance. Other species, including *Arius manillensis*, *Ilisha elongata* and several gobiid fishes, contributed relatively minor proportions of total abundance. Family-level analyses similarly showed strong dominance by Sciaenidae, followed by Engraulidae and Gobiidae. The two Bahaba-associated modules exhibited little taxonomic overlap. No species were shared between the red and yellow modules, and only a single family was common to both modules (**Figure 13C**). Furthermore, Shannon diversity differed significantly between modules, with the yellow module exhibiting significantly greater species diversity than the red module ($p < 0.001$). Collectively, these results indicate that Chinese Bahaba detections are associated with at least two distinct co-occurring assemblages that differ in species composition, family composition, and diversity characteristics. The separation between the red and yellow modules suggests that Chinese Bahaba may be associated multiple ecological assemblages within western Hong Kong waters rather than being associated with a single characteristic fish community. Although both modules were positively associated with Bahaba detections, they differed substantially in dominant taxa, family composition, and diversity, indicating that they may represent different habitat types or seasonal community states occupied by the species. The

identification of characteristic taxa within these modules provides a valuable ecological baseline and highlights potential indicator species that could be incorporated into future monitoring programs to identify habitats with a greater likelihood of supporting Chinese Bahaba. However, these species should not be interpreted as having direct biological interactions with Chinese Bahaba. Instead, their co-occurrence likely reflects shared environmental preferences or habitat requirements, making them potential ecological indicators of conditions favorable for Chinese Bahaba occurrence. The red and yellow WGCNA modules show two distinct fish assemblages that tend to co-occur with Chinese Bahaba, rather than as evidence that Bahaba is linked to one specific companion species or direct species interactions which is consistent with what is known about Chinese Bahaba habitat use. Chinese Bahaba occupies a broad estuarine–coastal habitat gradient, including open seas, shallow seas, subtidal beds, rocky shores, and estuaries, and its documented range extends from the Yangtze River southwards to Hong Kong (Wang et al., 2009), therefore, it is possible that Bahaba detections would align with more than one recurring community configuration rather than one fixed fish assemblage. The two Bahaba-associated modules with different dominant species suggests that Bahaba may use multiple suitable habitat states within western Hong Kong waters. Because the modules differ in composition, the key ecological signal is more likely the overall assemblage context than the identity of the single most abundant taxon in each module. The dominant species contributing roughly 80–85% of relative abundance to these modules indicate a strong compositional skew, but such dominance should not be assumed to identify the ecological driver of Bahaba occurrence. In rare coastal fishes, eDNA-based module dominance can reflect a mixture of true biomass, schooling behavior, habitat concentration, and signal-generation processes, so the dominant taxon is better treated as a marker of assemblage identity than as proof of a direct ecological association. This is especially important for Chinese Bahaba because its detections are likely to reflect transient use of favorable habitat windows rather than stable residence within a narrowly bounded species cluster (Wang et al., 2009; Sadovy & Cheung, 2003).

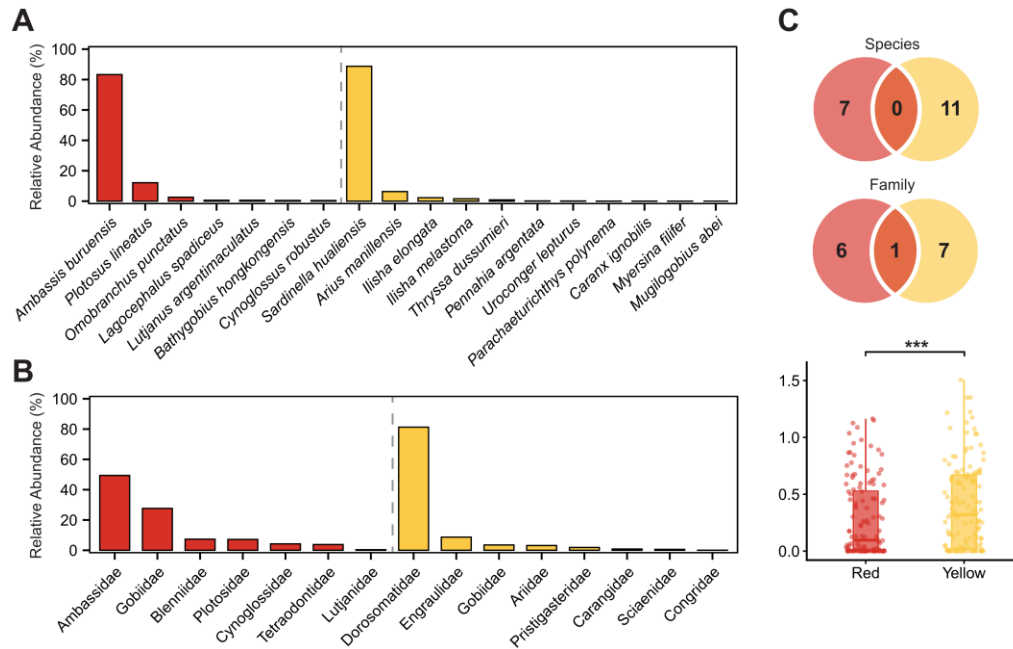


Figure 13. Relative abundance bar plots of species (A) and families (B) from significant module correlation from WGCNA analysis based off 12S-V5 reads. (C) Venn diagram of the species and family overlap between the two significant Bahaba-correlated modules and Shannon diversity of species from each module.

