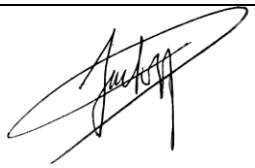
Marine Ecology Enhancement Fund (MEEF)
MEEF2024012 Completion Report

Funding Scheme:	Marine Ecology Enhancement Fund (MEEF)			
Project Number:	MEEF2024012			
Project Title:	Exploring the mystery of Chinese Bahaba in Hong Kong western waters using revolutionary eDNA techniques			
Name of Organisation:	Lingnan University			
Reporting Period:	From:	01/11/2024	To:	30/06/2025
Date of Report Submission:	30/06/2025			
Project Leader:	Dr. Chi-Ho IP, Assistant Professor			
Signature:				
Official Chop of Organisation, 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 authorisation 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 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.

We successfully developed species-specific primer assays for the Chinese Bahaba targeting four gene regions of the mitochondrial genome: 12S rRNA (12S), 16S rRNA (16S), NADH dehydrogenase subunit 5 (ND5), and D-loop (DLP). Their effectiveness was demonstrated through both *in-silico* (i.e., primer-BLAST) and *in-vitro* (i.e., closely related fish tissue DNA, tissue/synthetic DNA and aquaculture eDNA of Chinese Bahaba) validations, confirming the suitability of these primer sets for upcoming experiments.

From January to June 2025, five fieldwork sessions were conducted as scheduled: one in the dry season (January) and four in the wet season (from March to June). Water filtering and eDNA extraction for most samples were performed in UV hoods at the Lingnan University laboratory. A total of 345 eDNA samples were processed and subjected to qPCR assays for the Chinese Bahaba, alongside negative (autoclaved MQ water) and positive controls (tissue and synthetic Chinese Bahaba DNA). Additionally, we tested the eDNA metabarcoding methodology with new index barcodes and universal fish primers using both tissue and eDNA samples. An optimized data analysis workflow has also been successfully established, preparing us to proceed with Phase 2 (MEEF2024012A).

Highlights of key results

- Recruitment of essential research staff for the project.
- Completed project planning and scheduling for field and laboratory activities.
- Developed and tested four Chinese Bahaba specific primers and 2 barcoded universal fish primers for metabarcoding.
- Conducted eDNA water sampling with 345 seawater samples collected in total.
- Finished qPCR for Chinese Bahaba detecting of all 345 samples.
- Initiated eDNA metabarcoding analysis.
- Established a foundational protocol for ongoing eDNA monitoring efforts.
- Presented the MEEF project in South Asia Forum 2024 and the 7th conference on China' environmental impact assessment.

(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 (*B. 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.

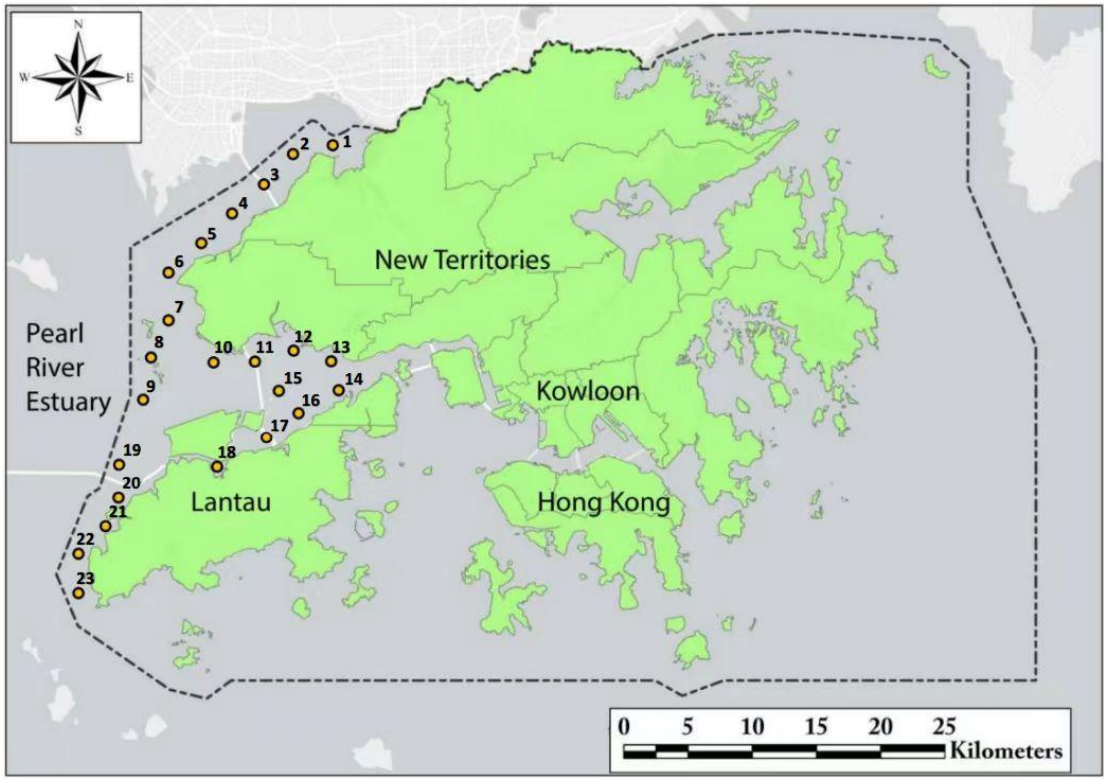


Figure 1. Map of the waters of Hong Kong and 23 sampling sites (green) of this study.

(iii) Completed activities against the proposed Work Schedule;

Table 1. The Funded Project work plan.

No.	Activity / Task for the Project	Date (Scheduled)	Details of the Activity
1	Project planning and recruitment (Phase 1)	Nov 2024 (**completed in Phase 1**)	Recruitment of one full-time and one part-time Research Assistant to implement this project. Scheduling and planning the field and wetlab items and consumables. This activity was completed and both RA and Part RA reported duty in Nov 2024.
2	Task 1 – Water sampling and qPCR (Phase 1 & 2)	Jan – Jun 2025 (**completed in Phase 1**) Sep 2025 (continuous in Phase 2)	Conducting field trips to collect water samples in western waters, completing according eDNA experiments and qPCR analysis for Task 1. In Phase 1, we completed five rounds of sampling for eDNA extraction and qPCR analysis. The sixth round, scheduled for September 2025, will continue into Phase 2.
3	Task 2-eDNA metabarcoding (Phase 1 & 2)	May-Jun 2025 (**completed in Phase 1**) July - Dec 2025 (continuous in Phase 2)	Conducting eDNA metabarcoding and integrating the data of Chinese Bahaba to investigate spatial-temporal distribution and species interaction for Task 2. In Phase 1, we successfully utilized the eDNA metabarcoding approach (see the details in Part iv). We will continue and complete Task 2 in Phase 2.

During the reporting period from November 1, 2024, to June 30, 2025, several key activities were successfully carried out to advance the project. From November to December 2024, the team focused on recruiting the Research Assistant, setting up the wetlab, and developing and validating the species-specific primers for Chinese Bahaba. In January 2025, we completed the first round of field sampling, including the collection and filtration of seawater samples. Following this, from January to February 2025, we conducted the first round of eDNA extraction, PCR amplification, and prepared the samples for Illumina sequencing. From March to June 2025, four surveys in wet season were successfully conducted, as well as the eDNA extraction and corresponding qPCR experiments. These activities mark a significant step forward in our ongoing research efforts.

Summary of Key Outcomes

- Recruitment of essential research staff for the project.
- Completed project planning and scheduling for field and laboratory activities.
- Developed and tested Chinese Bahaba specific primers and universal fish primers for metabarcoding.
- Conducted eDNA water sampling.
- Initiated Chinese Bahaba eDNA detecting and eDNA metabarcoding analysis
- Established a foundational protocol for ongoing eDNA monitoring efforts.
- Presented the MEEF project in two conferences.

Overall, progress was made from November 2024 to June 2025 concerning the work plan and outcomes. Notably, we developed Chinese Bahaba-specific eDNA primers and tested their specificity for **Task 1**. Additionally, we evaluated the performance of universal fish eDNA

primers for **Task 2**. These findings are crucial for the successful implementation of the entire project, ensuring that we meet the objectives of both Phase 1 and Phase 2. **Detailed results are presented in Section (iv).**

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

During the reporting period from **November 1, 2024**, to **June 30, 2025**, the following key activities were conducted:

- (November 2024 – December 2024): RA recruitment, wetlab items & consumables preparation, design & validation for species-specific primer of Chinese Bahaba
- (January 2025 – February 2025): Round 1 (dry season survey) field sampling, seawater filtering, eDNA extraction, PCR
- (March 2025 – June 2025): Round 2–5 (wet season survey) field sampling, seawater filtering, eDNA extraction, qPCR

1. Species-Specific Primer Design (Nov 2024 – Dec 2024)

To design species-specific primers for the detection of Chinese Bahaba (*Bahaba taipingensis*) eDNA, we selected and aligned the mitochondrial DNA (mtDNA) of Chinese Bahaba with that of 10 related species (*Collichthys lucidus*, *C. niveatus*, *Larimichthys crocea*, *L. polyactis*, *Parapristipoma trilineatum*, *Dendrophysa russelii*, *Pennahia argentata*, *Miichthys miiuy*, *Nibea albiflora*, and *N. coibor*). Then, Geneious Prime software was used to design the species-specific primers targeting the 12S-rRNA (12S), 16S-rRNA (16S), NADH dehydrogenase subunit 5 (ND5), and D-loop (DLP) gene regions (**Table 2; Figure 2**).

Table 2. Information on designed Chinese Bahaba's primers

Name	Sequence	Length	GC%	Tm	
Bhb_16S_F	CTTTAGACACCAAGACAGACCA	22	45.45	55.81	From Dr. Wang (Co-I)
Bhb_16S_R	TAAGAGCAGGTGGTGCTCTCA	21	52.38	57.57	
Bhb_DLP_F	TGTCACACATTCATACACCACCAT	24	41.67	56.15	
Bhb_DLP_R	CTTATGGACGGAACTGTTAGGAGC	24	50	59.57	
Bhb_ND5_F	CCTAGCCTACCCCATCCTTACAA	23	52.17	59.55	**This study**
Bhb_ND5_R	AGGAAAAGGGGAAGGAGGCTTAT	23	47.83	57.77	
Bhb_12S_F	TGTTGAAATACACGTCTGAAGGAGG	25	44	57.94	
Bhb_12S_R	CTGAGTTCAATTAGTTCGGGGAGA	24	45.83	57.86	

Figure 2. Alignment and primer design of the **(A)** 12S-rRNA, **(B)** 16S-rRNA, **(C)** NADH dehydrogenase subunit 5, and **(D)** D-loop gene regions.

To evaluate the amplification efficiency and specificity of the designed primers for Chinese Bahaba. The designed primers were first validated *in silico* using the NCBI Primer-BLAST tool to assess their specificity and potential off-target binding. Then, six closely related species (*L. crocea, C. lucidus, J. belangerii, P. argentata, N. albiflora, and N. squamosa*) were selected for *in-vitro* experimental validation. PCR amplification tests were performed in the laboratory using DNA extracted from these species, with Chinese Bahaba DNA serving as the target control. PCR products were analyzed through gel electrophoresis to evaluate amplification success and specificity.

The *in-silico* validation using Primer-BLAST confirmed that the designed primers successfully aligned with Chinese Bahaba mtDNA sequences as the top match (**Figure 3**). While some closely related species showed secondary alignments, significant nucleotide differences were observed in the binding regions, supporting the specificity of the primers.

Figure 3. Primer-BLAST result of **(A)** 12S-rRNA, **(B)** 16S-rRNA, **(C)** NADH dehydrogenase subunit 5, and **(D)** D-loop primers.

PCR analysis using fish tissue DNA demonstrated that all primer pairs successfully amplified DNA from the Chinese Bahaba. Non-specific amplifications were observed in 16S primer for *C. lucidus* and ND5 primer for *L. crocea*. After modifying the PCR conditions, annealing temperature at 57°C for 5 seconds, we were able to eliminate these non-specific amplification signals (**Figure 4**).

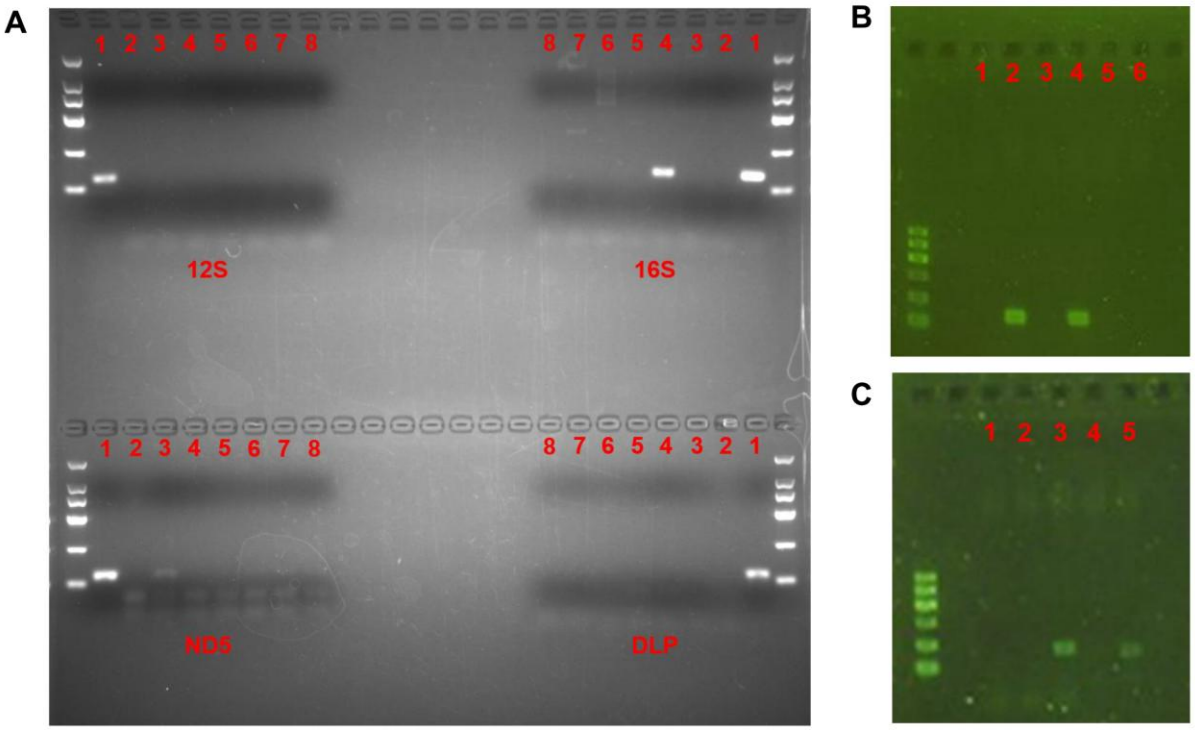
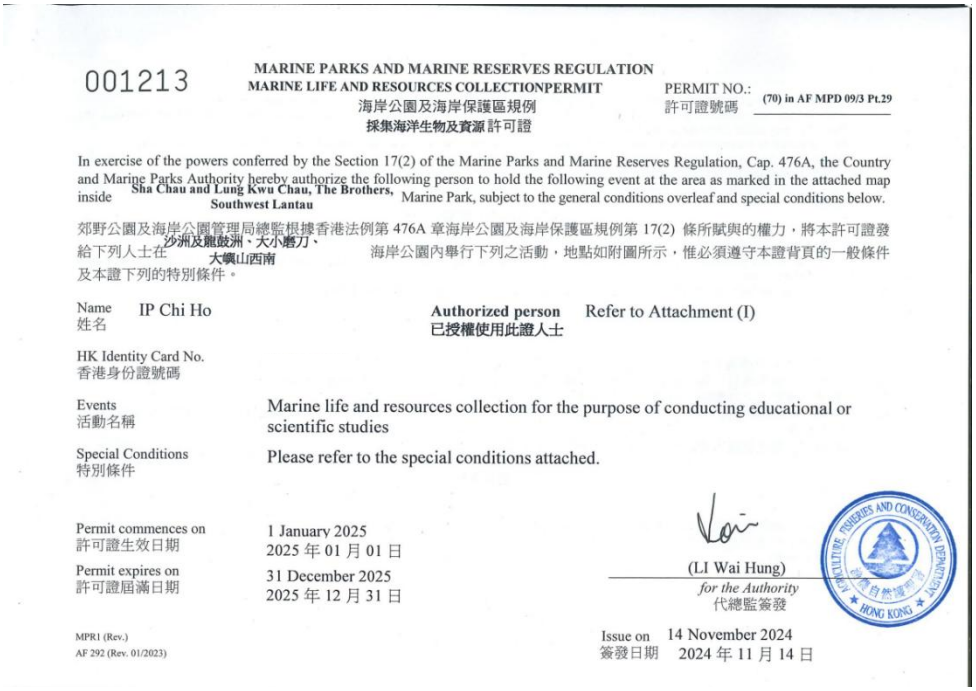


Figure 4. Gel electrophoresis of four species-specific primers. **(A)** #1 to #8: Positive control (*Bahaba taipingensis*), Negative control (MQ water), *Larimichthys crocea*, *Collichthys lucidus*, *Johnius belangerii*, *Pennahia argentata*, *Nibea albiflora*, and *N. squamosa*. **(B)** #1 to #6: 12S-Negative control, 12S- *B. taipingensis*, ND5-Negative control, ND5- *B. taipingensis*, ND5- *L. crocea*, 12S- *L. crocea*. **(C)** #1 to #5: 16S-Negative control, 16S- *C. lucidus*, 16S- *B. taipingensis*, DLP-Negative control, DLP- *B. taipingensis*.

2. First eDNA survey in western Hong Kong waters – Dry Season (Jan 2025 – Feb 2025)

To detect Chinese Bahaba eDNA in the western waters of Hong Kong and to gain a comprehensive understanding of the ecosystem associated with the Chinese Bahaba, we obtained the ACFD permit for water sampling in the marine park (**Figure 5**). We also rented the P4 boat for water sampling from Inner Deep Bay to southwestern Lantau, and completed water filtering in UV hoods at the Lingnan University laboratory (**Figure 6**).



001213

MARINE PARKS AND MARINE RESERVES REGULATION
MARINE LIFE AND RESOURCES COLLECTIONPERMIT
海岸公園及海岸保護區規例
採集海洋生物及資源許可證

PERMIT NO.: (70) in AF MPD 09/3 Pt.29
許可證號碼

In exercise of the powers conferred by the Section 17(2) of the Marine Parks and Marine Reserves Regulation, Cap. 476A, the Country and Marine Parks Authority hereby authorize the following person to hold the following event at the area as marked in the attached map inside Sha Chau and Lung Kwu Chau, The Brothers, Southwest Lantau Marine Park, subject to the general conditions overleaf and special conditions below.

郊野公園及海岸公園管理局總監根據香港法例第 476A 章海岸公園及海岸保護區規例第 17(2) 條所賦與的權力，將本許可證發給下列人士在沙洲及龍鼓洲、大小磨刀、大嶼山西南海岸公園內舉行下列之活動，地點如附圖所示，惟必須遵守本證背頁的一般條件及本證下列的特別條件。

Name 姓名: IP Chi Ho

Authorized person 已授權使用此證人士: Refer to Attachment (I)

HK Identity Card No. 香港身份證號碼:

Events 活動名稱: Marine life and resources collection for the purpose of conducting educational or scientific studies

Special Conditions 特別條件: Please refer to the special conditions attached.

Permit commences on 許可證生效日期: 1 January 2025 2025 年 01 月 01 日

Permit expires on 許可證屆滿日期: 31 December 2025 2025 年 12 月 31 日

(LI Wai Hung)
for the Authority
代總監簽發

AGRICULTURE, FISHERIES AND CONSERVATION DEPARTMENT 漁農自然護理署 HONG KONG

MPR1 (Rev.)
AF 292 (Rev. 01/2023)

Issue on 簽發日期: 14 November 2024 2024 年 11 月 14 日

Figure 5. AFCD permit for this MEEF project for one-year eDNA survey.

Figure 6. Fieldwork for water sampling and water filtering in the laboratory.

We have successfully eDNA extraction, and eDNA PCR testing in UV hoods (**Figure 7**). To further test the Chinese Bahaba-specific primer and universal primers, we conducted PCR analysis using pooled eDNA samples for a more cost-effective approach (**Figure 8**). The results indicated no detection of Chinese Bahaba eDNA in the dry season samples, aligning with Dr. Wang's findings in the PRE (submitted manuscript). Overall, we have further confirmed the

specificity of our Chinese Bahaba-specific primers, showing no non-target amplification in the eDNA samples, and successfully amplified using the MiFish-U and 12SV5 primers following the standard protocol.

Figure 7. eDNA extraction and PCR experiment in UV hoods.

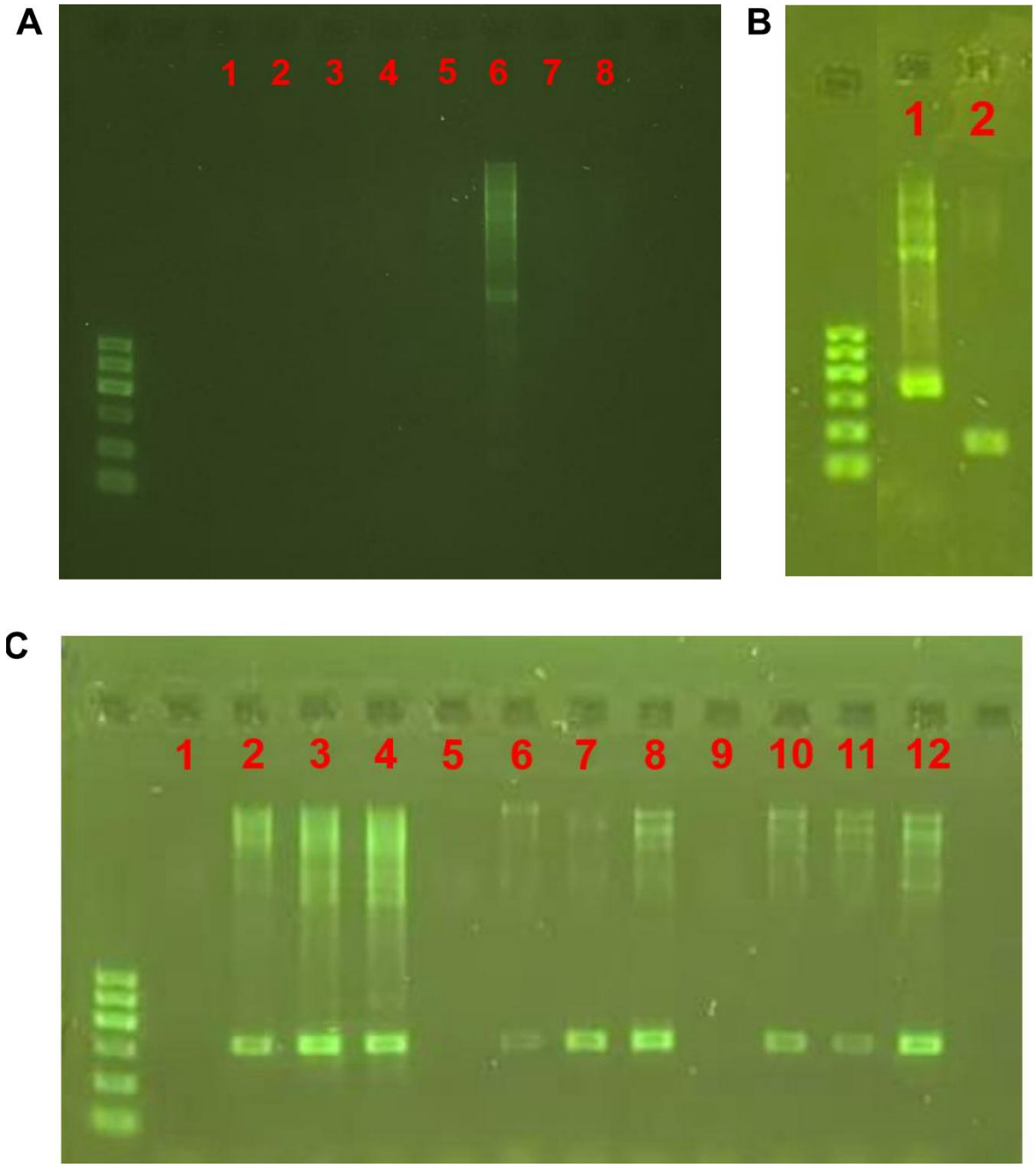


Figure 8. (A) Species-specific PCR results, with #1 to #4: Negative control (MQ water) for 12S, 16S, ND5, and DLP, #5 to #8: Jan 2025 eDNA pooled sample for 12S, 16S, ND5, and DLP. **(B)** PCR test result of #1: Mifish-U and #2: 12SV5, using the pooled eDNA. **(C)** Testing of indexed MiFish-U primer with #1, #5, and #9 as field controls.

3. Wet-Season eDNA surveys in western Hong Kong waters (March 2025 – June 2025)

From March to June, as known as the migration period of Chinese Bahaba, we successfully conducted monthly water sampling, water filtering, and eDNA extraction (**Figure9**), following the same protocol with former dry season survey.

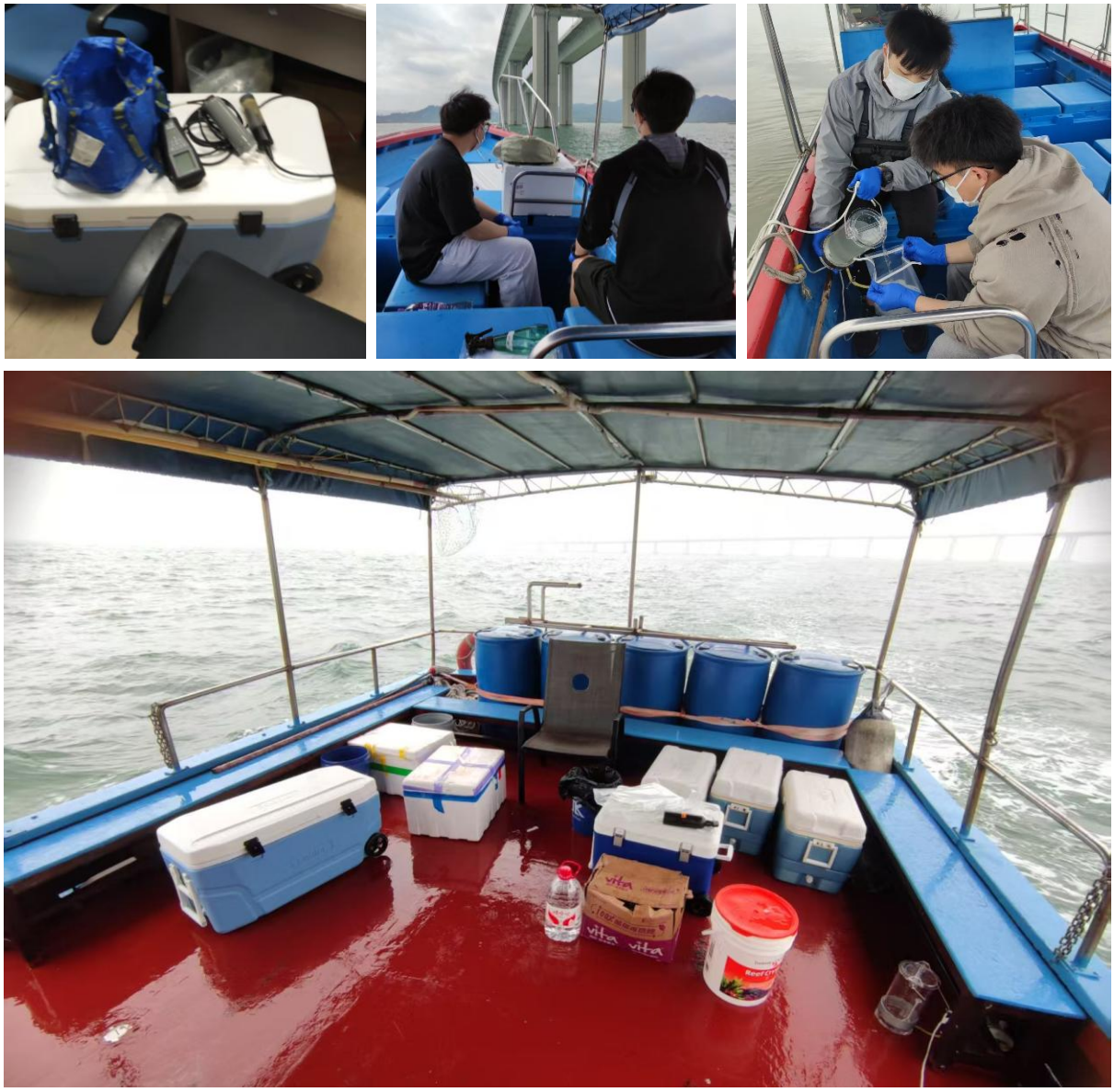


Figure 9. Fieldwork for water sampling during wet season.

After obtaining eDNA samples from wet season, we performed qPCR experiments for testing the effectiveness of our species-specific primer sets for qPCR method and in real eDNA samples, before exploring whether Chinese Bahaba eDNA exists in our field samples.

For the test qPCR experiment, we first conducted qPCR for eDNA sampled from the breeding pond of Chinese Bahaba in the aquaculture farm, with results showed the effectiveness of our primer sets (**Figure 10**); then, we divided samples from Round 4 (May survey) into 3 groups: Deep Bay (S1–S9, DB), The Brothers marine park (S10–S18, TB), and Tai Ao (S19–S23, TA); after that, pooled eDNA samples of each group were made, and different amount of Chinese Bahaba tissue DNA were added into these pooled samples, to prepare the dilution series samples with the DNA concentration of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} ng/μL, simulating natural and authentic state of Chinese Bahaba eDNA in west Hong Kong waters. Test qPCR result

confirmed the specificity of our Chinese Bahaba-specific primers, as well as their high sensitivity, in qPCR method (**Figure 11**).

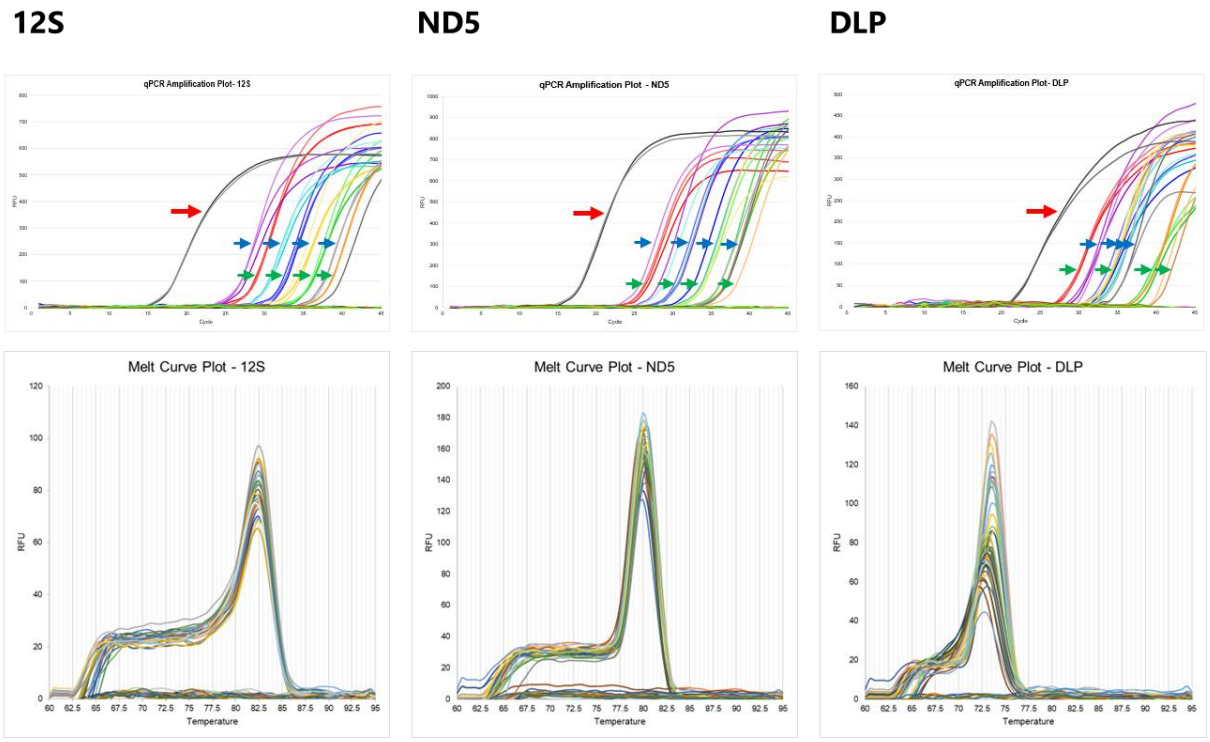


Figure 10. Test qPCR results of 3 primer sets designed in this study, with red arrow indicates amplification curve of positive control (Chinese Bahaba tissue DNA), and blue and green arrow shows the synthesis Chinese Bahaba DNA and breeding pond eDNA, respectively

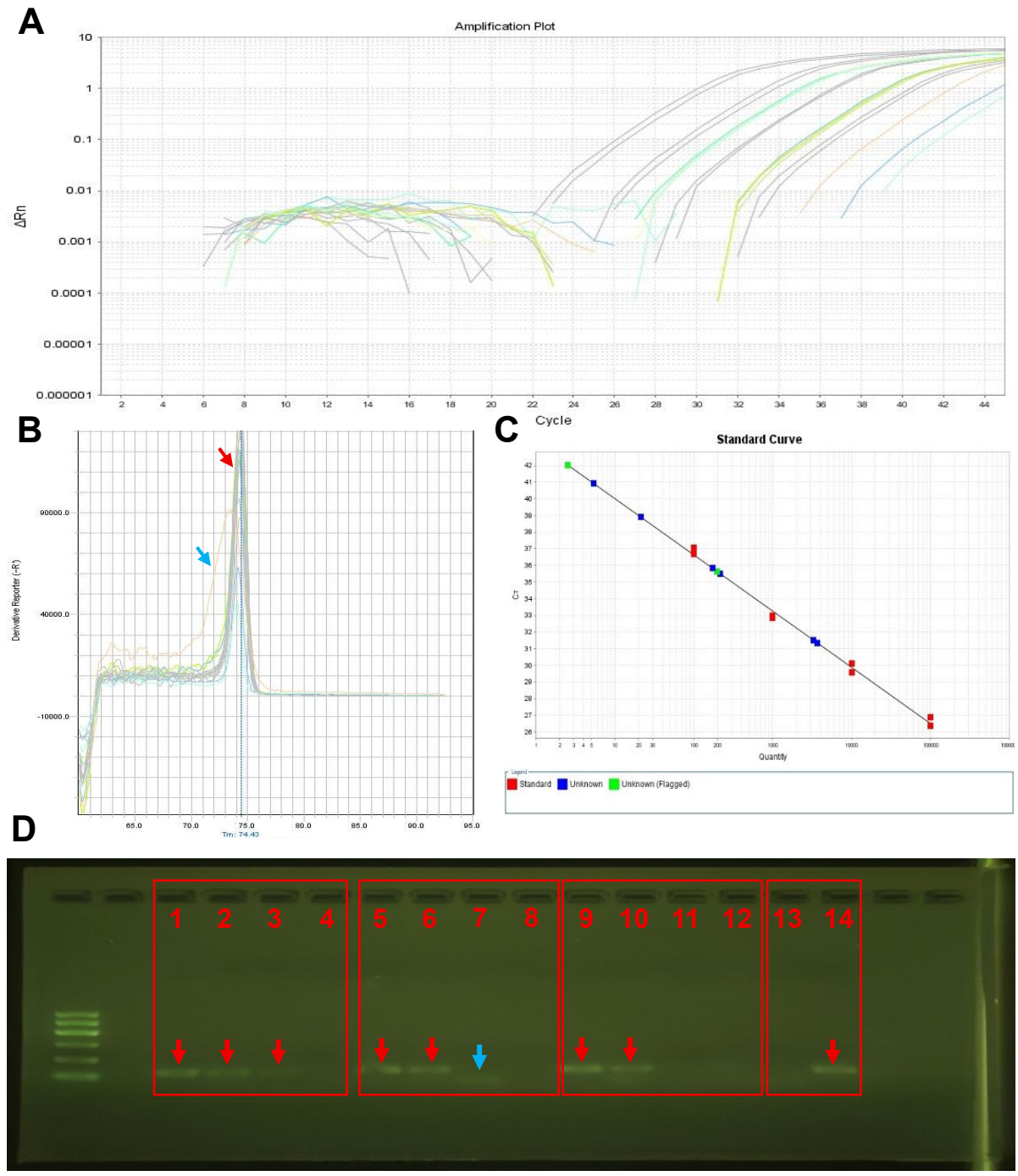


Figure 11. Test qPCR results of DLP primer set. **(A)** Amplification plot. **(B)** Melt curve plot, showing the identical melt curves were generated from most of test samples (coloured) and standard DNA (grey). **(C)** Standard Curve plot shows the lowest detecting limit of DLP primer has reached to ~5 copy/μL. **(D)** Gel electrophoresis result of test samples analysed above. with #1 to #4: DB with dilution series of Chinese Bahaba tissue DNA; #5 to #8: TB with same treatment; #9 to #12: TA with same treatment; #13 & #14: negative control (MQ water) and positive control (standard DNA). Blue arrow in plot B and D towards a different product in real sample, which is considered to be the primer dimer occurs occasionally when concentration of target DNA is too low.

To date, qPCR assays have been performed on all 345 field samples from rounds 1–5, collected during daytime high tide under clear weather conditions (**Figure 12; Table 3**). no Chinese Bahaba signal was detected (**Figure 13; Table 4**).

To ensure that the absence of detection was not due to methodological variation or human error, we standardized the sampling approach across all surveys to minimize potential inconsistencies (Figure 12; Table 3). Specifically, we ensured that: (1) all water samples were collected during daytime high tide (09:00–15:00) under clear weather conditions; (2) a consistent 2 L water volume was filtered for every sample to ensure representative biomass capture; and (3) a strict 8-hour cold-chain protocol (from collection to −80°C storage) was implemented to mitigate DNA degradation. The robustness of these results is further supported by the high sensitivity of our qPCR assay (approximately 6.58×10^{-10} ng/μL), which enables reliable detection of low-concentration eDNA signals even in complex estuarine environments.

Regarding environmental factors, we performed a Principal Component Analysis (PCA) on seven water quality parameters — Temperature (°C), Dissolved Oxygen (%), Dissolved Oxygen (mg/L), Conductivity (mS/cm), Total Dissolved Solids (mg/L), Salinity (ppt), and pH — measured during sampling. The analysis revealed significant differences among surveys (rounds) and sites (PERMANOVA $p < 0.01$; **Figure 14**), confirming strong seasonal and spatial variation in the western Hong Kong waters. The vector plot indicates that temperature, TDS and pH were the primary drivers of variation in environmental conditions, while the DO and pH contributed the difference with inner Deep Bay – Mai PO and other sites. Thus, environmental conditions alone are unlikely to fully explain the observed pattern.

The distribution of Chinese Bahaba is strongly associated with brackish estuarine habitats, where adults form large spawning aggregations. Adults are benthopelagic, typically inhabiting shallow coastal seas (<30–60 m depth), subtidal beds, and rocky shores. After spawning, adults tend to move offshore, while juveniles remain in nutrient-rich estuarine nursery grounds (Chen et al., 2025). Historically, the western waters of Hong Kong have served as an important fishing ground for Chinese Bahaba during their spawning migration from March to July (Sadovy and Cheung, 2003). However, no eDNA signals were detected throughout the Phase 1 project period. This absence is consistent with a parallel study by Co-I Dr. Wang, which similarly reported no detections across the Pearl River Estuary (PRE), from the inner PRE and the Dongguan Municipal Chinese Bahaba Nature Reserve to the outer PRE (manuscript in submission). Collectively, these results suggest that the annual spawning migration of Chinese Bahaba to western Hong Kong waters may have ceased, likely due to prolonged overexploitation and the resulting severe population decline. Overall, our findings highlight the need for a multi-year monitoring programme to determine whether the species has been locally extirpated from the region or whether the lack of detection simply reflects inter-annual variation.

Table 3. Detailed sample accounting table with information of each sample from Round 1 to Round 5.

Round	Date	Sites sampled	Replicates per site	Water filtered per replicate	Total number of water samples (volume)	Number of filtration replicates (total number)	eDNA extracts per replicate (total number)
R1	6/1/2025 7/1/2025	23	3	2L	69 (138L)	1 (69)	1 (69)
R2	11/3/2025 12/3/2025	23	3	2L	69 (138L)	1 (69)	1 (69)
R3	8/4/2025 9/4/2025	23	3	2L	69 (138L)	1 (69)	1 (69)
R4	13/5/2025 14/5/2025	23	3	2L	69 (138L)	1 (69)	1 (69)
R5	3/6/2025 4/6/2025	23	3	2L	69 (138L)	1 (69)	1 (69)

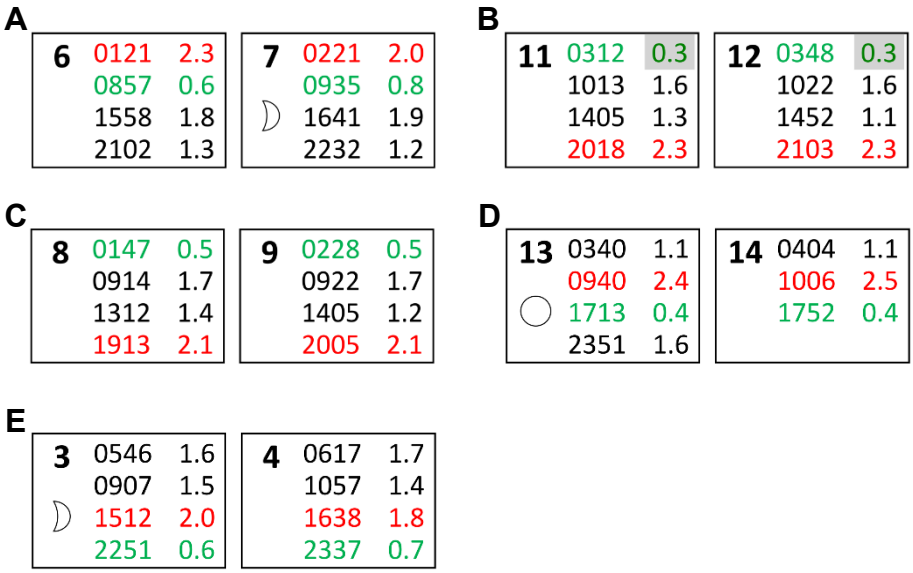
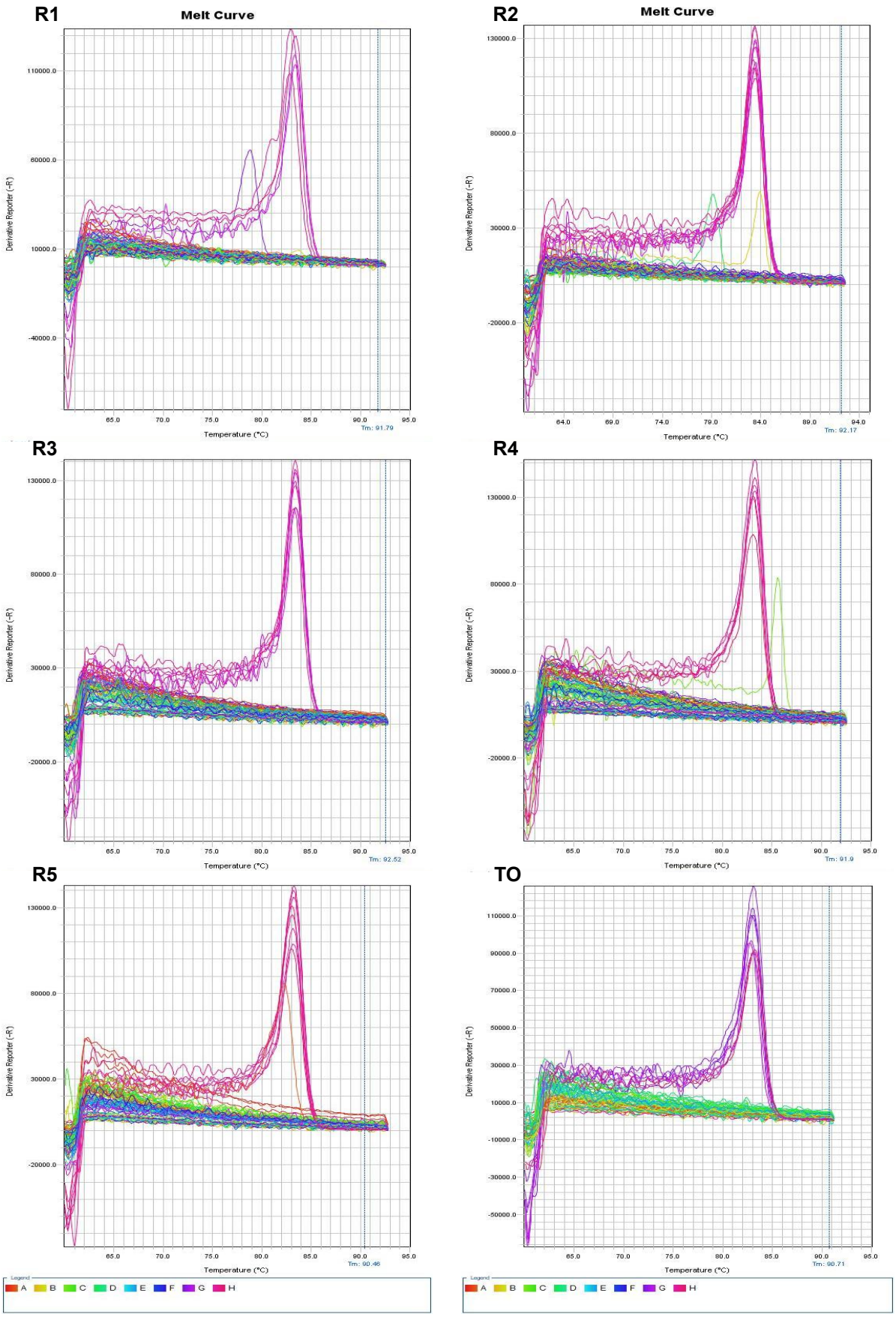


Figure 12. Timetable of high and low tides in western Hong Kong waters (Chek Lap Kok), 2025. Numbers in each cell indicate date, time, and sea level (m), respectively. Letters denote months: A = January, B = March, C = April, D = May, E = June. All samples were collected within a fixed time window (09:00–15:00) during high tide and under comparable weather conditions.