5. Other project progress

(1) Dr. Ip (PI) and the project's RA have been invited to participate in the following five conference/talk events:

1. “环境 DNA 与生态健康评估” Academic Seminar and the First “Water Ecosystem Integrity” Academic Conference, to be held August 28–31, 2025, in Kunming, Yunnan.
2. Symposium on Biodiversity Conservation at Nanjing University, and the conference “Sustainable Utilization of Fish in the Belt and Road Initiative” at Northwest Normal University on September 27–28, 2025.
3. The 13th National Conference on Environmental Chemistry, to be held November 28–December 2, 2025, at Wuyi University, Jiangmen, Guangdong.
4. The 3rd International Conference on Biodiversity, Ecology, and Conservation of Marine Ecosystems (19–23 August 2025), Hong Kong SAR, China.

(2) The project prepared an article in Wen Wei Po (II) on 2026-04-15, titled “環境 DNA 揭示生物留下‘隱形名片’” (嶺創新知).

(3) In addition, two manuscripts related to environmental DNA have been submitted:

- “Tracing the 'Panda of the Sea': Species-Specific qPCR Assays for eDNA Monitoring of Critically Endangered *Bahaba taipingensis* in the Pearl River Estuary” Status: reviewed and rejected; currently revising and preparing for resubmission to *Ecological Indicators*.
- “Life in the Turbid Zone: Environmental DNA Uncovers Hidden Biodiversity and Fishery Values of Mangrove Habitats in Urbanized Subtropical Estuary” Status: under review.

Both submissions acknowledge MEEF2024012 and MEEF2024012A.



Invitation Letter

Dear Dr. Jack CH Ip,
Affiliation: Lingnan University

We are writing to cordially invite you to participate in the seminar entitled *Marine and Coastal Environmental DNA (eDNA)*, which will be held by Nanjing University from 28 to 31 August 2025. This seminar is part of our ongoing efforts to advance interdisciplinary research in marine and coastal biomonitoring and ecosystem integrity assessment, particularly through the use of novel technologies such as eDNA.

You are invited to share your recent research and academic insights in the field of marine and coastal eDNA. We believe your contribution will be of great value to the seminar and will foster fruitful academic exchange and collaboration.

Please kindly note that international airfare and accommodation will be self-arranged by the participant. An expert consultation fee will be provided in recognition of your academic contribution.

We would be most grateful if you could accept this invitation. Should you have any questions regarding the seminar or travel arrangements, please feel free to contact us at any time.

Thank you in advance for your kind consideration.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'Yan Zhang'.

Dr. Yan Zhang
School of the Environment, Nanjing
University, Nanjing, Jiangsu Province,
210023, China

A handwritten signature in black ink, appearing to read 'Xiaowei Zhang'.

Prof. Dr. Xiaowei Zhang
School of the Environment, Nanjing
University, Nanjing, Jiangsu Province,
210023, China



第六届“环境DNA与生态健康评估”学术研讨会
暨第一届“水生态系统完整性”学术研讨会

*The 6th Symposium on Environmental DNA and Ecological Health Assessment
& The 1st Symposium on Aquatic Ecosystem Integrity*

大会专题报告
keynote talk

叶志豪

环境DNA技术用于监测香港水域丰富的生物多样性

特此感谢

南大学生态与环境学院

中国环境科学研究院

中国环境监测总站

昆明学院

2025年8月28-31日 云南·昆明

组委会

关于邀请出席“一带一路”鱼类生物多样性保护与 可持续利用学术研讨会的函

尊敬的专家:

西北师范大学承担的国家自然科学基金委员会(京港学术交流中心)的国际(地区)合作与交流项目——“一带一路”鱼类生物多样性保护与可持续利用学术研讨会,拟于2025年9月28日全天在西北师范大学国际交流中心召开,会议期间国家自然科学基金委员会相关处室负责人将出席会议,拟定邀请来自香港和内地高校和研究所的30位知名专家学者参加会议并做会议报告。

诚邀您能出席会议并作会议报告(附会议日程)。

专此函请,盼复。



(联系人: 李建真 联系电话: 13919775526)

鱼类生物学与前沿技术			
时间：2025年9月28日（下午） 地点：国际学术交流中心403会议室			
主持人：李铭源、赵 晖			
时间	报告题目	报告人	单位
13:30 -13:45	香港淡水生态系的生物入侵	曾憲發	岭南大学
13:45 -14:00	绿色鱼饲料对鱼类养殖的影响	黃詩韻	香港大学
14:00 -14:15	Exploring the hidden fish community and fishery value of estuarine Hong Kong waters using eDNA metabarcoding	叶志豪	岭南大学
14:15 -14:30	中国南部海域游泳动物条形码数据库构建	刘 敏	厦门大学
14:30 -14:45	斑马鱼在神经疾病与药物成瘾研究中的模型潜力	李铭源	香港理工大学
14:45 -15:00	Thyroid hormone receptor alpha a (Thraa) regulates heart regeneration and beyond	趙 暉	香港中文大学
15:00 -15:15	Elucidating the Role of the WRN Gene in Regulating Bone Development and Growth in Werner Syndrome Using Zebrafish Models	王武明	香港中文大学



第十三届全国环境化学大会 邀 请 函

尊敬的叶志豪教授，

您好！

“第十三届全国环境化学大会”将于2025年11月28日-12月2日在广东江门五邑大学举行。本届大会以“创新环境科学 推动绿色发展”为主题，围绕理论环境化学、环境与健康、环境催化、环境管理，以及水、土、气和固废相关的环境分析、界面过程、生态毒理、污染治理与修复技术等领域的最新研究进展设置90余个分会，并邀请各类相关企事业单位参展，同时组织行业企业“揭榜挂帅”专题研讨、研究生论坛、主编见面会及论文墙报展等学术活动和特色活动。

鉴于您在环境化学领域取得的创新成果和突出贡献，我们诚挚地邀请您届时拨冗出席本届大会并做分会邀请报告，同与会人员分享最新的研究成果。感谢您在百忙中关注、支持并指导本届大会。如蒙同意，请您于8月31日前登录会议网址（<https://www.ncec2025.com/>）注册参会，并上传论文摘要。

期待深秋时节与您在秀美侨乡江门相聚！

分会名称：第55分会场：大湾区环境与健康论坛

召集人：杨铸、伍健林、胡娣、黎永杰

分会秘书：朱林，13058170160，zhu_lin@hkbu.edu.hk；

胡晓兰，19120090238，huxiaolan-0403@163.com

第十三届全国环境化学大会组织委员会

五邑大学

2025年8月8日

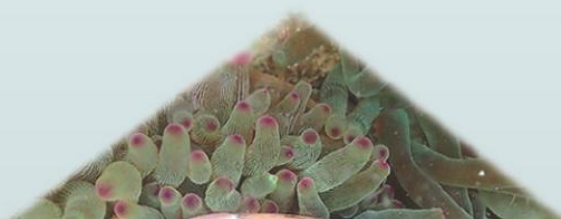


The 3rd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems 2025

Conference Programme

19-23 August 2025

City University of Hong Kong
Hong Kong SAR, China



3rd International Conference on Biodiversity, Ecology, and Conservation of Marine Ecosystems

14:40	O22 Marine Thomas <i>The Nature Conservancy, Hong Kong, China</i> Getting to Scale – Gaps for Restoring 30 x 30 of Hong Kong's Reef Ecosystems	O42 Zhongyue Wan <i>The University of Hong Kong, China</i> Autonomous Reef Monitoring Structures (ARMS) Reveal Human-Induced Biodiversity Shifts in Urban Coastal Ecosystems	O65 Song Ge <i>Zhejiang University, China</i> Mapping the Global Carbon Emissions of Marine Sectors
15:00	Panel Discussion Turning 30x30 into Reality: The Role of Science Moderator: Ander Cornish	O43 Leong Mei Kei Mikael <i>University of Saint Joseph, Macau</i> Exploring Biodiversity in Macao's Urban Wetland Ecosystems through e-DNA Analysis	O66 Syakirah Azmey <i>Universiti Brunei Darussalam, Brunei</i> Genetic Connectivity of <i>Pterobdella Arugamensis</i> in The Indo-West Pacific Region: Dispersal and Biosecurity Implication
15:20	Speakers: Jim Chu (Assistant Director, Agriculture Fisheries and Conservation Department of the HKSAR Government, China) Brendan Godley (Professor, Exeter University, UK)	O44 Yuzhuo Liao <i>Lingnan University, China</i> Development of Species-Specific Primer assays for eDNA Monitoring of the Critically Endangered Chinese Bahaba (<i>Bahaba taipingensis</i>)	O67 Tajra Smajic <i>The Chinese University of Hong Kong, China</i> Preserving the Area for Future Generations - The Legal Obligation to Protect the Common Heritage of Mankind
15:40	Kenneth Leung (Chair Professor, City University of Hong Kong, China) Lydia Pang (Head of Oceans Conservation, WWF-HK, China)	O45 Hong Zhou <i>Ocean University of China</i> Constructing A Regional DNA Barcode Reference Library of Polychaetes from the Coastal Waters of China	O68 Jiajia Lin <i>Shanghai Ocean University, China</i> Mainstreaming Biodiversity in Fishery Sector: China's Practice
16:00	Tea Break (Outside Joseph Lee Hall, 3/F, LI)		

腸道細菌增 恐令小鼠「變傻」

科學講堂

在人口結構日益高齡化的當代社會，年齡增長對認知功能的影響備受關注。近年來，一系列研究已揭示腸道微生物生態的改變，與實驗小鼠乃至人類的腦部活動變化之間存在關聯。那麼，腸道微生物生態與老年所導致的認知衰退之間，究竟存在何種聯繫？本文將綜述近年相關的研究發現。

認知功能的下降，主要源於腦部機能的衰退。尤其其體位於記憶形成、資訊處理及提取能力等。此現象可能歸因於神經元退化、腦血管病變、甚至腦神經等病理因素。除了基因調控外，飲食習慣、生活形態、感染、有微生物寄生及其他環境因素，都會對老年人的認知功能產生影響。而腸道微生物生態，是否亦為其中的一個相關因子？

短期記憶下降

近期有研究指出，將年輕與年老的實驗小鼠共同飼養，其腸道內會逐漸形成相似的微生物群落結構。研究人員隨後對這些小鼠進行新物體識別測試及迷宮空間長期記憶任務評估，結果顯示小鼠的整體健康狀況並無顯著差異，但其短期記憶表現受到負面影響。