Table 4. Progress of Chinese Bahaba eDNA detecting experiment. “N”: No signal in qPCR result; “-”: Samples to be collected.

Round / Site	R1	R2	R3	R4	R5	R6
S1	N	N	N	N	N	-
S2	N	N	N	N	N	-
S3	N	N	N	N	N	-
S4	N	N	N	N	N	-
S5	N	N	N	N	N	-
S6	N	N	N	N	N	-
S7	N	N	N	N	N	-
S8	N	N	N	N	N	-
S9	N	N	N	N	N	-
S10	N	N	N	N	N	-
S11	N	N	N	N	N	-
S12	N	N	N	N	N	-
S13	N	N	N	N	N	-
S14	N	N	N	N	N	-
S15	N	N	N	N	N	-
S16	N	N	N	N	N	-
S17	N	N	N	N	N	-
S18	N	N	N	N	N	-
S19	N	N	N	N	N	-
S20	N	N	N	N	N	-
S21	N	N	N	N	N	-
S22	N	N	N	N	N	-
S23	N	N	N	N	N	-


R1
Melt Curve
Derivative Reporter (-R')
Temperature (°C)
Tm: 91.79
R2
Melt Curve
Derivative Reporter (-R')
Temperature (°C)
Tm: 92.17
R3
Derivative Reporter (-R')
Temperature (°C)
Tm: 92.52
R4
Derivative Reporter (-R')
Temperature (°C)
Tm: 91.9
R5
Derivative Reporter (-R')
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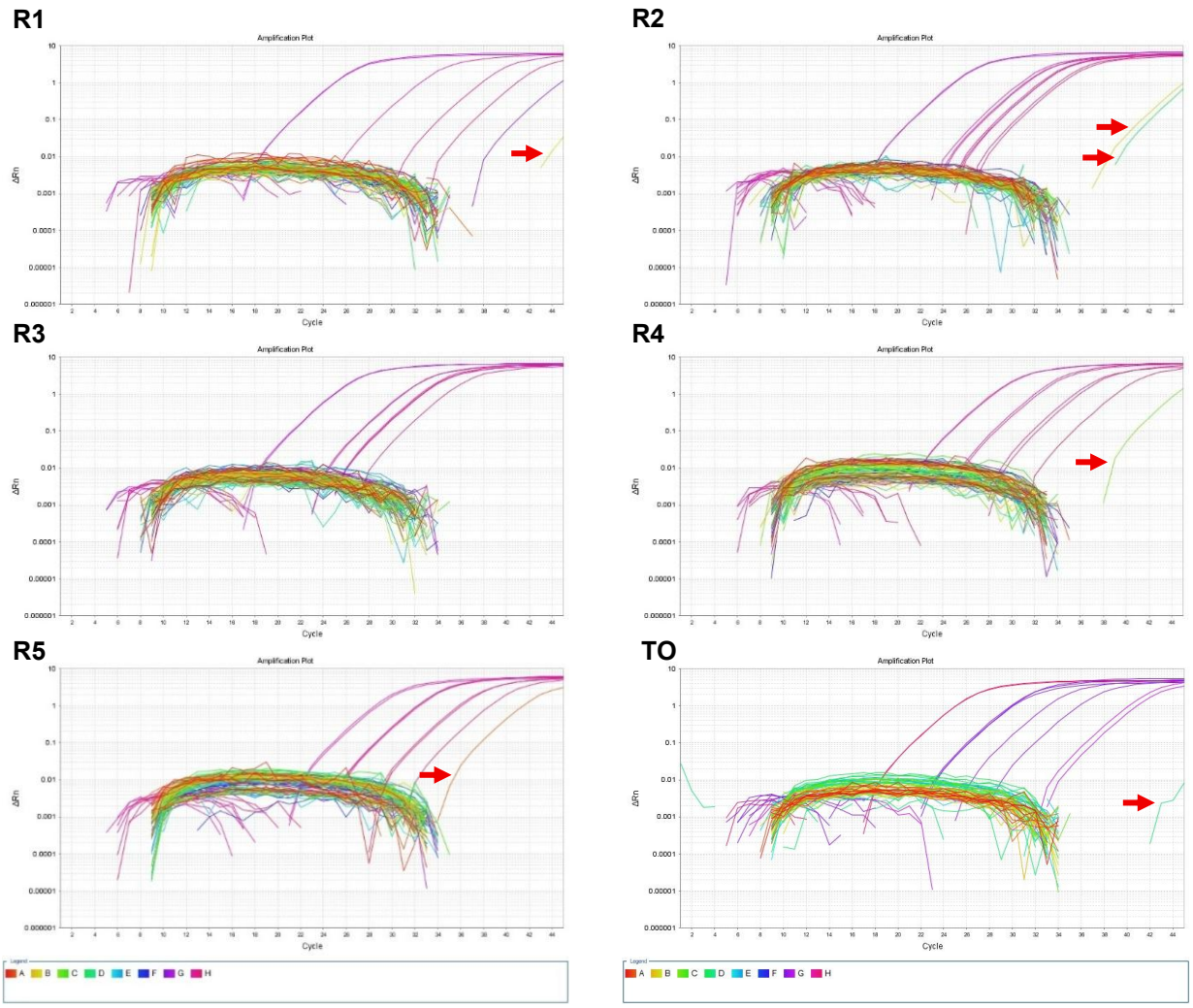


Figure 13. qPCR result for field samples from Round 1-5, TO = Tai O sites from Round 1-5. Amplification curves indicated by red arrows are considered non-target product, due to Ct value lower than threshold or/and different product size compared to reference samples.

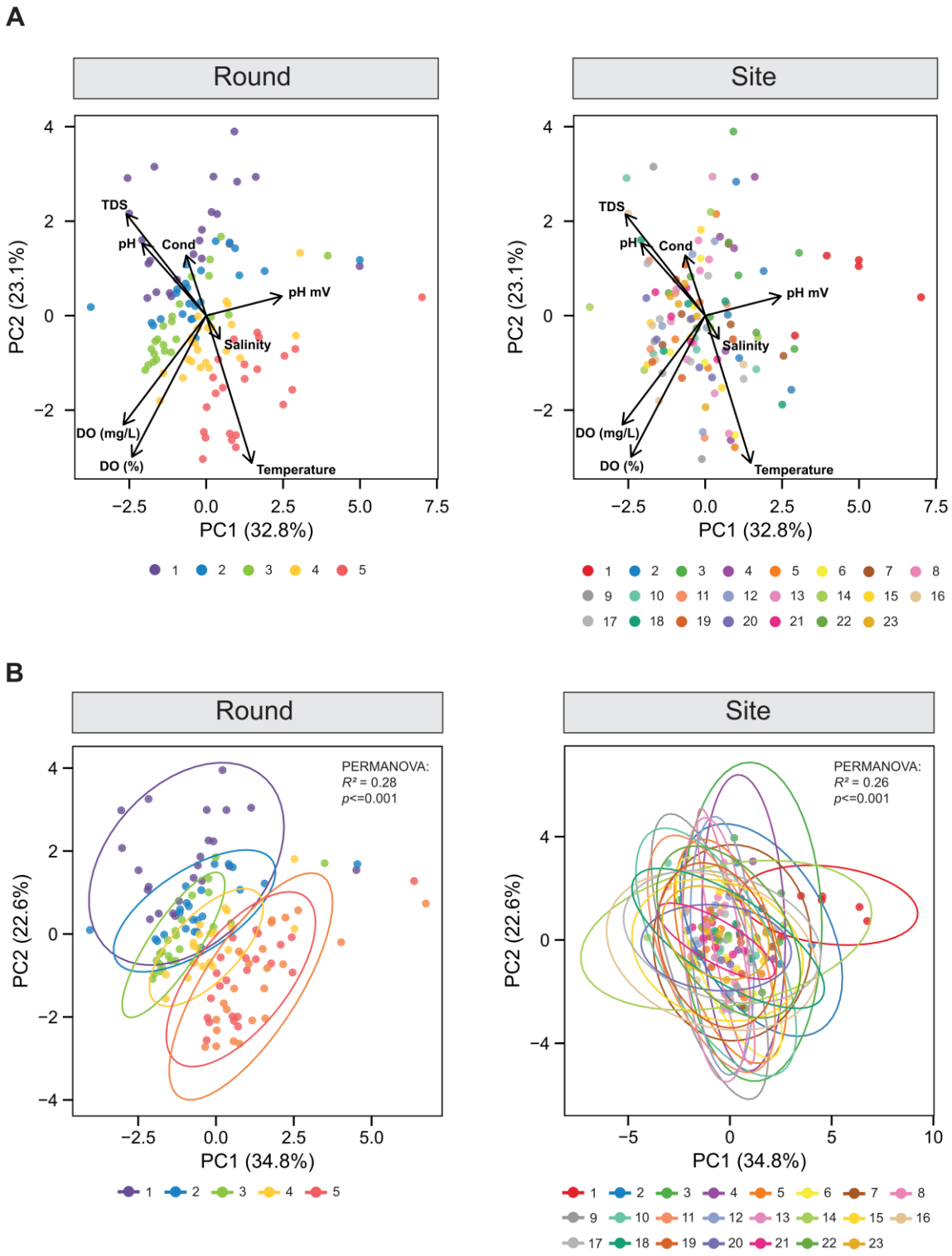


Figure 14. Principal Component Analysis (PCA) based on water quality metrics: Temperature (°C), Dissolved Oxygen (%), Dissolved Oxygen (mg/L), Conductivity (mS/cm), Total Dissolved Solids (mg/L), Salinity (ppt), pH, pH mV between sampling rounds (left) and sites (right). (A) Variable vector plots. (B) Samples colored by sampling round or site with 95% confidence ellipses indicating group clustering with PERMANOVA analysis.

4. eDNA metabarcoding

Since most of seawater samples of this project been successfully collected, preparation for eDNA metabarcoding in **Task 2** needs to be done in advance. As mentioned above, PCR test

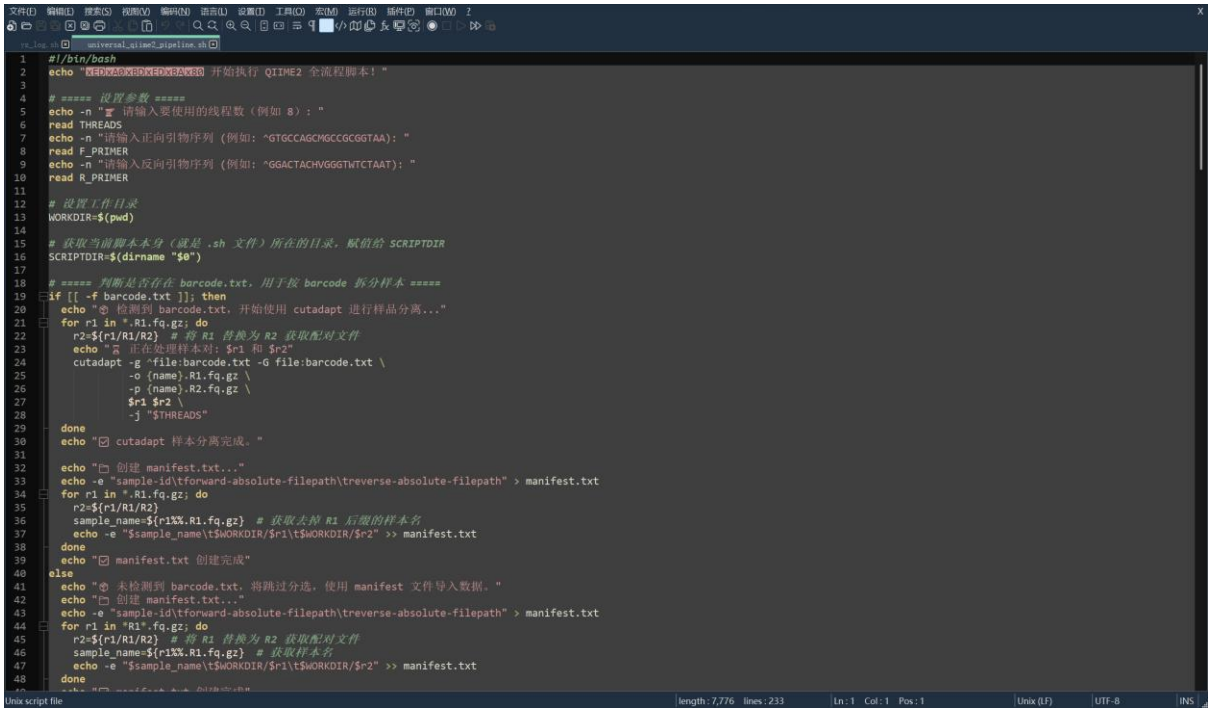
result of Mifish-U and 12S-V5 using the pooled round 1 eDNA shows the effectiveness of these refined universal primers along with other PCR materials in our lab. After confirming target bands by gel electrophoresis, we gel-extracted the PCR products for sequencing.

The first sequencing run (Magigene, Guangdong) showed that the metabarcoding workflow recovered eDNA from many common marine species in western Hong Kong waters, although the total number of detected taxa was low (**Table 5**). To improve taxonomic recovery, we performed a resequencing run at Novogene (Hong Kong) using the library preparation and analysis approach described in Ip et al. (2024; doi:10.1002/edn3.70031). To reduce primer bias and low sequencing rate of rare taxa associated with pooling many eDNA samples, we limited resequencing to a single site (Tai O, S21) from Round 1. Resequencing produced a substantial improvement in detection: we recovered 102 taxa, of which 55 were identified to species level, 29 to genus, and 18 to family (**Table 6**), following the methods in Ip et al. (2024). Notably, we detected two marine mammals — the Chinese white dolphin (*Sousa chinensis*) and the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) — in all three water sample replicates.

In parallel, we optimized a bioinformatics pipeline for sequence data processing, including quality control, trimming, denoising, merging, and taxonomic annotation. Relevant scripts were written and generalized to improve processing efficiency and reproducibility for future field samples (**Figure 14**).

Table 5. Species occurred in test eDNA metabarcoding PCR results

Scientific Name	Mifish-U	12SV5
Acanthopagrus pacificus	√	
Acanthopagrus schlegelii		√
Amblygaster sirm	√	
Butis gymnopomus	√	
Channa bankanensis		√
Chascanopsetta micrognatha	√	√
Cynoglossus bilineatus	√	
Drepane punctata	√	
Johnius belangerii	√	√
Lutjanus rivulatus	√	
Moolgarda cunnesius	√	
Mugil cephalus	√	√
Oostethus brachyurus	√	√
Paraplagusia japonica	√	√
Pristipomoides typus	√	√



```
#!/bin/bash
echo "🚀 开始执行 QIIME2 全流程脚本！"

# ===== 设置参数 =====
echo -n "🧵 请输入要使用的线程数（例如 8）："
read THREADS
echo -n "请输入正向引物序列（例如：^GTGCCAGCMGCCGCGGTAA）："
read F_PRIMER
echo -n "请输入反向引物序列（例如：^GGACTACHVGGGTWTCTAAT）："
read R_PRIMER

# 设置工作目录
WORKDIR=$(pwd)

# 获取当前脚本本身（就是 .sh 文件）所在的目录，赋值给 SCRIPTDIR
SCRIPTDIR=$(dirname "$0")

# ===== 判断是否存在 barcode.txt，用于按 barcode 拆分样本 =====
if [[ -f barcode.txt ]]; then
  echo "🔍 检测到 barcode.txt，开始使用 cutadapt 进行样品分离..."
  for r1 in *.R1.fq.gz; do
    r2=${r1/R1/R2}  # 将 R1 替换为 R2 获取配对文件
    echo "🧬 正在处理样本对：$r1 和 $r2"
    cutadapt -g ^file:barcode.txt -G file:barcode.txt \
             -o {name}.R1.fq.gz \
             -p {name}.R2.fq.gz \
             $r1 $r2 \
             -j "$THREADS"
  done
  echo "☑ cutadapt 样本分离完成。"

  echo "📁 创建 manifest.txt..."
  echo -e "sample-id\tforward-absolute-filepath\treverse-absolute-filepath" > manifest.txt
  for r1 in *.R1.fq.gz; do
    r2=${r1/R1/R2}
    sample_name=${r1%%.R1.fq.gz}  # 获取去掉 R1 后缀的样本名
    echo -e "$sample_name\t$WORKDIR/$r1\t$WORKDIR/$r2" >> manifest.txt
  done
  echo "☑ manifest.txt 创建完成"
else
  echo "🔍 未检测到 barcode.txt，将跳过分选，使用 manifest 文件导入数据。"
  echo "📁 创建 manifest.txt..."
  echo -e "sample-id\tforward-absolute-filepath\treverse-absolute-filepath" > manifest.txt
  for r1 in *R1*.fq.gz; do
    r2=${r1/R1/R2}  # 将 R1 替换为 R2 获取配对文件
    sample_name=${r1%%.R1.fq.gz}  # 获取样本名
    echo -e "$sample_name\t$WORKDIR/$r1\t$WORKDIR/$r2" >> manifest.txt
  done
```

Figure 14. Customized universal script for automatically processing raw sequencing data

Table 6. Sequencing results from Novogene HK for Round 1 Tai O site (S21), showing presence/absence of eDNA signal using MiFish and 12S-V5 assays.

Taxonomic Level	Taxa	Family	Genus	Species	MiFish			12S-V5		
					R1_S21_1	R1_S21_2	R1_S21_3	R1_S21_1	R1_S21_2	R1_S21_3
species	Marine Mammal	Delphinidae	*Sousa*	*Sousa chinensis*	×	×	×	✓	✓	✓
species	Marine Mammal	Phocoenidae	*Neophocaena*	*Neophocaena phocaenoides*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Clupeidae	*Sardinella*	*Sardinella hualiensis*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Clupeidae	*Sardinella*	*Sardinella zunasi*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Clupeidae	*Nematalosa*	*Nematalosa japonica*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Ambassidae	*Ambassis*	*Ambassis buruensis*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Gerreidae	*Gerres*	*Gerres decacanthus*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Siganidae	*Siganus*	*Siganus doliatus*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Sillaginidae	*Sillago*	*Sillago sihama*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Engraulidae	*Encrasicholina*	*Encrasicholina heteroloba*	✓	✓	✓	✓	×	×
species	Neopterygii	Pristigasteridae	*Ilisha*	*Ilisha elongata*	×	×	×	✓	✓	✓
species	Neopterygii	Mugilidae	*Planiliza*	*Planiliza haematocheilus*	×	×	×	✓	✓	✓
species	Neopterygii	Engraulidae	*Thryssa*	*Thryssa chefuensis*	×	×	×	✓	✓	✓
species	Neopterygii	Gobiidae	*Tridentiger*	*Tridentiger trigonocephalus*	✓	✓	✓	×	×	×
species	Neopterygii	Sparidae	*Acanthopagrus*	*Acanthopagrus latus*	×	×	×	✓	✓	✓
species	Neopterygii	Carangidae	*Alepes*	*Alepes vari*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Pristigasteridae	*Ilisha*	*Ilisha melastoma*	×	×	×	✓	✓	✓
species	Neopterygii	Sebastidae	*Sebastiscus*	*Sebastiscus marmoratus*	×	×	×	×	✓	×
species	Neopterygii	Sparidae	*Sparus*	*Sparus aurata*	×	✓	✓	✓	✓	✓
species	Neopterygii	Eleotridae	*Oxyeleotris*	*Oxyeleotris marmorata*	×	✓	✓	×	×	×
species	Neopterygii	Plotosidae	*Plotosus*	*Plotosus lineatus*	✓	✓	✓	×	✓	×
species	Neopterygii	Nemipteridae	*Nemipterus*	*Nemipterus virgatus*	×	×	×	✓	✓	✓
species	Neopterygii	Lateolabracidae	*Lateolabrax*	*Lateolabrax japonicus*	×	×	×	✓	✓	✓
species	Neopterygii	Gobiidae	*Tridentiger*	*Tridentiger nudicervicus*	×	×	×	×	✓	×
species	Neopterygii	Engraulidae	*Thryssa*	*Thryssa hamiltonii*	×	✓	×	✓	✓	✓
species	Neopterygii	Blenniidae	*Omobranchus*	*Omobranchus fasciolatoceps*	×	✓	×	✓	×	×
species	Neopterygii	Clupeidae	*Konosirus*	*Konosirus punctatus*	×	×	×	✓	✓	✓
species	Neopterygii	Scatophagidae	*Scatophagus*	*Scatophagus argus*	×	×	×	✓	✓	✓
species	Neopterygii	Blenniidae	*Omobranchus*	*Omobranchus punctatus*	×	✓	✓	✓	✓	✓
species	Neopterygii	Mugilidae	*Mugil*	*Mugil cephalus*	×	×	×	✓	×	✓
species	Neopterygii	Engraulidae	*Thryssa*	*Thryssa dussumieri*	×	×	×	✓	×	×
species	Neopterygii	Sillaginidae	*Sillago*	*Sillago aeolus*	✓	✓	✓	✓	✓	✓
species	Neopterygii	Gobiidae	*Tridentiger*	*Tridentiger radiatus*	×	×	×	✓	✓	✓
species	Neopterygii	Elopidae	*Elops*	*Elops hawaiensis*	×	×	×	✓	✓	×

species	Neopterygii	Platycephalidae	*Platycephalus*	*Platycephalus cultellatus*	✗	✗	✗	✓	✗	✗
species	Neopterygii	Terapontidae	*Terapon*	*Terapon jarbua*	✓	✓	✓	✗	✗	✗
species	Neopterygii	Gobiidae	*Bathygobius*	*Bathygobius hongkongensis*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Tetraodontidae	*Lagocephalus*	*Lagocephalus spadiceus*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Gobiidae	*Tridentiger*	*Tridentiger bifasciatus*	✗	✗	✗	✗	✗	✓
species	Neopterygii	Carangidae	*Caranx*	*Caranx ignobilis*	✓	✓	✓	✗	✗	✗
species	Neopterygii	Belonidae	*Tylosurus*	*Tylosurus crocodilus*	✗	✗	✗	✓	✓	✗
species	Neopterygii	Ariidae	*Arius*	*Arius manillensis*	✗	✗	✗	✓	✓	✓
species	Neopterygii	Congridae	*Uroconger*	*Uroconger lepturus*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Cyprinidae	*Carassius*	*Carassius auratus*	✓	✓	✓	✗	✗	✗
species	Neopterygii	Paralichthyidae	*Pseudorhombus*	*Pseudorhombus oligodon*	✗	✗	✗	✓	✗	✗
species	Neopterygii	Ophichthidae	*Muraenichthys*	*Muraenichthys gymnopterus*	✗	✓	✓	✗	✗	✗
species	Neopterygii	Sparidae	*Pagrus*	*Pagrus major*	✗	✗	✗	✗	✗	✓
species	Neopterygii	Gobiidae	*Taenioides*	*Taenioides snyderi*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Blenniidae	*Parablennius*	*Parablennius yatabei*	✗	✗	✗	✓	✗	✗
species	Neopterygii	Engraulidae	*Stolephorus*	*Stolephorus commersonnii*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Cynoglossidae	*Cynoglossus*	*Cynoglossus semilaevis*	✗	✗	✗	✓	✓	✗
species	Neopterygii	Sillaginidae	*Sillago*	*Sillago parvisquamis*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Platycephalidae	*Platycephalus*	*Platycephalus indicus*	✗	✗	✗	✗	✓	✗
species	Neopterygii	Gobiidae	*Oxyurichthys*	*Oxyurichthys saru*	✗	✓	✗	✗	✗	✗
species	Neopterygii	Engraulidae	*Coilia*	*Coilia mystus*	✗	✗	✓	✗	✗	✗
genus	Neopterygii	Clupeidae	*Konosirus*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Clupeidae	*Nematalosa*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Clupeidae	*Sardinella*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Carangidae	*Trachinotus*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Mugilidae	*Mugil*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Sparidae	*Acanthopagrus*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Mugilidae	*Chelon*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Apogonidae	*Ostorhinchus*		✗	✗	✗	✓	✓	✓
genus	Neopterygii	Sciaenidae	*Nibea*		✗	✗	✗	✗	✓	✗
genus	Neopterygii	Ambassidae	*Ambassis*		✗	✗	✗	✓	✓	✗
genus	Neopterygii	Ariidae	*Arius*		✗	✗	✗	✗	✓	✓
genus	Neopterygii	Pomacentridae	*Neopomacentrus*		✗	✓	✗	✗	✗	✗
genus	Neopterygii	Gobiidae	*Drombus*		✗	✗	✗	✓	✓	✗
genus	Neopterygii	Gerreidae	*Gerres*		✗	✗	✗	✗	✓	✗
genus	Neopterygii	Siganidae	*Siganus*		✗	✗	✗	✓	✗	✓
genus	Neopterygii	Gobiidae	*Oxyurichthys*		✗	✗	✗	✗	✓	✗
genus	Neopterygii	Lutjanidae	*Lutjanus*		✗	✗	✗	✗	✓	✓
genus	Neopterygii	Sparidae	*Sparus*		✗	✗	✗	✗	✗	✓
genus	Neopterygii	Drepaneidae	*Drepane*		✗	✗	✗	✓	✗	✗

genus	Neopterygii	Tetrarogidae	*Hypodytes*		✓	✓	✓	×	×	×
genus	Neopterygii	Gobiidae	*Scartelaos*		×	×	×	×	✓	×
genus	Neopterygii	Sillaginidae	*Sillago*		✓	✓	×	×	×	×
genus	Neopterygii	Muraenesocidae	*Muraenesox*		✓	✓	✓	✓	×	×
genus	Neopterygii	Salangidae	*Neosalangichthys*		×	×	×	×	✓	×
genus	Neopterygii	Serranidae	*Hyporthodus*		✓	✓	✓	×	×	×
genus	Neopterygii	Nemipteridae	*Nemipterus*		✓	✓	×	×	×	×
genus	Neopterygii	Gobiidae	*Tridentiger*		×	×	×	×	✓	×
genus	Neopterygii	Ophichthidae	*Ophichthus*		×	×	×	×	✓	×
genus	Neopterygii	Gobiidae	*Acentrogobius*		✓	✓	✓	×	×	×
family	Neopterygii	Paralichthyidae			×	×	×	✓	✓	✓
family	Neopterygii	Cobitidae			×	×	×	✓	✓	✓
family	Neopterygii	Synanceiidae			×	×	×	✓	✓	✓
family	Neopterygii	Sphyraenidae			×	×	×	✓	✓	✓
family	Neopterygii	Sciaenidae			×	×	×	✓	✓	✓
family	Neopterygii	Labridae			×	×	×	✓	✓	✓
family	Neopterygii	Sillaginidae			×	×	×	✓	✓	✓
family	Neopterygii	Gobiidae			×	×	×	✓	✓	✓
family	Neopterygii	Clupeidae			×	×	×	✓	✓	✓
family	Neopterygii	Engraulidae			×	×	×	✓	✓	✓
family	Neopterygii	Trichiuridae			×	×	×	×	×	✓
family	Neopterygii	Gerreidae			×	×	×	✓	×	✓
family	Neopterygii	Polynemidae			×	×	×	×	✓	×
family	Neopterygii	Tetrarogidae			×	×	×	✓	×	×
family	Neopterygii	Blenniidae			×	×	×	✓	×	✓
family	Neopterygii	Cynoglossidae			×	×	×	✓	×	×
family	Neopterygii	Sparidae			×	×	×	×	✓	×
family	Neopterygii	Syngnathidae			×	×	×	✓	×	×

After the establishment of eDNA metabarcoding workflow, we have commenced the analyse of samples from former surveys (**Figure 15**). To date, PCR with two metabarcoding primers, gel recovery, and eDNA pooling for sequencing, for round 5 samples, were all completed.

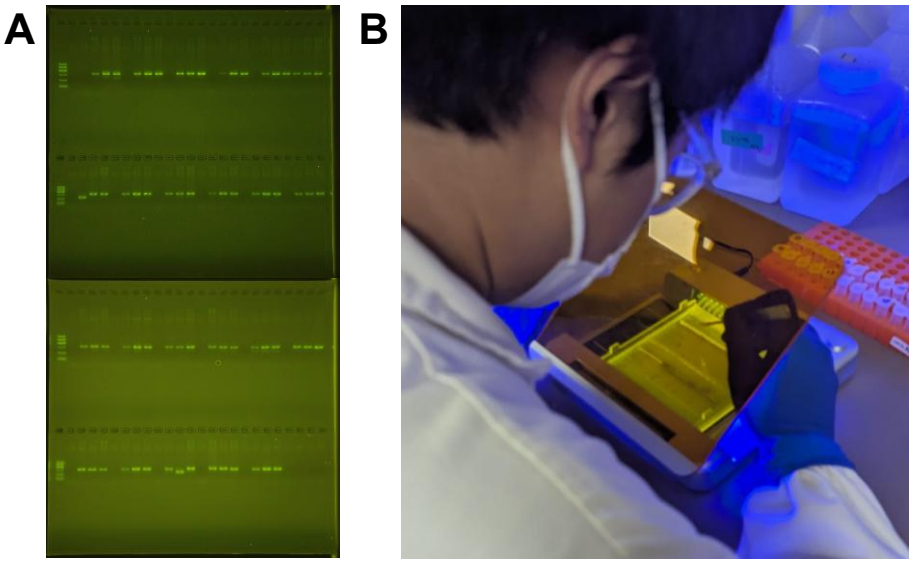


Figure 15. (A) PCR result of samples from round 5 with Mifish-U primer. (B) RA doing gel recovery for round 5 eDNA metabarcoding PCR products

5. Other project progress

Dr. Ip (PI) has been invited to join the South Asia Forum 2024 hosted by South China Normal University and the 7th conference on China' environmental impact assessment, jointly hosted by the Chinese University of Hong Kong, Nankai University, and the Hong Kong Institute of Environmental Impact Assessment, where he presented MEEF Phase 1 project, with a particular focus on species-specific primer design. During the symposium, Dr. Ip discussed the MEEF project with researchers in the GBA and officials from HKEPD, highlighting how MEEF results can contribute to the Centralized Environmental Database (CED) launched in 2022. Additionally, we are currently working on the manuscript related to the species-specific primer design, which includes *in-silico* and *in-vitro* testing, design for specificity, and qPCR benchmarking to assess primer sensitivity. To date, all lab experiments were done with expected results acquired successfully, and according part in manuscript are being fulfilled (around 30% of manuscript completed); scheduled time for finishing and submitting this manuscript is mid of December, which will be after experiments of task 2 (metabarcoding) finished. These efforts contribute to the project objectives, outcomes, and benefits for marine conservation in Hong Kong and the PRE regions.

Invitation Letter

Dear Dr. IP Chi Ho,

The Center for Southeast Asian Study of South China Normal University, as a national base for country and regional studies filed with the Ministry of Education, in collaboration with Guangdong International Culture Exchange Center and Cross-Strait and Hong Kong and Macau Collaborative Innovation Alliance, plans to hold the **"Southeast Asia Forum" 2024 International Conference from November 15th to 18th, 2024**. The theme of this conference is **Connectivity and Coordinated Development from the Linkage Perspective of Guangdong-Hong Kong-Macao Greater Bay Area - Hainan Free Trade Port - Southeast Asia**.

We cordially invite you to attend the conference given your profound expertise and remarkable accomplishments in your field. We look forward to meeting you at the conference.Wish you all the best!

Annex:

1. Registration Form
2.Advance Notice of "Southeast Asia Forum" 2024 International Conference

Southeast Asia Forum Secretariat
School of Life Sciences
South China Normal University
Phone: 020-85210103
Sept. 23, 2024

Peer-review paper: We are currently working on the manuscript for a species-specific qPCR assay of the Chinese Bahaba. Our goal is to submit the manuscript in Q1 2026 to the journal Environmental DNA. The tentative title is "Silent Echoes of eDNA: Establishing the Missing Link for Chinese Bahaba Via Species-Specific qPCR Assay".

The manuscript will cover the following topics: (1) Primer design, (2) in silico and in vivo testing, (3) qPCR efficiency testing, and (4) field applications and results.

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

From **November 2024 to June 2025**, significant progress has been made in understanding the current status and conservation of Chinese Bahaba in the western waters of Hong Kong. **The design and validation of Chinese Bahaba species-specific primers** were successfully completed. These activities represent crucial milestones in the project timeline, advancing our first research objective: tracing the Chinese Bahaba eDNA and assessing its distribution, seasonality, and habitat preferences in western Hong Kong waters.

Additionally, the **successful implementation of the dry/wet season eDNA survey and qPCR screening** provides a solid foundation for expanding the study. **We also constructed the species-specific eDNA detecting protocol for Chinese Bahaba and streamlined the eDNA metabarcoding protocol for universal fish eDNA primers, 12S-V5 and MiFish-U**, which supports the implementation of Objectives 1 and 2. These advancements will facilitate more effective monitoring of the target species, enhancing our understanding of its distribution and abundance. The application of eDNA technology in this project offers significant potential

benefits for conservation efforts, providing a non-invasive and highly sensitive method for monitoring species in aquatic environments.

In summary, the progress made thus far underscores our commitment to achieving the project's objectives. These activities will deepen our understanding of the distribution and abundance of Chinese Bahaba, which is crucial for its conservation. As the project continues, the results will contribute to a greater knowledge of this species and marine biodiversity, informing conservation strategies in the western waters of Hong Kong.

(vi) Summary and Way Forward;
Overall, the expected outcomes of the first phase of this project have been achieved smoothly.

With the efforts of team members, including key research assistant, we successfully completed the preparations for the whole project, including the preparation of laboratory instruments, reagents, consumables, and the determination of experiment protocols. The testing part for the qPCR experiment to detect Chinese Bahaba for task 1 and the eDNA metabarcoding experiment to reveal fish biodiversity for task 2 have also been carried out successfully; the specific primers, sequencing results, and data analysis methods developed during this process also provide an important baseline for future related scientific research.

In fieldwork part, five of six in total proposed surveys of seawater sampling have been conducted as scheduled; all data related to these surveys, including sampling information, environmental data, special incident log (such as sea-bloom, Chinese White Dolphin witness), were well recorded and archived; to date, qPCR experiments for all R1-5 surveys were completed, and external conditions are being optimized to enable efficient, accurate, and highly sensitive detection of Chinese Bahaba eDNA.

For the coming phase 2, we are heading forward to finish eDNA metabarcoding for obtained samples as well as rest of qPCR for Chinese Bahaba detecting. Further steps such as sequencing, sequence data analysing, will be performed once finish PCR/qPCR product preparation. Meanwhile, the baseline information, e.g. DNA barcode sequences of fish species including Chinese Bahaba detected in Hong Kong western waters, distribution status of threatened marine species in Hong Kong western waters, and protocol of non-invasive eDNA methods, are expected to be organized and archived in suitable platforms, to support future conservation strategies and fishery management in western Hong Kong waters and the nearby regions of the PRE.

(vii) Audited statement of account (Audited statement of account is not disclosed due to confidentiality reasons.)

Integrating eDNA and Conventional Surveys in Diverse Vertebrate and Crustacean Communities of Urban Subtropical Estuaries

结合环境DNA与传统调查研究城市亚热带河口区的多样脊椎动物和甲壳动物群落

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Introduction

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Estuary Ecosystem 河口生态系统

- Estuaries are one of the most productive and nutrient-rich ecosystems.
- Providing diverse habitats, feeding grounds, recruitment, and nursery areas for many aquatic and commercial species
- Unfortunately, human activities have threatened these ecosystem.
- As a result, in some urban estuaries, > 90% of economically important species, including fish and crustaceans, have been depleted, and the overall biomass is < 50% of historical levels.

Source: https://english.news.cn/

Introduction

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Hong Kong - an urbanized subtropical estuary 香港—城市化的亚热带河口

- Hong Kong is a major city within the mouth of Pearl River Estuary (PRE), and thus is strongly influenced by freshwater and sediment discharge
- The complex hydrographic environments resulted in high marine diversity (~6000 species), with 1,192 fish species from 148 families.
- **Human activities and climate changes** have threatened the local marine ecosystems in past decades

Morton and Morton 1983

Introduction

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Hong Kong - an urbanized subtropical estuary 香港—城市化的亚热带河口

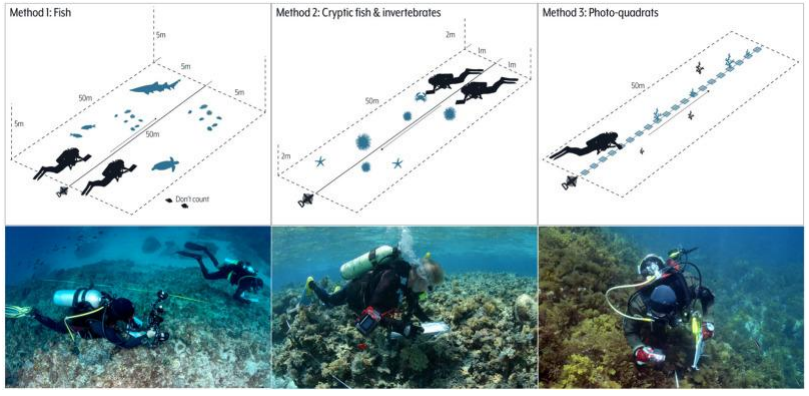
- Recognizing the importance of marine conservation, HK government has implemented a number of management measures, such as **9 marine protected areas** (since 1996)**, trawling ban** (Dec 31, 2012), and **regulator monitoring.**

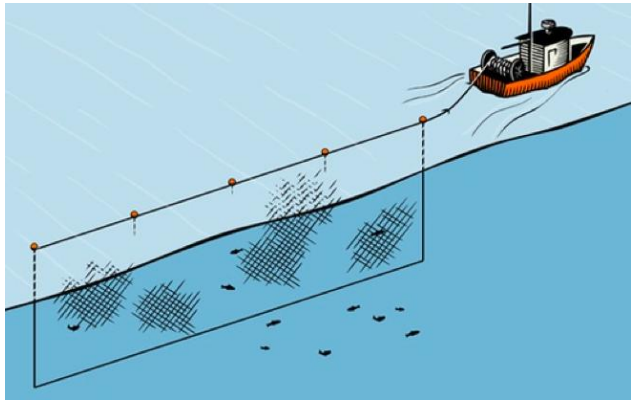
AFCD HKSAR

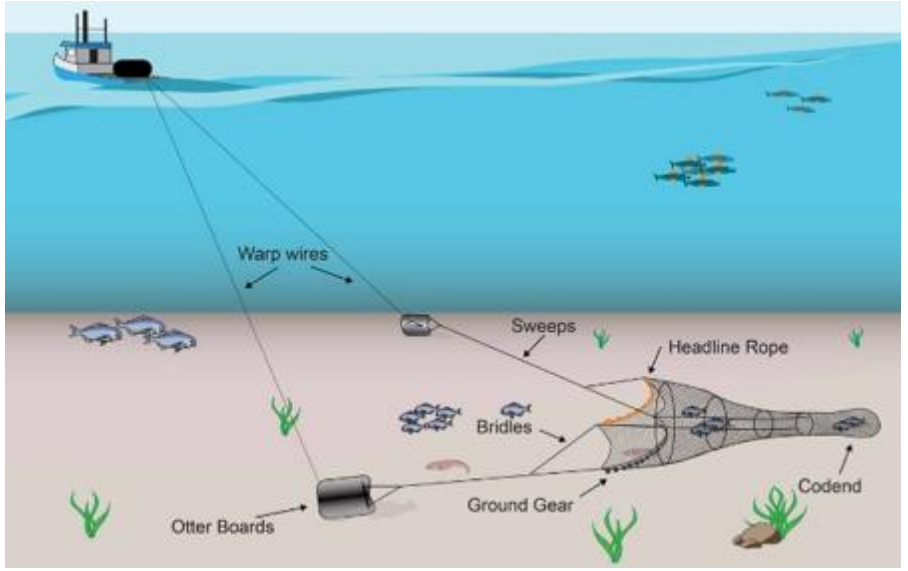
Introduction

Biomonitoring and limitation in estuary Ecosystem 河口生态系统的生物监测与限制

- Biomonitoring aquatic organisms in estuaries is challenging due to their complex environmental conditions (e.g., salinity and turbidity) and diverse habitats.
- Traditional surveys like trawling and gillnetting have negative effects on fishery resources and ecosystems.
- Non-destructive sampling methods like underwater visual censuses and echo sounder surveys have **limitations in data quality and habitats**.
- These conventional surveys are **cost and labor intensive**.







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eDNA study in Hong Kong waters 香港水域的eDNA研究 (2024 年之前)

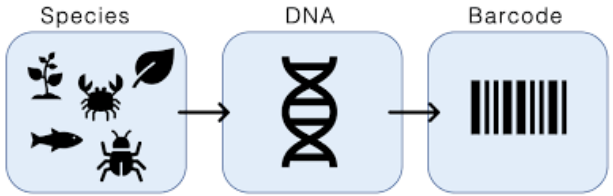
Sampling Year	Region	Conventional method	No. of fish	eDNA	No. of fish	References
2017-2018	PRE	Bottom trawling	32	MiFish-U	57	Zou et al. 2020
2018	PRE	Bottom trawling	11	Teleo	49	(method paper) Ruan et al. 2022
2019	PRE	Gill netting	94	MiFish-U	115	Li et al. 2023
2020	PRE	Bottom trawling	47	MiFish-U	175	Jiang et al.2022.
2020-2021	PRE	Bottom trawling	90	MiFish-U	214	Jiang et al. 2023
2018	W HK waters	--	--	mICOIint	22	Cheang et al. 2020

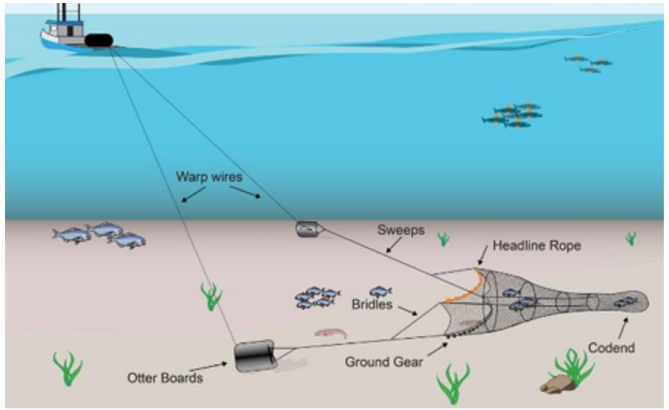
- In PRE, several studies have included and compared eDNA techniques with trawling and gillnetting surveys, but this kind of integrated approach necessitates further investigation in Hong Kong waters.