進一步對小鼠腸道微生物的基因組進行分析發現，隨著年齡增長，若干特定微生物的豐度確實有所上升，其中尤以戈氏副擬桿菌 (*Parabacteroides goldsteinii*) 最為顯著。後續實驗證實，僅需在年輕小鼠的腸道內引入並增殖戈氏副擬桿菌，便足以導致其認知功能減退，更何況其他微生物種類變化的疊加效應。

那麼，小鼠腦部的哪個區域受到了影響？研究發現，腸道中戈氏副擬桿菌的增多，會抑制實驗小鼠腦內海馬體的神經元活動，而海馬體正是掌管學習與記憶功能的關鍵腦區；反之，當老年小鼠腸道中的戈氏副擬桿菌數量減少（例如藉由抗生素處理），其記憶能力則獲得改善。

由此可見，腸道微生物組成的改變與腦部活動之間確實存在密切聯繫。

阻礙腦道與大腦「接線」

腸道功能又是如何影響腦部運作的呢？研究人員發現，部分感覺神經元會表達名為 PHOX2B 及 TRPV1 的蛋白質。若抑制這些蛋白質的活性，亦會導致海馬體神經活動減弱，進而影響認知表現。這些神經元屬於迷走神經感覺纖維的一部分，負責將來自各內臟器官的信號傳遞至腦部，極有可能構成腸道向大腦發送訊息的關鍵傳導路徑。

然而，並非所有研究結論皆指向同一方向。部分研究觀察到，隨著小鼠年齡增長，其腸道中戈氏副擬桿菌的數量反而呈現下降趨勢；亦有研究指出，該菌種能增強小鼠的抗炎反應，未必會導致腦功能衰退。

此種分歧可能源於不同研究分析了該菌種下的不同菌株，尚待後續更精確的鑑定與探究。此外，當前在小鼠模型中所觀察到的現象，仍需要進一步於人體研究中加以驗證。

小結

年齡增長與遺傳基因，是導致腦功能衰退的兩項



● 研究人員測試小鼠在迷宮中對空間長期記憶的能力。

較為明確的成因。本文所探討的研究，則開拓了腸道微生物對腦功能的潛在調節作用。冀望在不久的將來，相關研究成果能為我們開發應對認知相關疾病的有效策略，提供嶄新的方向與啟示。

● 杜子航 教育工作者
早年學理理工科，一直致力推動科學教育與科研工作，近年開始關注電腦發展對社會的影響。

論應科大發展：應用為本，產業導向

東華論衡

香港中學文憑考試 (DSE) 開考，考生傾盡全力，爭取好成績。本屆政府多管齊下推動應用科學大學在香港發展，大力提升專專教育，鼓勵年輕人投入的能力和興趣，選擇升學出路，加入不同行業，為有志在專業技術發展的青年建立康莊大道，達到行出致用的目標。

有別於傳統研究型的大學，應用科學大學注重結合理論和實踐的應用學位課程，與專業技術界緊密合作，以及在課程融入豐富的實習和職場學習機會，培養具備應用能力和知識的學生。

東華學院亦是香港應用科學大學聯盟的新成員之一，辦學定位是應用型、職業型，課程一直具備「應科大」特色，著重將科學研究應用於產業與社會。課程設計由專業專業人士深度參與、結合理論和實踐，所有學位均為學生提供實業教育，培訓學生的應用能力，為人力資源密切的行業培育專才。

東華學院亦是全港唯一擁有五個專專認可專職醫療及護理學位課程的自資專上院校，醫數基礎扎實，畢業生在業界業績良好口碑，學院2025年畢業生的平均入位數達3.5萬港元，於本港多間專專院校中居前列，94%畢業生投身就讀課程相關行業，印證了課程的實用性與競爭力。

放眼海外，應用科學大學在歐洲的發展尤為成熟，在德國、芬蘭等國家，應用科學大學數量遠超傳統研究型大學，成為中專畢業生升學的主選導流之一，其學位不僅具高度社會受性，亦與商業需求緊密

密銜接，充分印證應用科學大學在現代高等教育體系中的關鍵地位與長遠價值。東華學院應運而上去年底首屆畢業生畢業應用科學大學，推動香港、瑞士教育界分工合作，綜合性大學專才基礎科研，而應用科學大學及職業教育機構則負責為企業培育專才，推動科研成果商品化的重任。

為產業發展提供人才支撐

內地已建成世界規模最大的職業教育體系，中職、高職與職業本科結構比例持續優化，專業布局緊密對接國家戰略和區域經濟需求，基本覆蓋國民經濟各關鍵領域。據統計，職業教育為現代產業體系輸送了超過七成新增長技能人才，為製造業升級與實體經濟高質量發展提供了強有力的人才支撐。

特區政府正積極打造國際專專教育樞紐，這既是香港教育發展的新機遇，亦是應用科學大學應運發展的重要契機。香港應主動把握這一機遇，積極參考借鑒內地及海外應科大的成功經驗，推動應用科學大學與傳統大學建立互為支撐的社會地位。東華學院將繼續發揮應用科學教育的優勢，培育更多切合社會發展所需的專業人才，為香港的長遠發展注入持久動力。