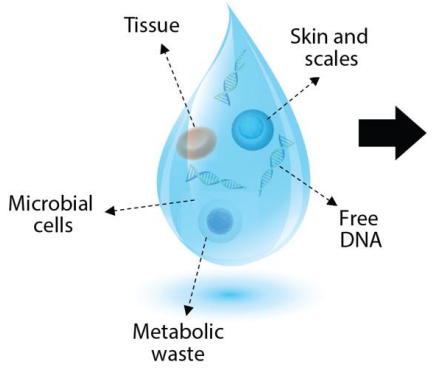
Objectives

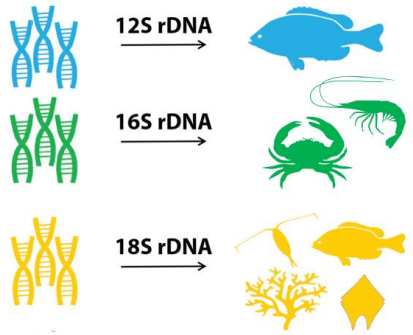
To facilitate the application of eDNA monitoring in Hong Kong waters, we aims to study the following factors:

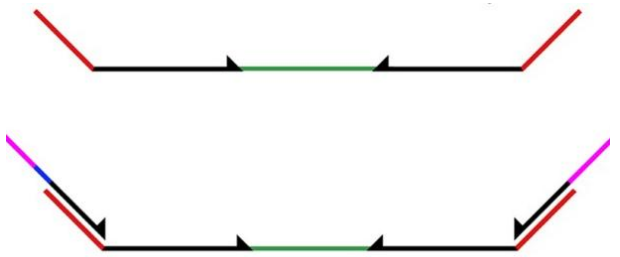
- **Comparison of eDNA and convectional surveys**
- **Reference DNA barcode database**
- **Evaluation the effectiveness and specific eDNA primers**
- **Streamlined eDNA Methods**









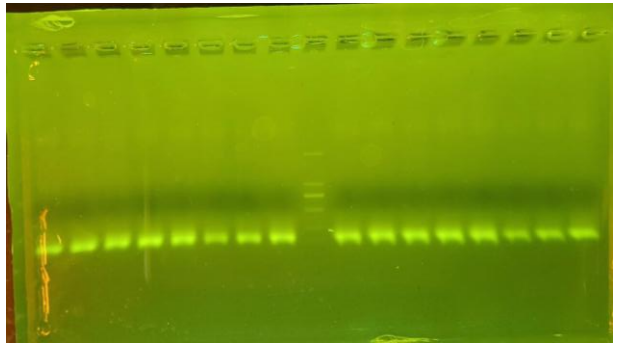


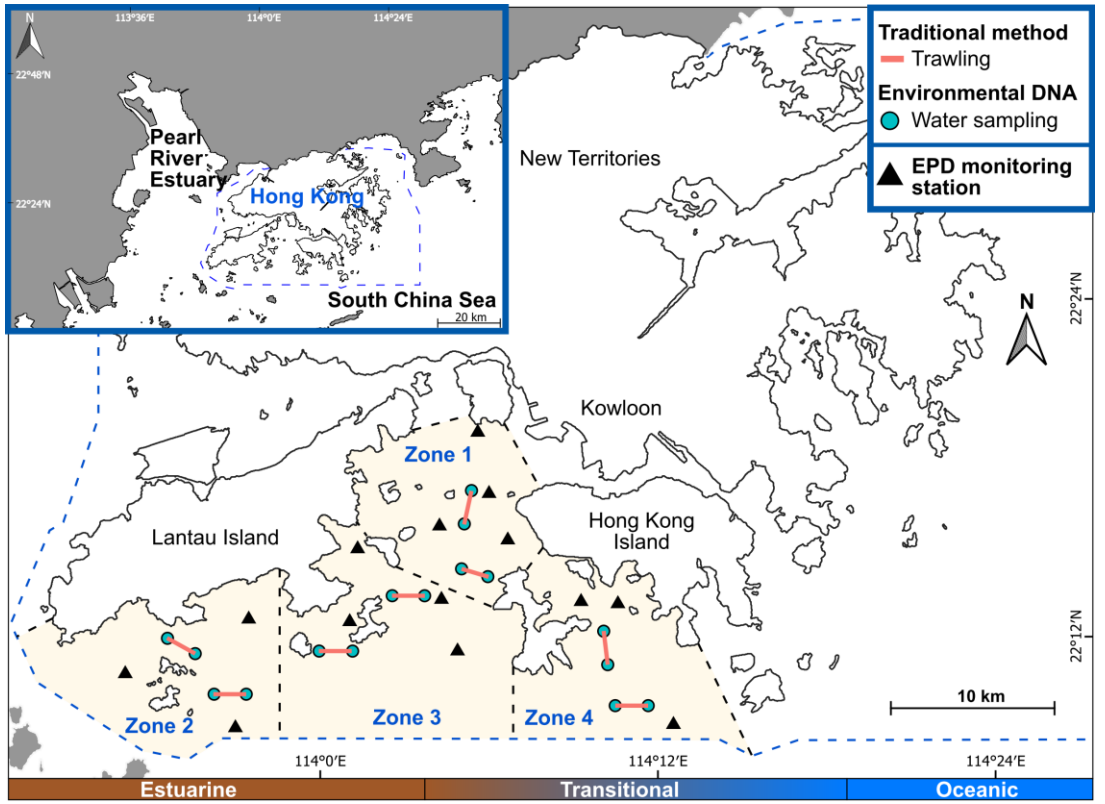
(1) Comparison of eDNA and convectional surveys

Bottom trawling in Southern waters

eDNA sampling in Southern waters

- 8 trawl sites (start and end)
- 2-L seawater x 2 replicates
- Target taxa: marine vertebrates (12S-V5, MiFish-U, Berry-Fish), and crustacean (MiDeca)



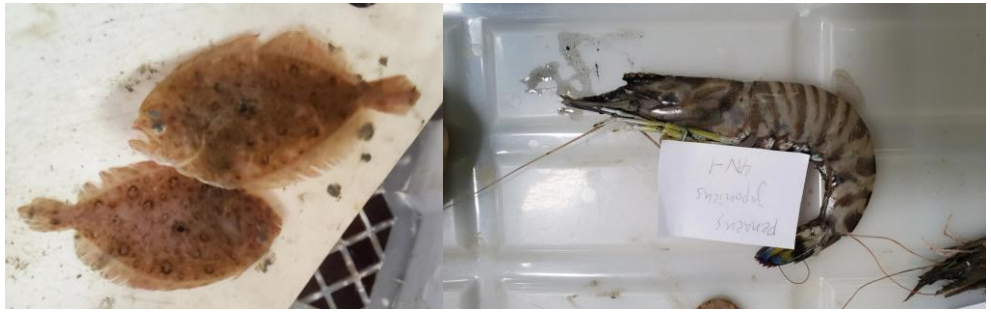


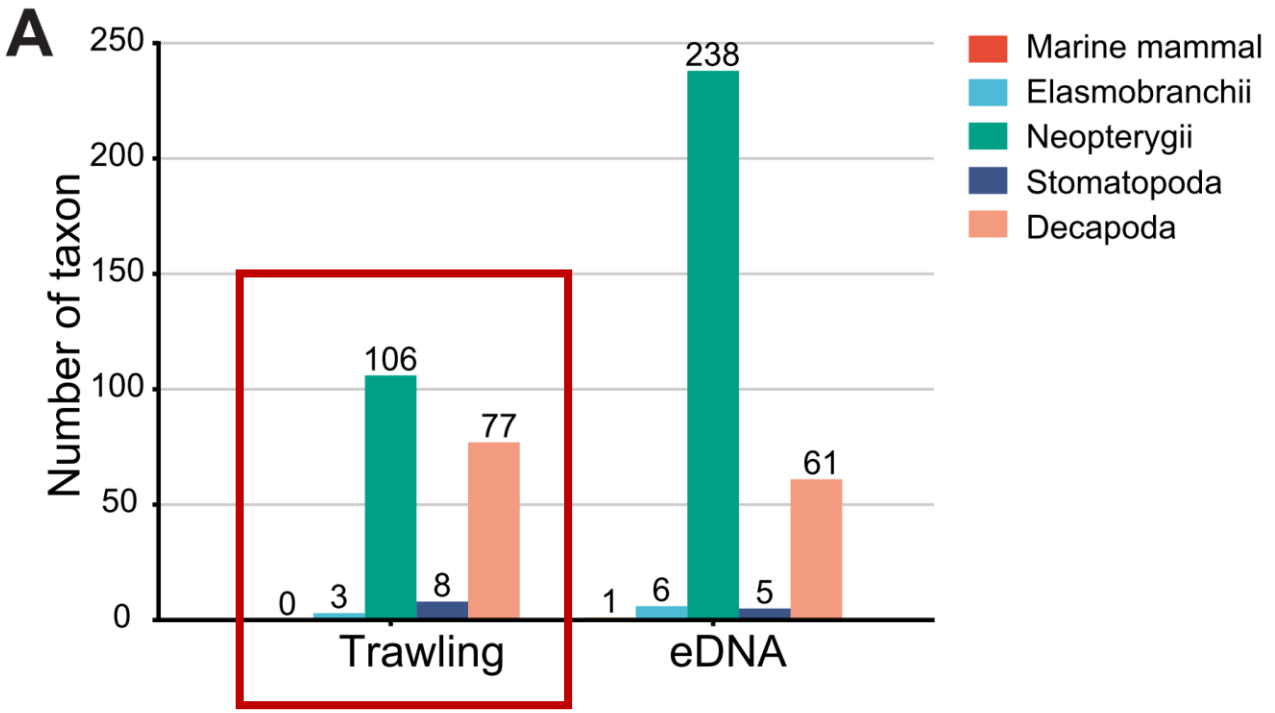
LCF project, HKSAR 2022-2025; SKLMP project 2022-2024

(1) Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

- Workload for trawling: **Eight researchers** and experts for sampling on the boat and subsequent laboratory works, taking > **two months** to process.
- Trawling captured a total of **236 taxa from 8 trawl sites**
  - ❑ 3 elasmobranchs
  - ❑ 106 bony fishes
  - ❑ 85 crustaceans

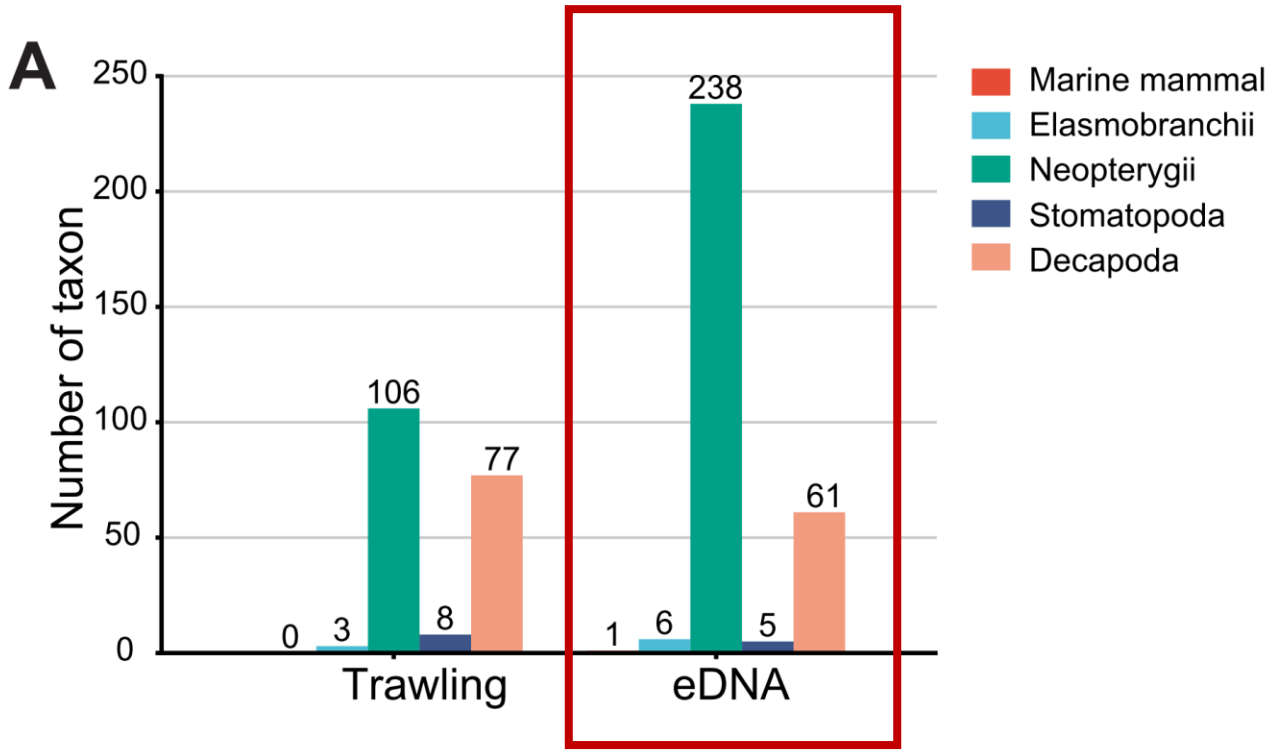




(1) Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

- Workload for eDNA: **One researcher** for sampling on the boat and subsequent laboratory works, taking ~**one month** to process.
- eDNA identified a total of **311 taxa from trawl sites**
  - ❑ 1 marine mammal
  - ❑ 6 elasmobranchs
  - ❑ 238 bony fishes
  - ❑ 66 crustaceans
- Primer performances
  - ❑ 12S-V5 detected more vertebrates
  - ❑ BerryFish (16S) more specific on bony fishes
  - ❑ **Muti-assays** enhance the detection

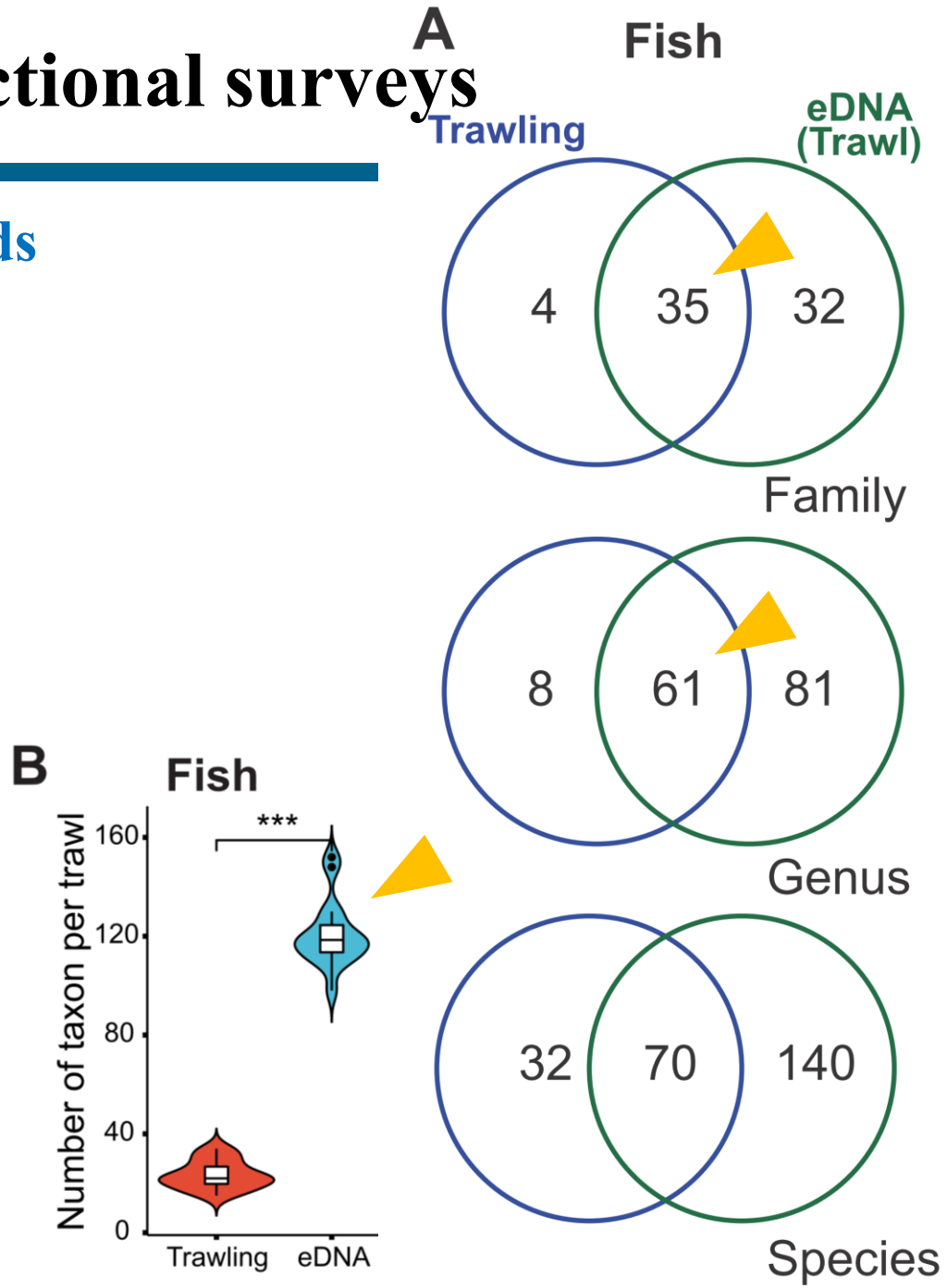


(1) Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

Fish community

- Trawling: 109 taxa, **102 species** from 39 families
- eDNA: 238 taxa, **210 species** from 67 families
- eDNA data covered most of the family (35/39) and genera (61/69) detected in trawling
- eDNA detected higher diversity and more fish per sample

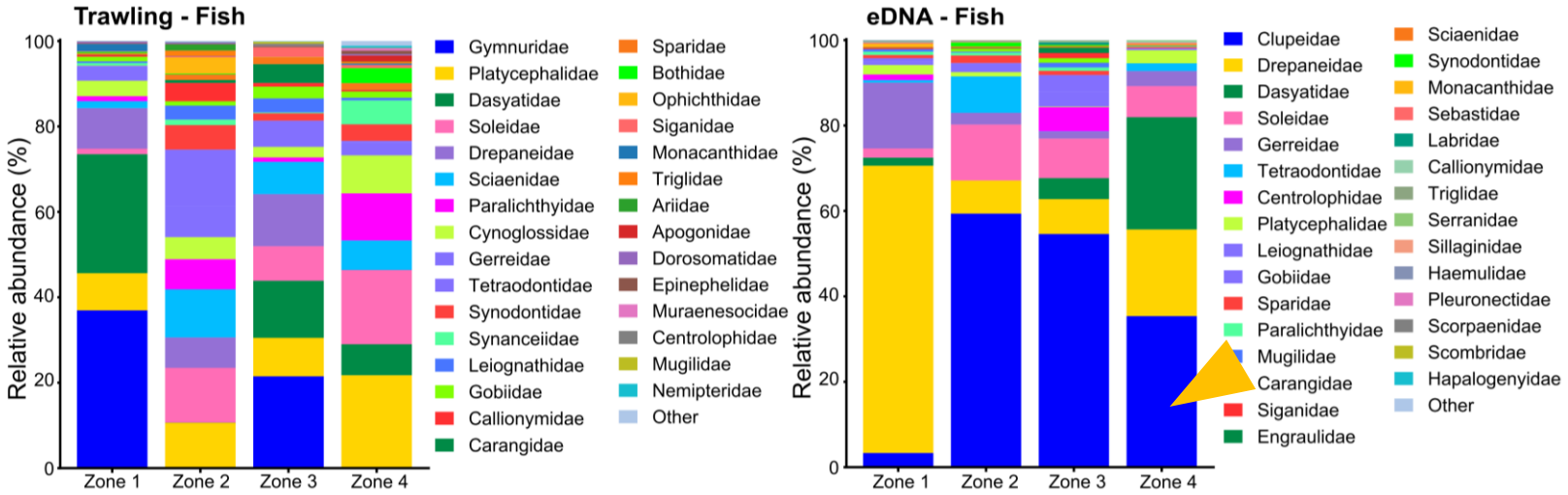


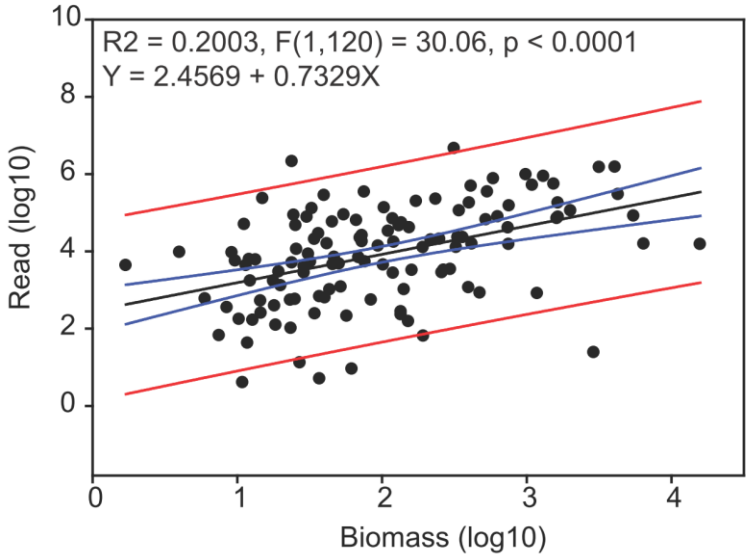
(1) Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

Fish community

- Trawling was selectivity on epibenthic fish
- eDNA detected **both epibenthic and pelagic fish species**
- eDNA metabarcoding via conventional method for abundance and biomass analysis.