東華學院

東華學院自2010年創立以來，致力培育符合社會發展所需、具備專業素養的優秀人才。本專欄旨在與各界交流分享真知灼見，攜手打造香港成為國際教育樞紐。

● 東華學院校長 陳慧慈

中國軍事科技如何以「無人」換「無損」？

科技暢想

最近，我們協會帶領二十多位年輕人到北京訪問，其中一站是參觀中國人民革命軍事博物館，館內展示了許多先進的軍事裝備，讓我們大開眼界，也深深感受到國家軍事科技的快速進步。以下就用最簡單的方式，為大家介紹近兩三年中國在軍事科技上的新發展。特別是您可能更有興趣的無人機和機械人（尤其是四足機械人，也就是俗稱的「機械狗」）。

激光武器聽起來很像科幻電影裏的東西，但其實中國已經把它變成現實了：「激光武器就像一支超級強力的「光槍」，它射出高能量的光束，瞬間把敵人的無人機、導彈甚至衛星燒穿。

例如，有款叫「光箭-21A」的國產激光武器，可以安裝在軍車上，一邊移動一邊射擊，做到「發現目標，立即摧毀」。另外還有一款「光箭-11E」，專門用激光干擾敵方無人機的視圖，讓他們變成「盲頭烏龜」，無法偵察或攻擊。

簡單來說，激光武器的好處是：速度極快（光速）、成本低（不用浪費昂貴的導彈），而且非常準確。以後如果敵方派出大量無人機來騷擾，激光武器就可以像「打蚊」一樣，輕鬆把它們全部擊落。

機械狗探路 無人機蜂群出擊

您可能在網上看到過四足機械人，它們像狗一樣有四條腿，可以行走、跑步、跳躍，甚至爬樓梯。在軍事上，這些「機械狗」可以發揮很大的作用！

機械狗體型小巧，行動安靜，可以悄悄走進危險的建築物、山洞或森林，代替士兵去偵察敵情。它身上裝有攝影機、熱成像儀和雷達，能即時把畫面傳回指揮部。如果遇到敵人埋伏，機械狗可以充當「誘餌」，吸引敵人開火，暴露他們的位置。

士兵在戰場上要攜帶很多裝備，例如彈藥、水、糧食、通訊設備等，非常耗費體力。機械狗可以背幾十公斤至上百公斤的物資，跟在士兵後面行走，甚至翻越崎嶇的地形，這比士兵搬運儲存體力、專心作戰。

如果發現敵方有炸彈或地雷，機械狗可以走上前去，用機械臂進行檢查和拆除，或者直接引爆危險物品。

此外，中國已經研發出多款軍用四足機械人，例如「紅翼」系列，它們正在部隊中進行測試和訓練。未來，我們可能會看到機械狗和士兵並肩作戰的場景。

無人機是近年軍事科技的一大熱點。中國研發了很多不同種類的軍用無人機，有的負責偵察，有的負責攻擊，有的甚至可以組成「蜂群」一起行動。

有句老話「蜂群」？想像一下，你一個人拿著一個遙控器，就可以同時操控幾十甚至上百架無人機。這些無人機會互相溝通、自動編隊，就像一群蜜蜂一樣跟隨你攻擊敵人。

中國的「阿特拉斯」系統就是這樣的裝備：一輛車可以在3秒內發射一架無人機，一個人就能控制96架。它們可以以幾分鐘的速度飛出敵人的位置，再派出子機編隊攻擊敵方，最後派出攻擊機直接打擊目標。即使沒有衛星導航或通訊信號，這些無人機也能靠自己的「智慧大



中國人民革命軍事博物館中的「機械狗」。

腦」完成任務，非常厲害。

除了小型蜂群，中國還有像「九天」這樣的大型無人機。它的「肚子」裏藏著一個「蜂軍團」，可以一次釋放上百架小型無人機或巡邏彈（一種可以發射尋找目標的炸彈）。

另外，《三馬1000》和《巨龍8》是專門用來運送物資的無人運輸機，它們可以在崎嶇的山路上起降，自動裝卸貨物，為前線士兵快速運送彈藥、食物和藥品。這樣就不需要冒險派出有人駕駛的運輸機了。

打仗除了要前線士兵，後方的補給也同樣重要。中國現在大量使用無人車和無人機來運送物資。例如，在高原哨所或偏遠海島，傳統卡車很難到達，但無人運輸機可以直接空投或降落，把新鮮食物和藥品送到士兵手中。

有些無人車是六輪或履帶式的，它們可以跟著部隊前進，自動巡邏和觀察。這些尖端科技的核心目的，從來不是為了侵略或挑起衝突，恰恰相反，在當前複雜多變的國際形勢下，擁有足夠強大的防衛力量，是維護國家主權與領土完整的根本保障。

● 洪正文
香港新網科技教育協會培育人才，提高各界對科技創新的認識，為香港青年提供更多機會參與國際性及大中華地區的科技創新活動，詳情可瀏覽 www.hknetec.org。

環境DNA揭示生物留下「隱形名片」

● 中國人民革命軍事博物館中的「機械狗」。

作者：張國權

● 當科學家不用潛水，卻能知道海洋裏住着誰，你是否好奇他們怎麼做到的？甚至連幾根隨風飄的動物是否仍留在某個角落悄然生存，也能從一杯海水中找到答案。這就是環境DNA (eDNA) 的威力。生態學家如同偵探，透過水樣分析，便能揭示「誰曾來過」，徹底革新我們觀察與守護生物多樣性的方式。