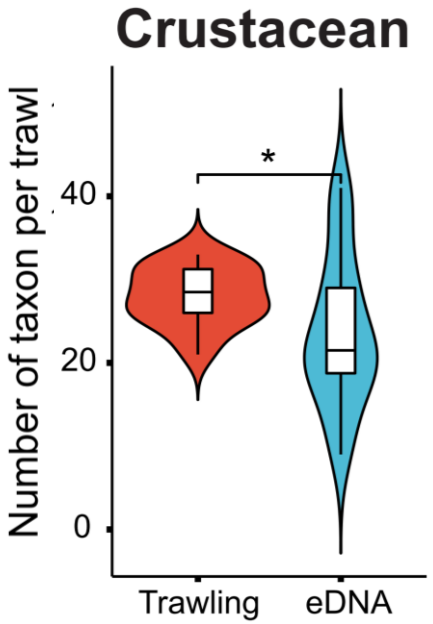
(1) Comparison of eDNA and convectional surveys

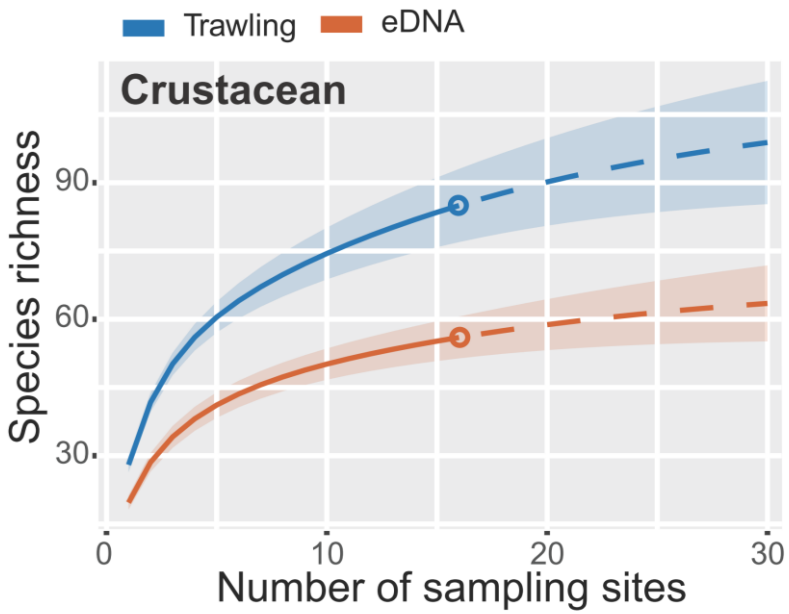
Comparison between trawling and eDNA methods

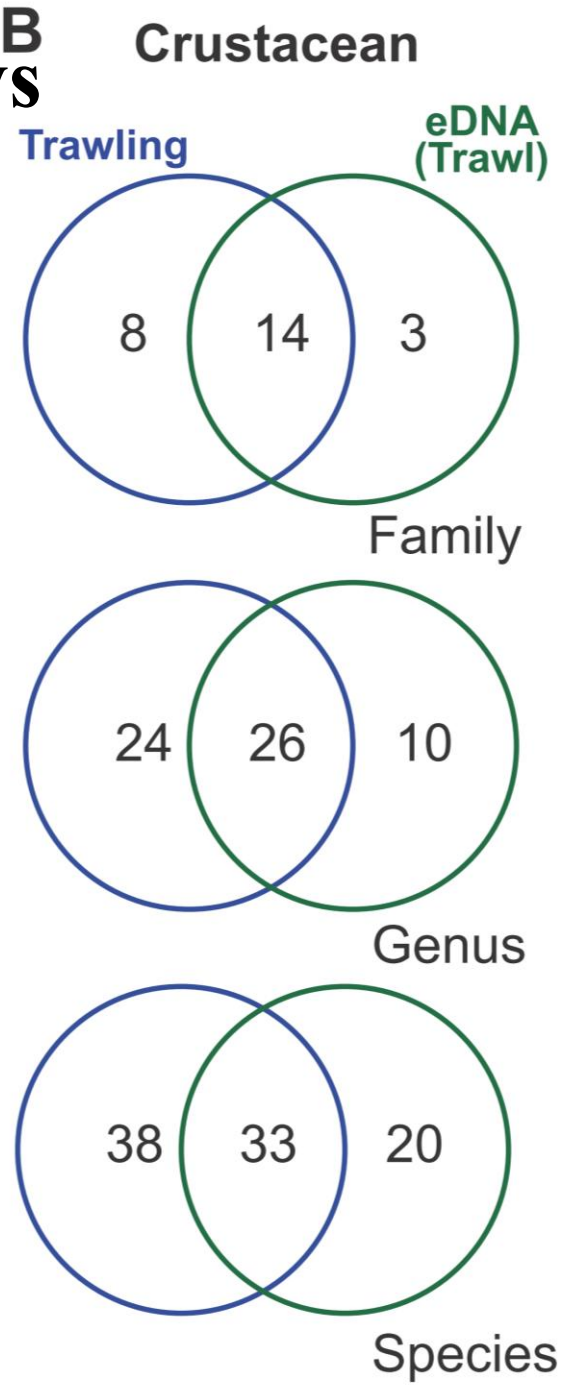
Crustacean community

- Trawling (85 species) captured more crustacean than eDNA (66 species)
- Possible reasons: replicates, primer, DNA reference database

Dorippoides facchino
No available 16S sequence





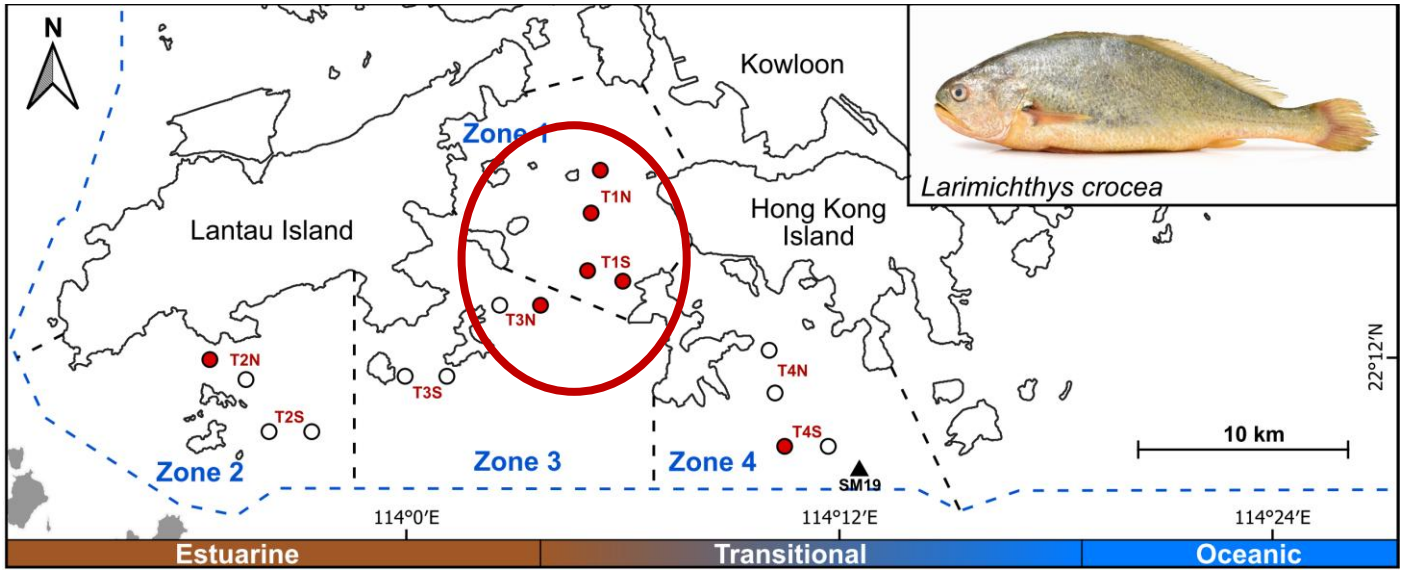


(1) Comparison of eDNA and convectional surveys

eDNA as a sustainable tool for monitoring rare and threatened species

- Trawling captured **three threatened species**: two Vulnerable elasmobranchs (*Gymnura japonica* and *Telatrygon zugei*), Endangered Threadfin Porgy (*Evynnis cardinalis*).
- eDNA identified **nine threatened species**, including one marine mammals *N. phocaenoides*, two elasmobranchs, and Critically Endangered *Larimichthys crocea*, and Endangered *E. cardinalis*.

Large yellow croaker



(1) Comparison of eDNA and convectional surveys

Summary

- eDNA method provides a rapid and comprehensive biodiversity assessment, with muti-assays
- Non-invasive eDNA provides a sustainable tool for detecting threatened species
- Demonstrated the efficiency of eDNA metabarcoding as a **complementary method** in Hong Kong waters
- **Limitation:** the completeness of the DNA reference database, the need of specific eDNA primers

ORIGINAL ARTICLE | Open Access

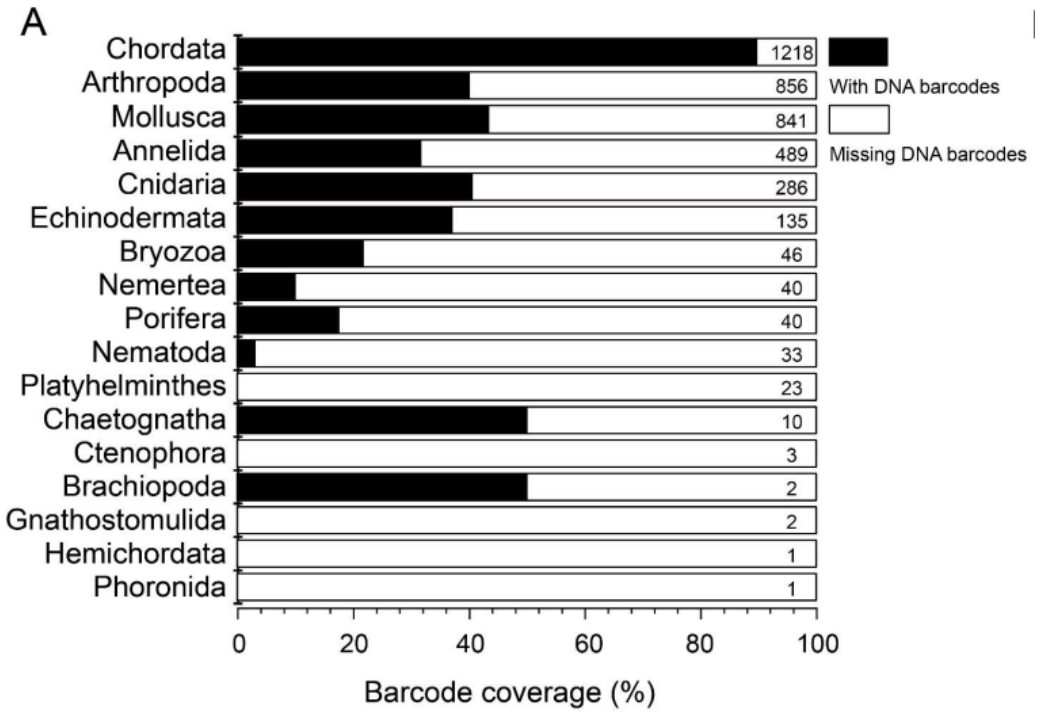
Bottom Trawling and Multi-Marker eDNA Metabarcoding Surveys Reveal Highly Diverse Vertebrate and Crustacean Communities: A Case Study in an Urbanized Subtropical Estuary

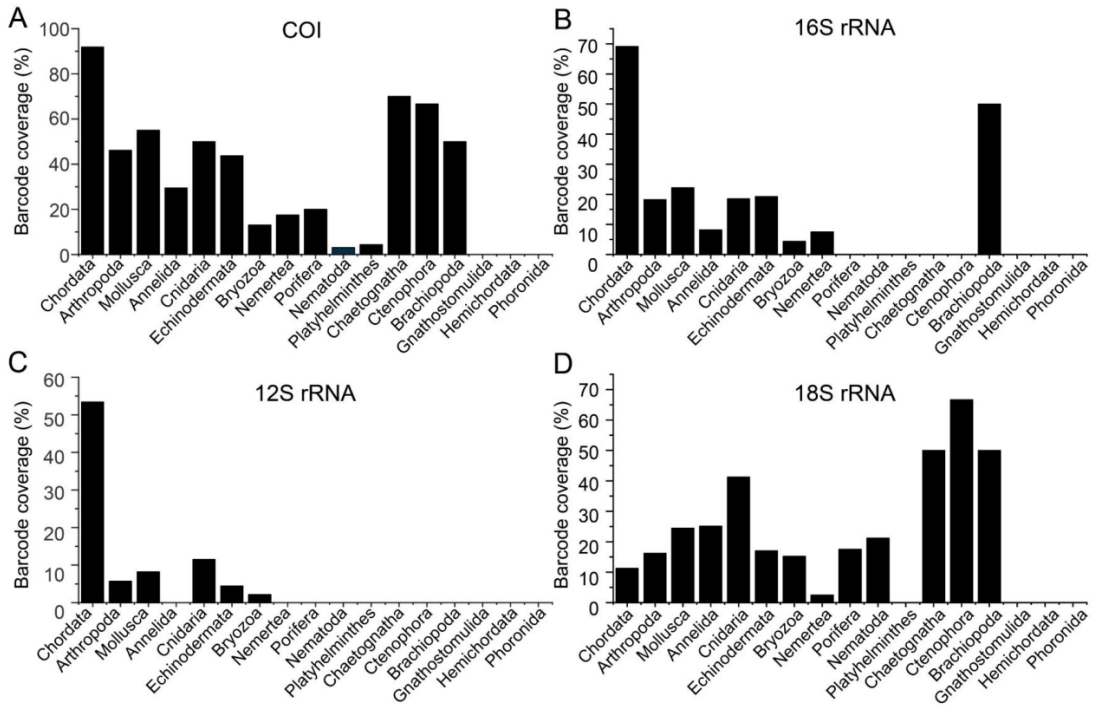
Jack Chi-Ho Ip, Hai-Xin Loke, Sam King Fung Yiu, Meihong Zhao, Yixuan Li, Yitao Lin, Chun-Ming How, Jiezhang Mo, Meng Yan, Jinping Cheng, Vincent Chi-Sing Lai, Leo Lai Chan ... See all authors

First published: 04 November 2024 | https://doi.org/10.1002/edn3.70031

(2) Reference DNA barcodes

- Download the Hong Kong marine species list from HKRMS, and DNA barcodes from BOLD and NCBI GenBank
- COI Barcoding coverage: 90% of Chordata; 55% of Mollusca; 46% of Arthropoda
- For the gene markers (12S, 16S, and 18S), **most of marine invertebrates** has less than **30% coverage**.

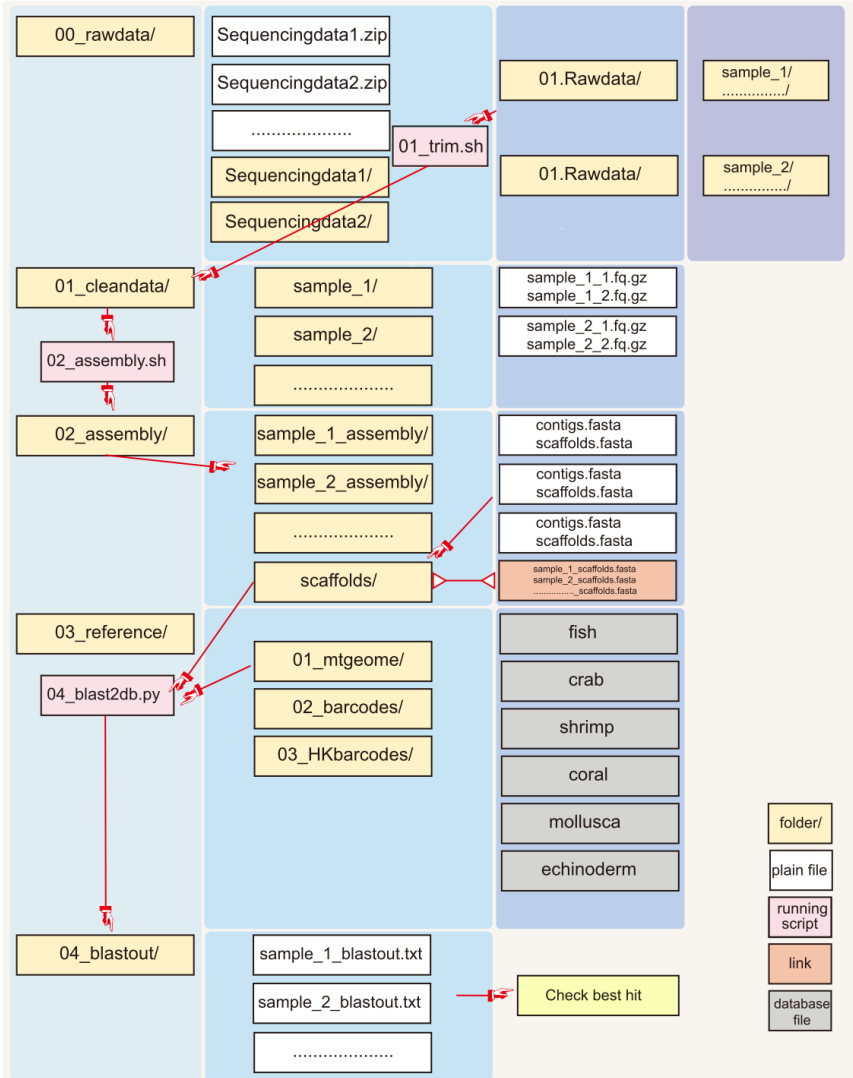




Zhao et al., minor revision in Reg Stud Mar Sci

(2) Reference DNA barcodes

Enhance the reference database via 500 genome skimming

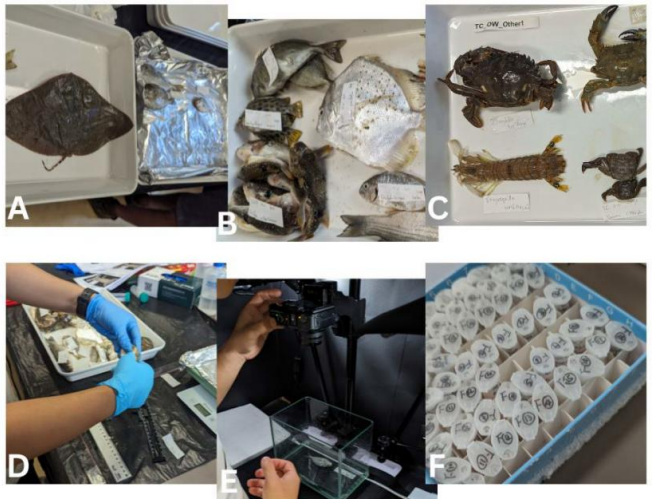


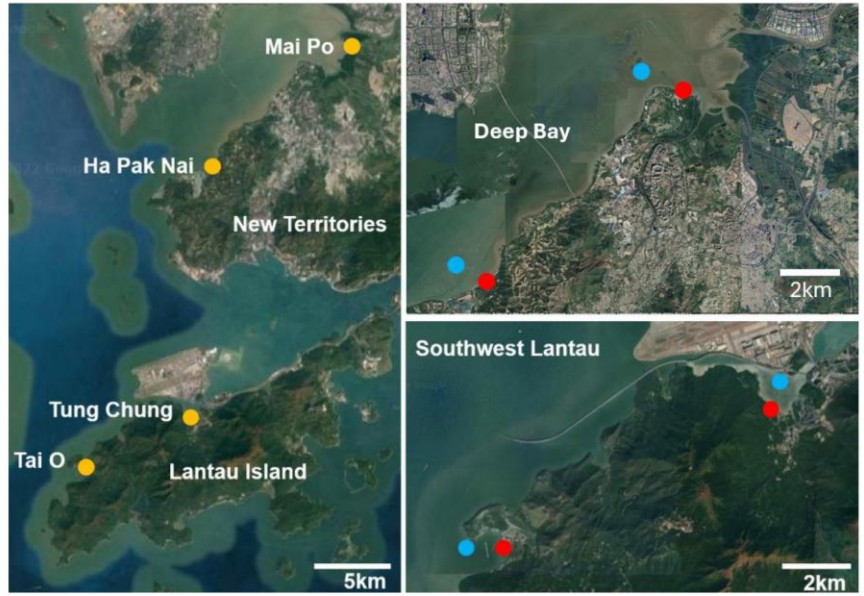
- Obtain the tissue sample from previous and ongoing fishery and biodiversity projects
- Low-coverage Illumina sequencing (10Gb)
- Assembly and recovering the MT genome and nuclear marker genes
- Now we have already generated >250 fishes and > 210 invertebrates

Ongoing projects -eDNA and conventional surveys

(1) Monitoring mangrove communities using eDNA and gill netting surveys

- Survey the biodiversity of fish and crustaceans in mangrove habitat
- Integrating the eDNA, gillnetting, caging for species detection





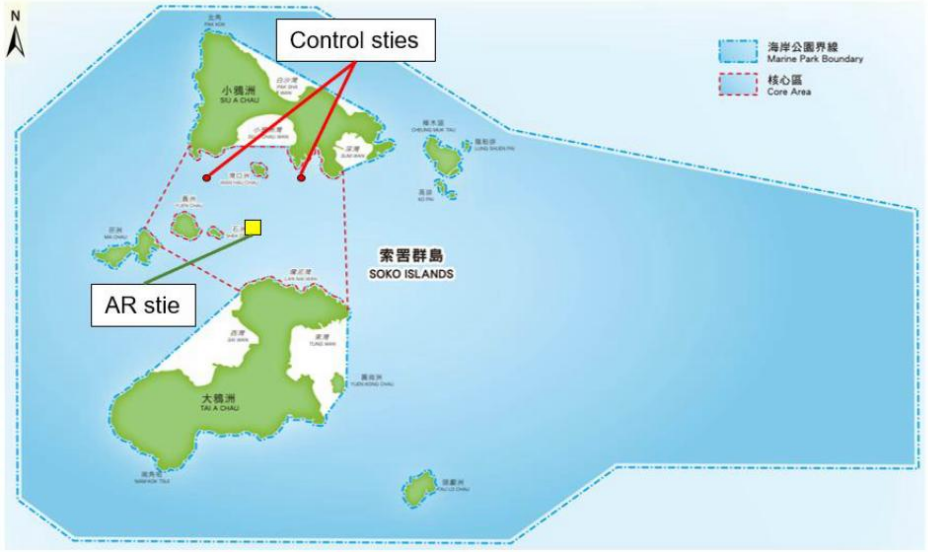
LNG-MCEF project 2024-2025

PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Prof. Jian-wen Qiu (HKBU), Dr. Meng Yan (SKLMP)

Ongoing projects -eDNA and conventional surveys

(2) Monitoring Artificial reef communities in South Lantau Marine Park

- Monitoring the species communities' changes before and after the deployment of AR in SLMP
- Evaluate the AR performance using eDNA and convectional approaches

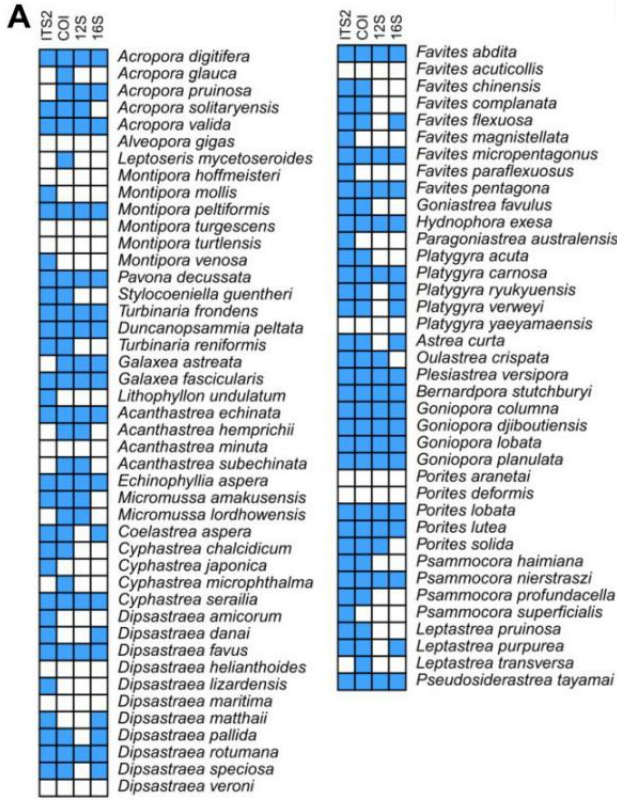


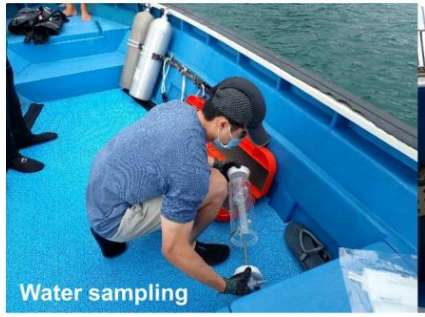
ECF, HKSAR project 2024-2026
PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Prof. Jian-wen Qiu (HKBU)

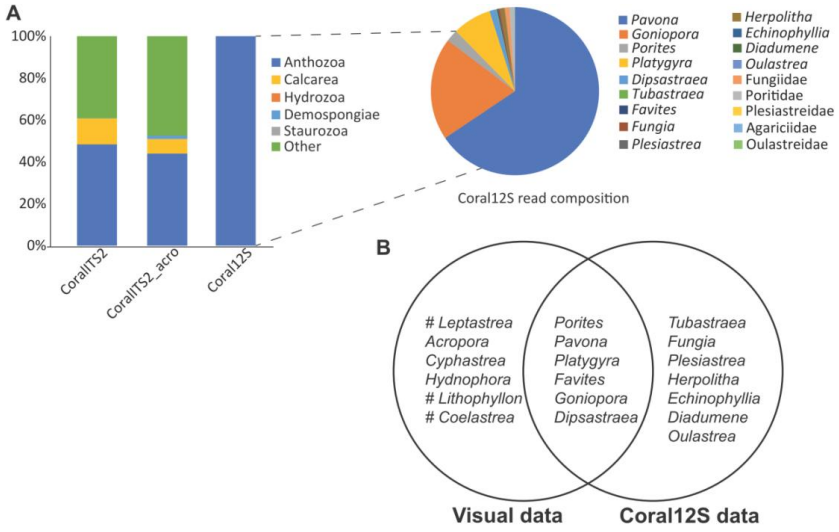
Ongoing projects -eDNA primers and methods

(3) Monitoring coral communities using eDNA and SCUBA

- Enhance scleractinian DNA reference database
- developing new scleractinian-specific primers
- Assess the performance of eDNA method by comparing eDNA results with visual data
- establish a comprehensive baseline of spatial and temporal variation in scleractinian assemblages







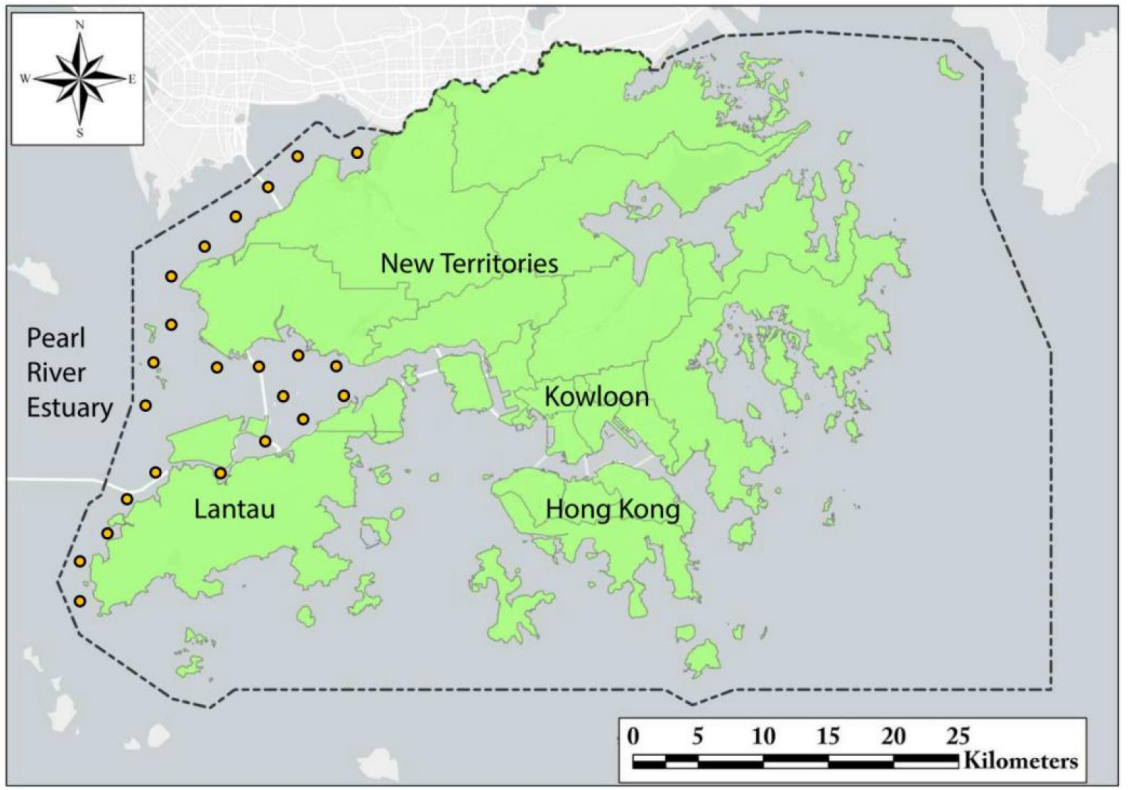
ECS, UGC, HKSAR project 2025-2027

PI: Dr. Jack Chi-Ho Ip (LU)

Ongoing projects -eDNA in marine conservation

(4) Monitoring Chinese bahaba 黃唇魚 in Hong Kong western waters

- Conduct eDNA surveys using a species-specific qPCR approach to trace any Chinese Bahaba in western Hong Kong waters.
- Utilize eDNA metabarcoding to monitor the overall biodiversity of the ecosystem, which provide insights into the species composition, interactions, and potential threats within the habitats associated with the Chinese Bahaba.



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Ongoing projects -eDNA in marine conservation

(4) Monitoring Chinese bahaba

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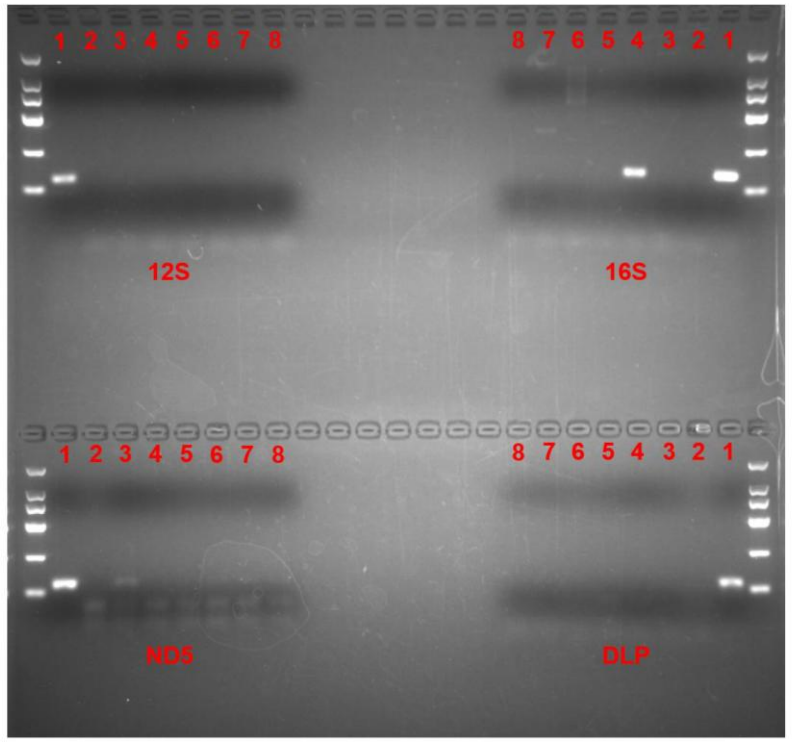

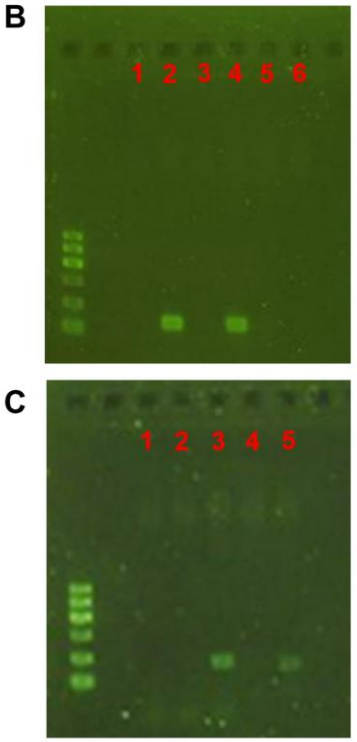

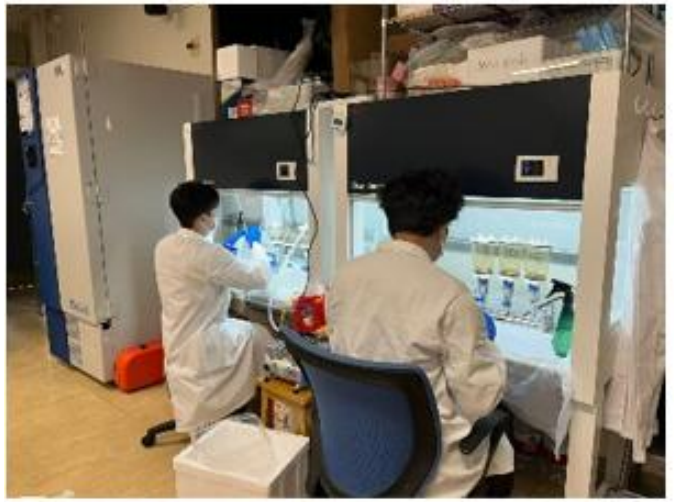

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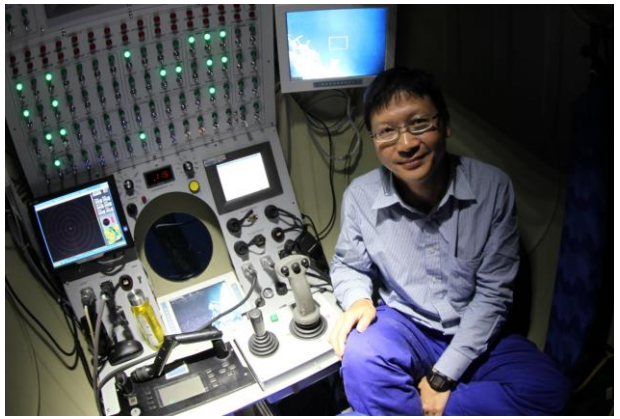
Acknowledgements



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Environmental DNA Metabarcoding Reveals Highly Diverse Vertebrate and Crustacean Communities in Hong Kong Waters

环境DNA应用于香港水域脊椎动物和甲壳类的生物多样性监测

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Research Areas

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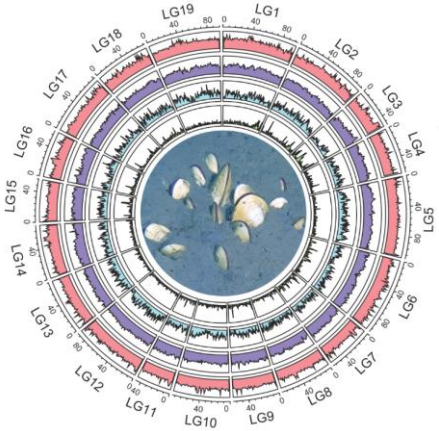
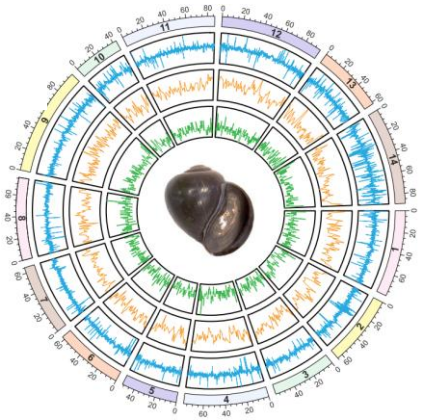
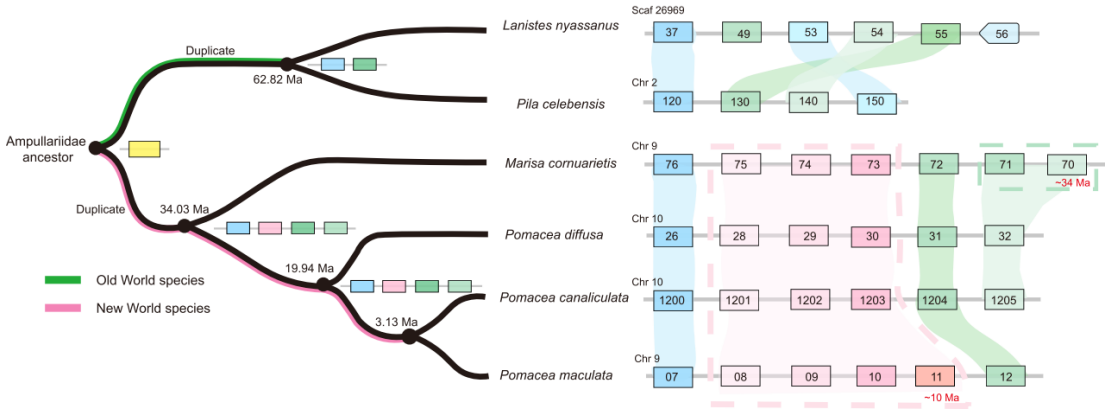
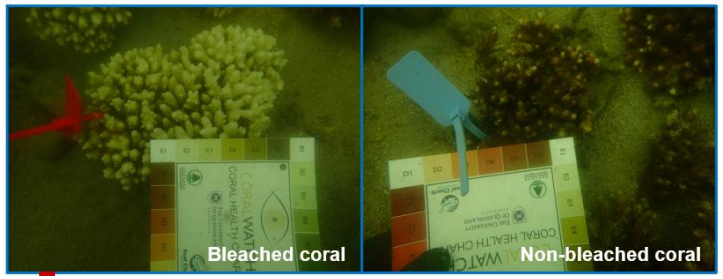
- *Genome Evolution and Adaptation*
- ***Aquatic Biodiversity via Climate Change***
- *Sustainable Aquaculture*







Molluscan genomics



Coral genomics

Contributes towards the SDGs:

Research Areas

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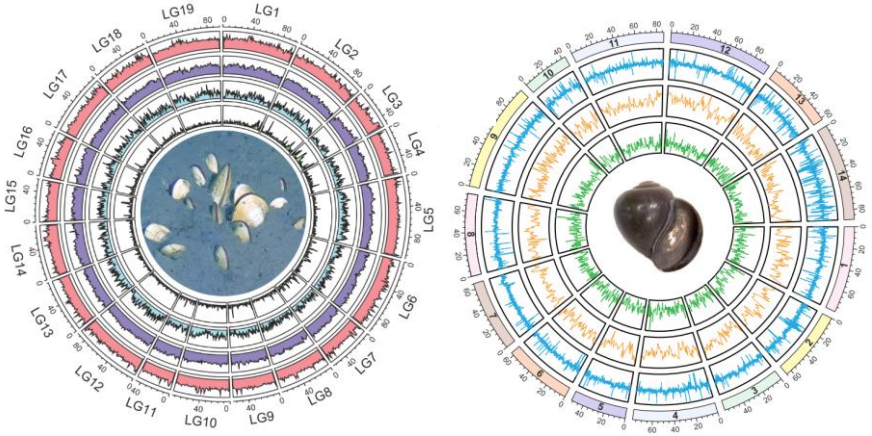
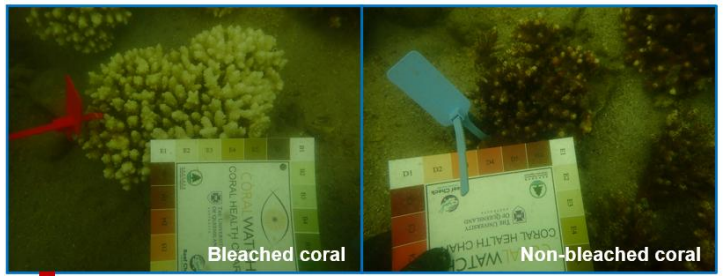
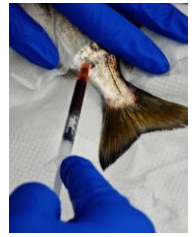
- *Genome Evolution and Adaptation*
- ***Aquatic Biodiversity via Climate Change***
- *Sustainable Aquaculture*

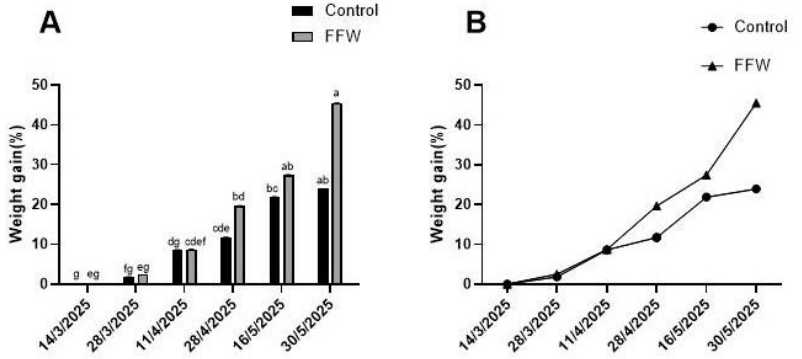


Molluscan genomics



Coral genomics





Contributes towards the SDGs:

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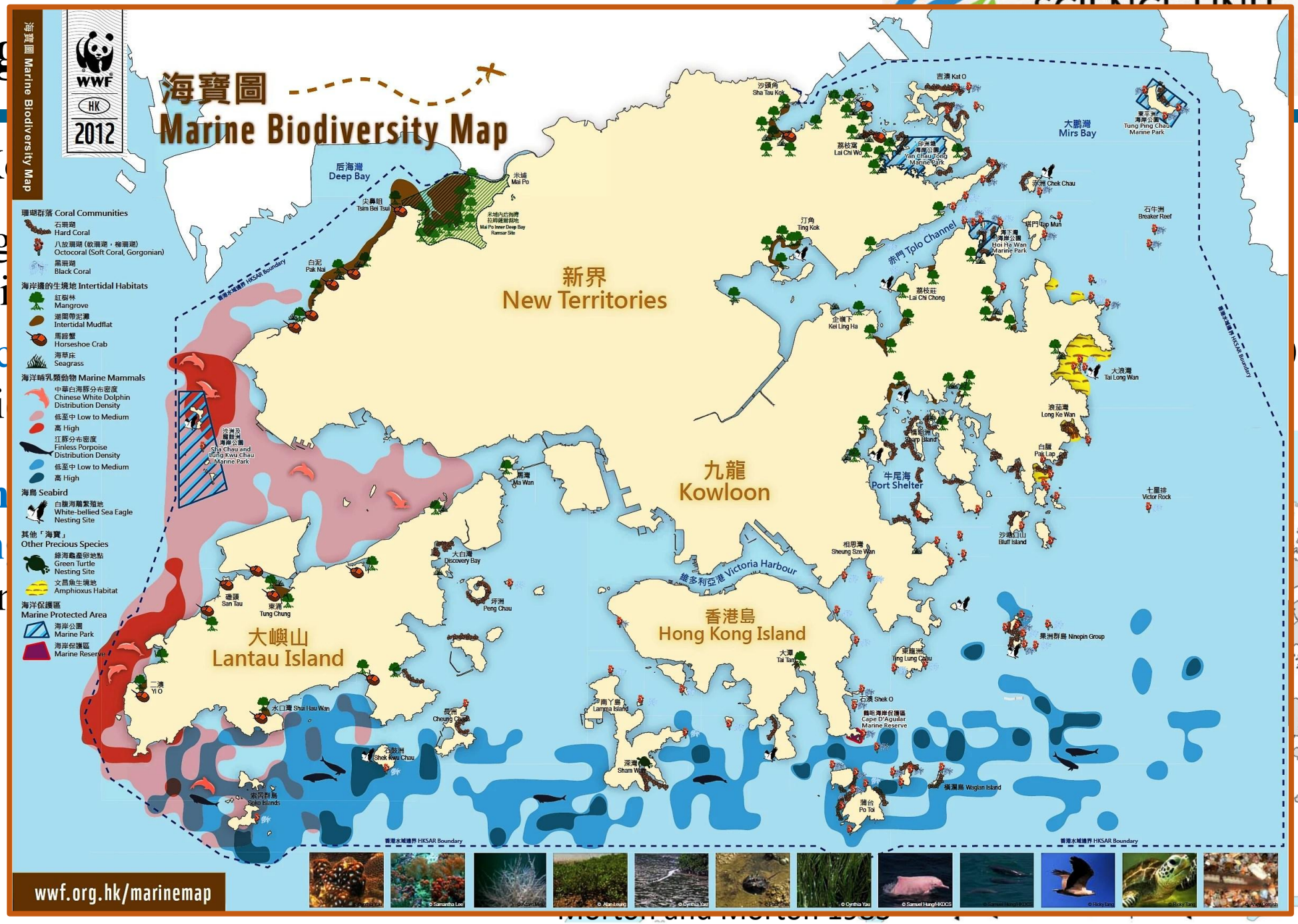