想像每隻動物活動時，都會悄悄留下生命足跡：脫落的皮膚、黏液，或排泄物。這些痕跡散佈著獨一無二的DNA，飄散在水、土、空氣中的DNA，就是eDNA，宛如生物散落的隱形名片。科學家只需收集環境樣本，就能提取這些名片，分析出「誰」曾造訪，實現非侵入式探測。

傳統生物調查往往需要大規模作業，如出海捕撈清道及長期觀察，這些方法不僅耗時費力，還可能破壞生態，更易遺漏稀有或隱蔽物種。

一杯海水看世界

相較之下，eDNA技術猶如一種先進的「生物偵測系統」，且具備三大優勢：非侵入性，不會傷害生物及其環境友善；高效便捷，僅一杯水樣即可探測數十種生物；靈敏度高，能有效識別稀有或瀕危物種。eDNA的出現，正重新定義我們探索與守護自然的方式。

進行eDNA研究就像完成一個嚴謹的科學實驗，步驟環環相扣：

1. 採樣：從目標環境收集樣本。
2. 過濾保存：用濾膜過濾水中DNA，低溫保存防分解。
3. DNA提取：純化並分離環境上的DNA。
4. 解碼分析：利用分子生物學技術來解讀這些遺傳密碼。如尋找特定物種會使用qPCR (即DNA擴增技術，可高效及精準地探測物種)，等技術。僅用一把網匙去開一把特定的鎖，若想了解整個生物群落，則採用「物種鑒別大分析 (Metabarcoding)」技術，像超市結賬時用的掃描器，一掃全識別所有「隱形名片」，零干擾閱讀生物群落。

eDNA技術主要應用於三大領域：生物多樣性監測，通過物種輪廓追蹤生態變化；生物安全管理，監控入侵物種，強化邊境生態；生態保育，為保護區、保育物種與特定棲息地提供科學依據。該技術已在全球生態研究中發揮關鍵作用，以下為幾項具體案例。

● 案例一：香港水域的生物多樣性調查
科學家在香港南部水域以抽測eDNA同步調查，抽測獲236種生物，eDNA則偵測出311種，基芬發現難以捕捉的江豚，展現其在辨識稀有物種上的優勢 (Ip et al. 2024)。

● 案例二：尋找黃唇魚
黃唇魚是中國特有的極危物種，被譽為「水中大熊貓」。科學家正透過eDNA技術，在香港與珠江口展開搜尋，並成功發現了其蹤跡。這項技術為保育工作帶來了新的希望改善海洋生態基MIEEP2024012)。

儘管eDNA技術充滿潛力，仍面臨挑戰。其準確性依賴完整的DNA數據庫，若物種未被記錄便無法識別。環境因素如紫外線與水流可能導致DNA降解，殘留的舊DNA可能干擾即時、全面的生態監測，成為守護地球生態的關鍵工具。

eDNA如同生態隱形鏡，讓我們以更細緻、高效的方式解開生物多樣性的隱藏密碼。它並非取代傳統方法，而是成為互補利器，在生態保育道路上扮演不可或缺的角色。

● 葉志豪
嶺南大學科學教育助理教授 (校長卓越青年學者)



Original Article

Tracing the 'Panda of the Sea': Species-Specific qPCR Assays for eDNA Monitoring of Critically Endangered Bahaba taipingensis in the Pearl River Estuary

Submission ID ba9a8de6-ff8e-4085-9842-4bbd81385bd2

Manuscript ID 9942925

Submission Version Initial Submission

PDF Generation 12 Mar 2026 07:36:09 EST by Atypon ReX

Funders

Is this research supported by funding?

- Marine Ecology Enhancement Fund
Organization is not listed
Grant numbers
 - MEEF2024012
 - MEEF2024012A
- Marine Conservation Enhancement Fund
Organization is not listed
Grant numbers
 - MCEF22116
- Project of Financial Funds of Ministry of Agriculture and Rural Affairs
Organization is not listed
Grant numbers
 - ZJZX-06
 - 441901202109379



Original Article

Life in the Turbid Zone: Environmental DNA Uncovers Hidden Biodiversity and Fishery Values of Mangrove Habitats in Urbanized Subtropical Estuary

Submission ID 69a2ce73-9b6a-41a5-9f2f-d0f368d22f25

Submission Version Initial Submission

PDF Generation 07 May 2026 08:13:59 EST by Atypon ReX

Funders

Is this research supported by funding?

- Marine Conservation Enhancement Fund

Organization is not listed

Grant numbers

- MCEF22116

- Marine Ecology Enhancement Fund

Organization is not listed

Grant numbers

- MEEF2024012
- MEEF2024012A

(v) Evaluation of the project effectiveness in achieving the proposed objectives as well as the impact (benefits) of the Project

The project successfully achieved all of its proposed objectives and demonstrated the effectiveness of integrating species-specific eDNA detection with community-level eDNA metabarcoding for the conservation of the Critically Endangered Chinese Bahaba. For Objective 1 (Task 1), species-specific qPCR assays targeting three mitochondrial regions (12S, ND5 and D-loop) were successfully designed, validated, and applied to environmental samples, providing the first validated molecular tools for non-invasive monitoring of this species. The field application of the developed assays resulted in the first successful detections of Chinese Bahaba eDNA in western Hong Kong waters in September 2025, providing important baseline information on its contemporary occurrence and potential habitat use.

In Objective 2 (Task 2), large-scale eDNA metabarcoding generated a comprehensive assessment of fish biodiversity, detecting 266 vertebrate taxa and demonstrating substantial spatial and seasonal variation in fish community composition. By integrating species-specific qPCR detections with community-level metabarcoding data, the project was able to investigate not only the occurrence of Chinese Bahaba but also the environmental conditions and fish assemblages associated with its detection. Chinese Bahaba was detected only during Round 6 (Sep 2025) and occurred within relatively species-rich communities associated with warmer environmental conditions, suggesting a pronounced seasonal occurrence and potential links to migration, reproductive behavior, or periods of increased habitat use. Environmental ordination and ecological network analyses identified distinct fish assemblages associated with Chinese Bahaba occurrence and highlighted several potential indicator species that may assist future monitoring efforts.

This study demonstrates that species-specific qPCR and community-level eDNA metabarcoding provide complementary information. While qPCR allows for the highly sensitive detection of rare species, metabarcoding simultaneously informs us about the surrounding fish community. Integrating these approaches allowed the ecological context of Chinese Bahaba detections to be investigated, providing insights into associated fish assemblages and environmental conditions that would not have been possible using either method independently.

Overall, the project has significantly improved our understanding of Chinese Bahaba ecology within western Hong Kong waters while establishing a robust and transferable eDNA monitoring framework for threatened marine fishes. The findings provide valuable baseline information and eDNA approach for future biodiversity monitoring, evaluation of restocking programs, identification of priority conservation habitats, and long-term management of fish communities within Hong Kong and the Pearl River Estuary.

(vi) Summary and Way Forward

Overall, all planned project activities were successfully completed within the proposed timeframe. The project successfully developed and validated the first species-specific qPCR assays for Chinese Bahaba, completed six rounds of seasonal eDNA sampling, generated comprehensive fish biodiversity datasets using dual-primer metabarcoding, and completed the associated bioinformatic, ecological, and statistical analyses which provide a comprehensive eDNA-based assessments of fish biodiversity and Chinese Bahaba ecology conducted in western Hong Kong waters.

The integration of species-specific qPCR detections with community-level eDNA metabarcoding allowed the investigation of the environmental conditions and fish assemblages associated with Chinese Bahaba occurrence. Multivariate analyses and weighted species co-occurrence network analyses identified environmentally structured fish assemblages and several potential indicator species associated with Chinese Bahaba, providing ecological insights that would not have been possible using targeted qPCR or metabarcoding alone. Although these ecological relationships are based on a limited number of Chinese Bahaba detections and should therefore be considered preliminary, they provide a foundation for future studies on habitat use and species associations.

The datasets, analytical workflows, DNA reference sequences, and validated molecular protocols generated during this project establish an important baseline for future conservation monitoring in Hong Kong and the Pearl River Estuary. Future work should focus on long-term and multi-year monitoring to determine whether the observed associations with higher temperatures, environmental gradients, and co-occurring fish assemblages remain consistent across years. Expanding surveys throughout the Pearl River Estuary and adjacent coastal waters would improve understanding of migration patterns and habitat connectivity, while repeated monitoring before and after future restocking programs could provide a sensitive, non-invasive method for evaluating conservation effectiveness. Future studies should also validate the identified indicator species and ecological assemblages using larger datasets, develop predictive habitat models by integrating environmental variables with eDNA observations, and further investigate the ecological mechanisms that drive Chinese Bahaba occurrence. This study establishes a framework for monitoring Chinese Bahaba and other threatened marine fishes and supporting evidence-based conservation planning, fisheries management, and biodiversity monitoring throughout the region.

References

- Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). Environmental DNA (eDNA) From the Wake of the Whales: Droplet Digital PCR for Detection and Species Identification. *Frontiers in Marine Science*, 5, 133. <https://doi.org/10.3389/fmars.2018.00133>
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Chen, Y., Guo, Y., Li, M., & Zhang, X. (2025). Decadal population depletion, size class reduction, and range contraction of the giant yellow croaker in China: Implications for conservation and management. *Ocean & Coastal Management*, 265, 107659. <https://doi.org/10.1016/j.ocecoaman.2025.107659>
- Ficetola, G. F., Taberlet, P., & Coissac, E. (2016). How to limit false positives in environmental DNA and metabarcoding? *Molecular Ecology Resources*, 16(3), 604–607. <https://doi.org/10.1111/1755-0998.12508>
- Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences*, 286(1915), 20191409. <https://doi.org/10.1098/rspb.2019.1409>
- He, X., Jeffery, N. W., Stanley, R. R. E., Hamilton, L. C., Rubidge, E. M., & Abbott, C. L. (2023). eDNA metabarcoding enriches traditional trawl survey data for monitoring biodiversity in the marine environment. *ICES Journal of Marine Science*, 80(5), 1529–1538. <https://doi.org/10.1093/icesjms/fsad083>
- Hong, C. G., Kim, K. Y., Heo, J. S., Choi, B. H., Park, J. H., Kim, S. Y., & Yang, S. G. (2025). Molecular Monitoring of *Leiocassis longirostris* Using Species-Specific qPCR Assays from Environmental DNA. *Animals*, 15(23), 3451. <https://doi.org/10.3390/ani15233451>
- Ip, J. C., Loke, H., Yiu, S. K. F., Zhao, M., Li, Y., Lin, Y., How, C., Mo, J., Yan, M., Cheng, J., Lai, V. C., Chan, L. L., Leung, K. M. Y., & Qiu, J. (2024). Bottom Trawling and Multi-Marker eDNA Metabarcoding Surveys Reveal Highly Diverse Vertebrate and Crustacean Communities: A Case Study in an Urbanized Subtropical Estuary. *Environmental DNA*, 6(6), e70031. <https://doi.org/10.1002/edn3.70031>
- Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, 2(3), 271–282. <https://doi.org/10.1002/edn3.29>
- Kralik, P., & Ricchi, M. (2017). A Basic Guide to Real Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.00108>

- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, 2(7), 150088. <https://doi.org/10.1098/rsos.150088>
- Osathanunkul, M., & Suwannapoom, C. (2023). Sustainable fisheries management through reliable restocking and stock enhancement evaluation with environmental DNA. *Scientific Reports*, 13(1), 11297. <https://doi.org/10.1038/s41598-023-38218-2>
- Osathanunkul, M., & Suwannapoom, C. (2025). eDNA confirms presence of critically endangered big-headed turtle to support community conservation. *Biological Conservation*, 310, 111329. <https://doi.org/10.1016/j.biocon.2025.111329>
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). REVIEW: The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51(5), 1450–1459. <https://doi.org/10.1111/1365-2664.12306>
- Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). ecoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, 39(21), e145. <https://doi.org/10.1093/nar/gkr732>
- Robinson, C. V., Garcia De Leaniz, C., Rolla, M., & Consuegra, S. (2019). Monitoring the eradication of the highly invasive topmouth gudgeon (*Pseudorasbora parva*) using a novel eDNA assay. *Environmental DNA*, 1(1), 74–85. <https://doi.org/10.1002/edn3.12>
- Sadovy, Y., & Cheung, W. L. (2003). Near extinction of a highly fecund fish: The one that nearly got away. *Fish and Fisheries*, 4(1), 86–99. <https://doi.org/10.1046/j.1467-2979.2003.00104.x>
- Sahu, A., Singh, M., Amin, A., Malik, M. M., Qadri, S. N., Abubakr, A., Teja, S. S., Dar, S. A., & Ahmad, I. (2025). A systematic review on environmental DNA (eDNA) Science: An eco-friendly survey method for conservation and restoration of fragile ecosystems. *Ecological Indicators*, 173, 113441. <https://doi.org/10.1016/j.ecolind.2025.113441>
- Thalinger, B., Deiner, K., Harper, L. R., Rees, H. C., Blackman, R. C., Sint, D., Traugott, M., Goldberg, C. S., & Bruce, K. (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA*, 3(4), 823–836. <https://doi.org/10.1002/edn3.189>
- Tsuji, S., Iguchi, Y., Shibata, N., Teramura, I., Kitagawa, T., & Yamanaka, H. (2018). Real-time multiplex PCR for simultaneous detection of multiple species from environmental DNA: An application on two Japanese medaka species. *Scientific Reports*, 8(1), 9138. <https://doi.org/10.1038/s41598-018-27434-w>
- Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T., & Yamanaka, H. (2017). Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. *PLoS ONE*, 12. <https://doi.org/10.1371/journal.pone.0176608>

- Ushio, M., Ozawa, S., Oka, S., Sado, T., Kisero, R. O., Porter, L., Matrai, E., & Miya, M. (2025). μ Ceta: A Set of Cetacean-Specific Primers for Environmental DNA Metabarcoding With Minimal Amplification of Non-Target Vertebrates. *Environmental DNA*, 7(5), e70193. <https://doi.org/10.1002/edn3.70193>
- Wang, Y., Hu, M., Sadovy, Y., Cheung, S., & Shin, P. (2009). Threatened fishes of the world: Bahaba taipingensis Herre, 1932 (Sciaenidae). *Environmental Biology of Fishes*, 85, 335–336. <https://doi.org/10.1007/s10641-009-9507-2>
- Yang, W., Hu, W., Chen, B., Tan, H., Su, S., Ding, L., Dong, P., Yu, W., & Du, J. (2023). Impact of climate change on potential habitat distribution of Sciaenidae in the coastal waters of China. *Acta Oceanologica Sinica*, 42(4), 59–71. <https://doi.org/10.1007/s13131-022-2053-x>
- Yu, D., Shen, Z., Chang, T., Li, S., & Liu, H. (2021). Using environmental DNA methods to improve detectability in an endangered sturgeon (*Acipenser sinensis*) monitoring program. *BMC Ecology and Evolution*, 21(1), 216. <https://doi.org/10.1186/s12862-021-01948-w>
- Zhang, S., Zhao, J., & Yao, M. (2020). A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. *Methods in Ecology and Evolution*, 11(12), 1609–1625. <https://doi.org/10.1111/2041-210X.13485>
- Zhao, L., Gao, T., & Lu, W. (2015). Complete mitochondrial DNA sequence of the endangered fish (*Bahaba taipingensis*): Mitogenome characterization and phylogenetic implications. *ZooKeys*, 546, 181–195. <https://doi.org/10.3897/zookeys.546.5964>
- Zou, K., Chen, J., Ruan, H., Li, Z., Guo, W., Li, M., & Liu, L. (2020). eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Science of The Total Environment*, 702, 134704. <https://doi.org/10.1016/j.scitotenv.2019.134704>