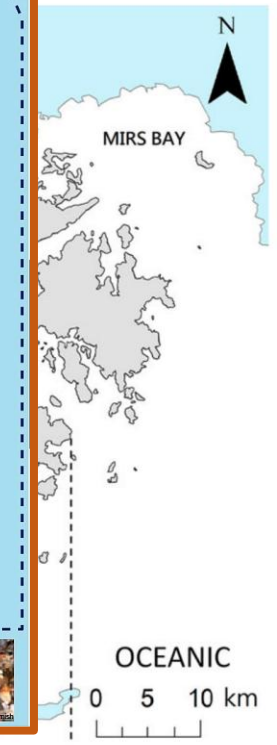

Background

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科學教研組

Hong Kong - an urbanized subtropical estuary 香港—城市化的亚热带河口

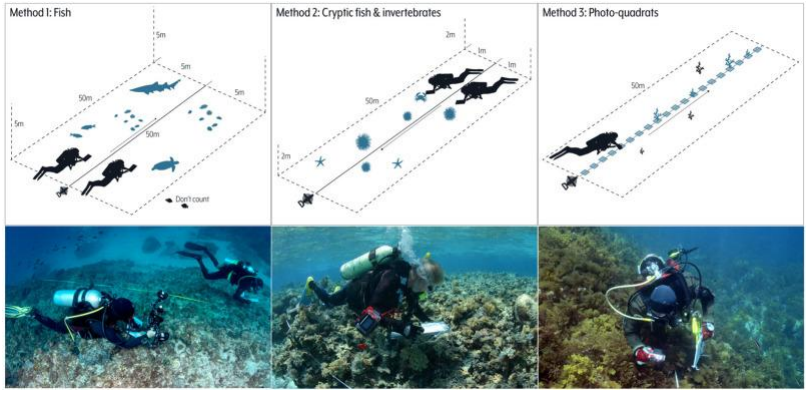
- Recognizing the importance of marine conservation, HK government has implemented a number of management measures, such as **9 marine protected areas** (since 1996**), restoration, trawling ban** (Dec 31, 2012), and **regulator monitoring.**
- Biodiversity Strategy and Action Plan (**BSAP**)
- EPD marine water quality monitoring

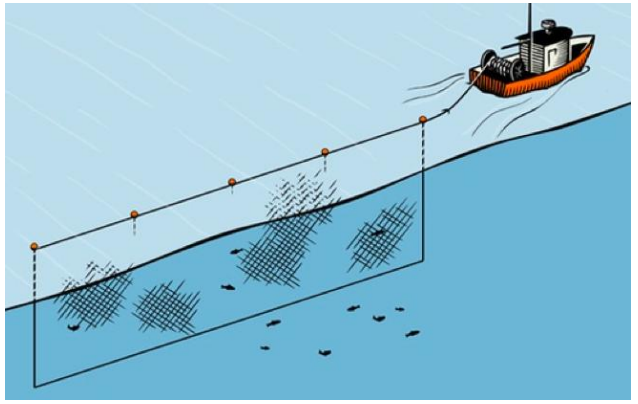
AFCD HKSAR

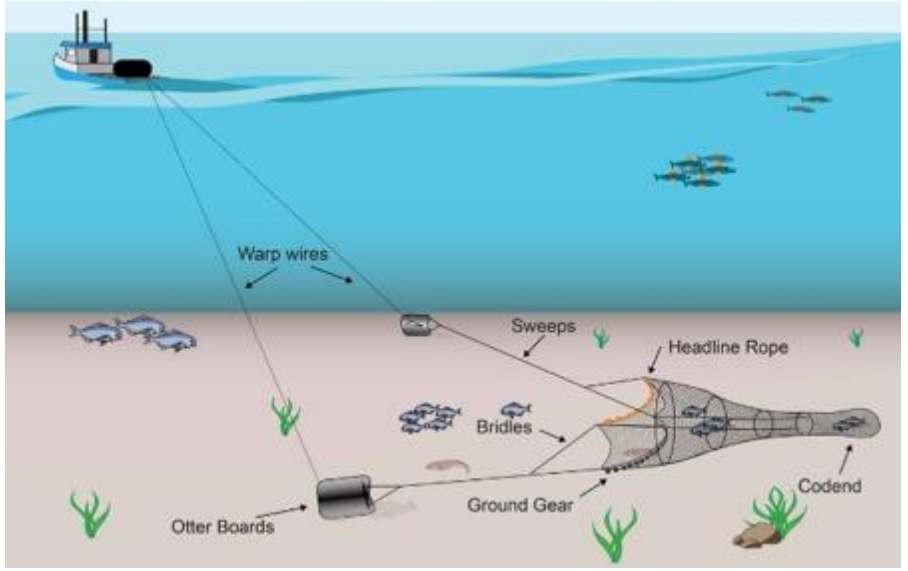
Background

Biomonitoring and limitation in estuary Ecosystem 河口生态系统的生物监测与限制

- Biomonitoring aquatic organisms in estuaries is challenging due to their complex environmental conditions (e.g., salinity and turbidity) and diverse habitats.
- Traditional surveys like trawling and gillnetting have negative effects on fishery resources and ecosystems.
- Non-destructive sampling methods like underwater visual censuses and echo sounder surveys have **limitations in data quality and habitats**.
- These conventional surveys are **cost and labor intensive**.







Background

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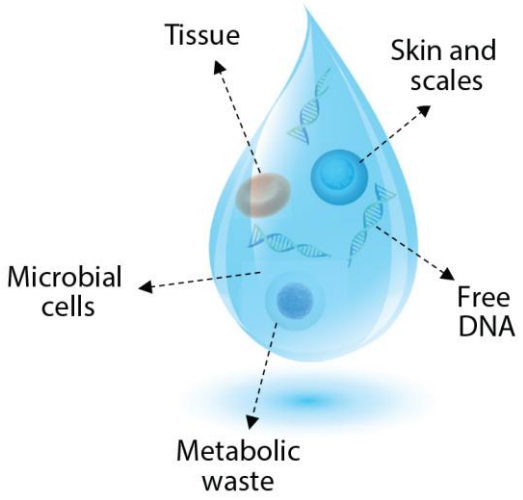
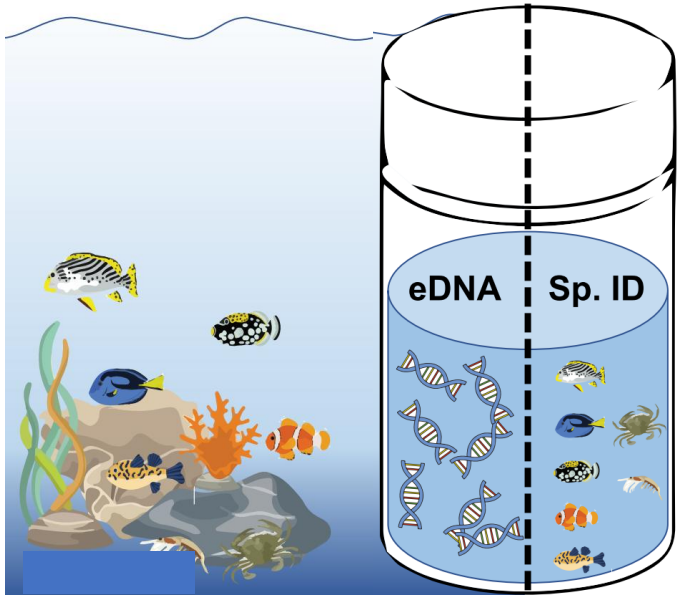
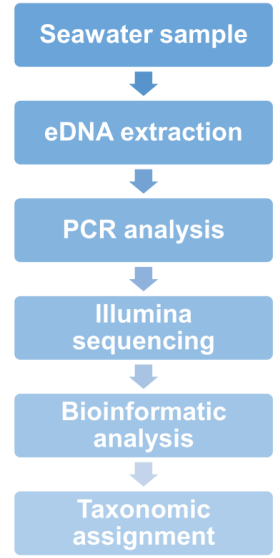
Environmental DNA

- A new method called environmental DNA (eDNA) metabarcoding has revolutionized the way we monitor fish and other aquatic communities.
- Instead of physically capturing organisms, this method collects and analyzes DNA left behind by organisms in the water.
- eDNA metabarcoding allows for comprehensive biomonitoring without causing harm or disturbing the ecosystem.





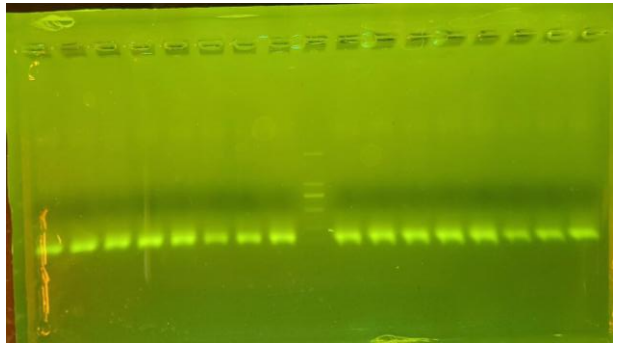


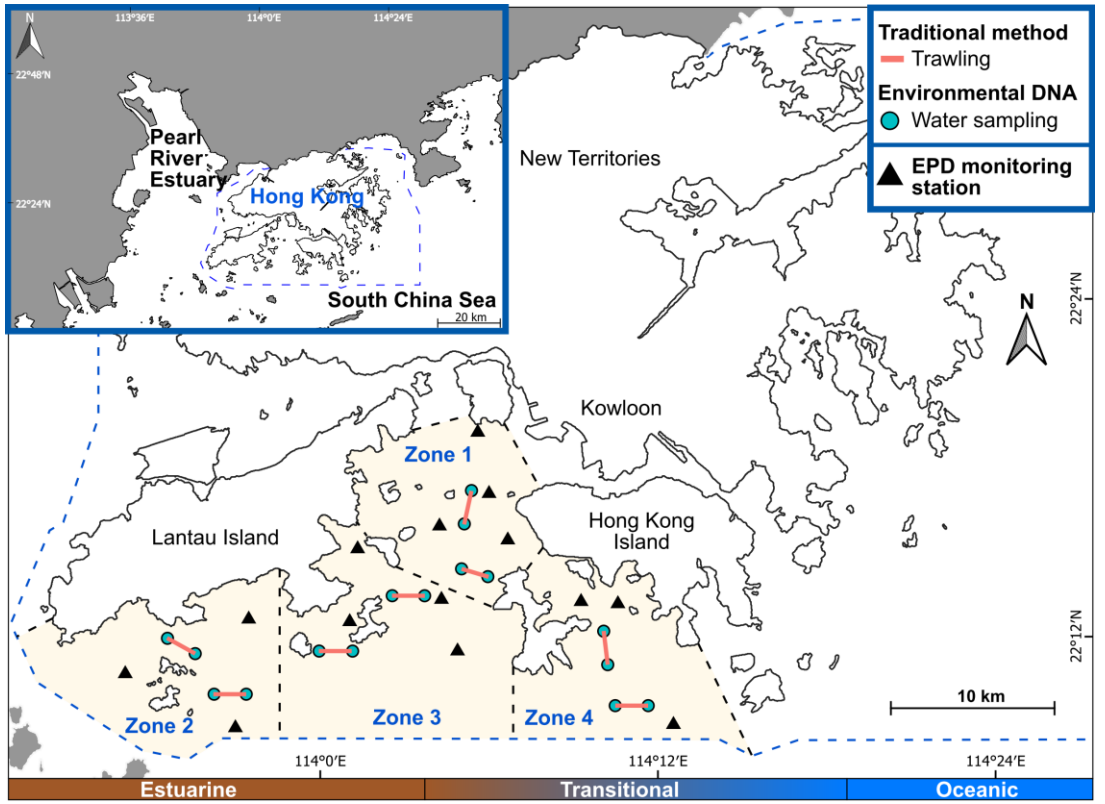
Comparison of eDNA and convectional surveys

In 2022, Bottom trawling in Southern waters

eDNA sampling

- 8 trawl sites (start and end)
- 2-L seawater x 2 replicates
- Target taxa: marine vertebrates (12S-V5, MiFish-U, Berry-Fish), and crustacean (MiDeca)



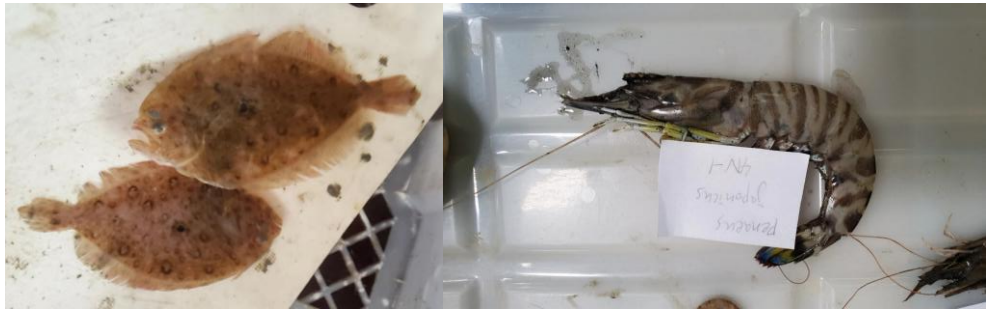


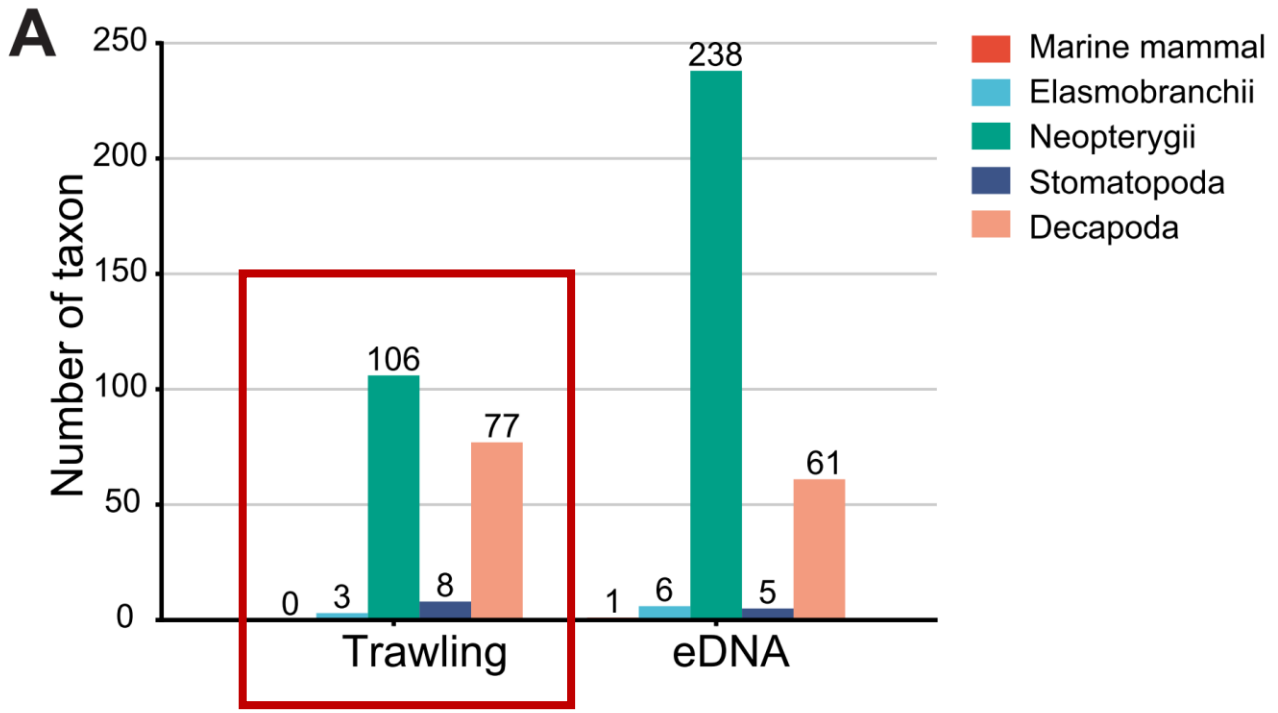
LCF project, HKSAR 2022-2025; SKLMP project 2022-2024

Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

- Workload for trawling: **Eight researchers** and experts for sampling on the boat and subsequent laboratory works, taking > **two months** to process.
- Trawling captured a total of **236 taxa from 8 trawl sites**
  - ❑ 3 elasmobranchs
  - ❑ 106 bony fishes
  - ❑ 85 crustaceans

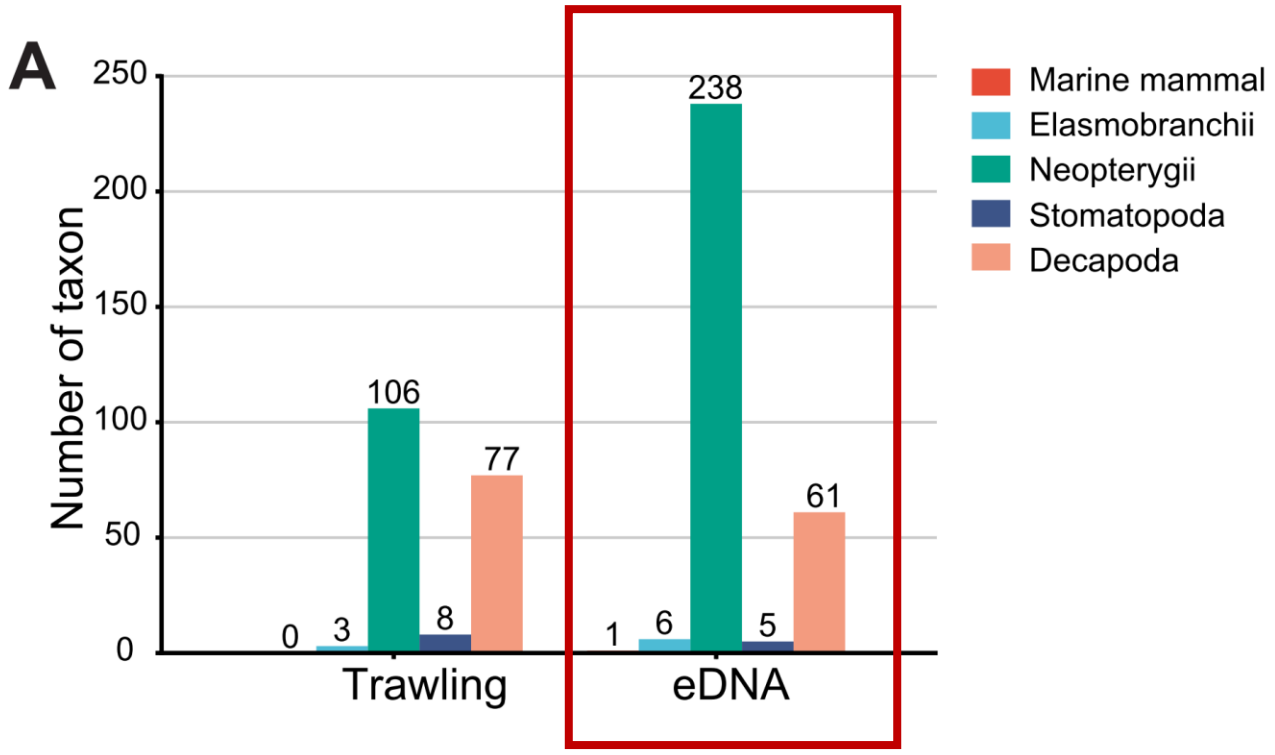




Comparison of eDNA and convectional surveys

Comparison between trawling and eDNA methods

- Workload for eDNA: **One researcher** for sampling on the boat and subsequent laboratory works, taking ~**one month** to process.
- eDNA identified a total of **311 taxa from trawl sites**
  - ❑ 1 marine mammal
  - ❑ 6 elasmobranchs
  - ❑ 238 bony fishes
  - ❑ 66 crustaceans
- Primer performances
  - ❑ 12S-V5 detected more vertebrates
  - ❑ BerryFish (16S) more specific on bony fishes
  - ❑ **Muti-assays** enhance the detection



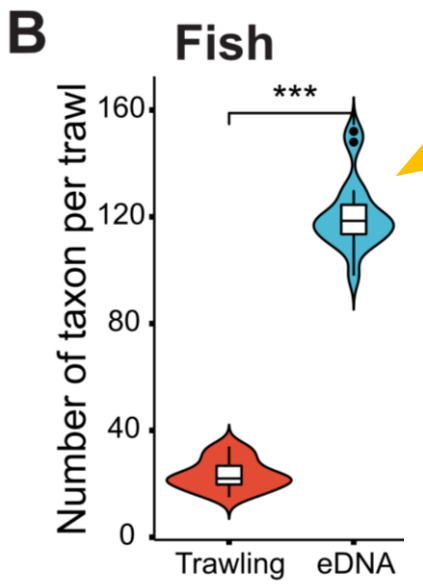
Comparison of eDNA and convectional surveys

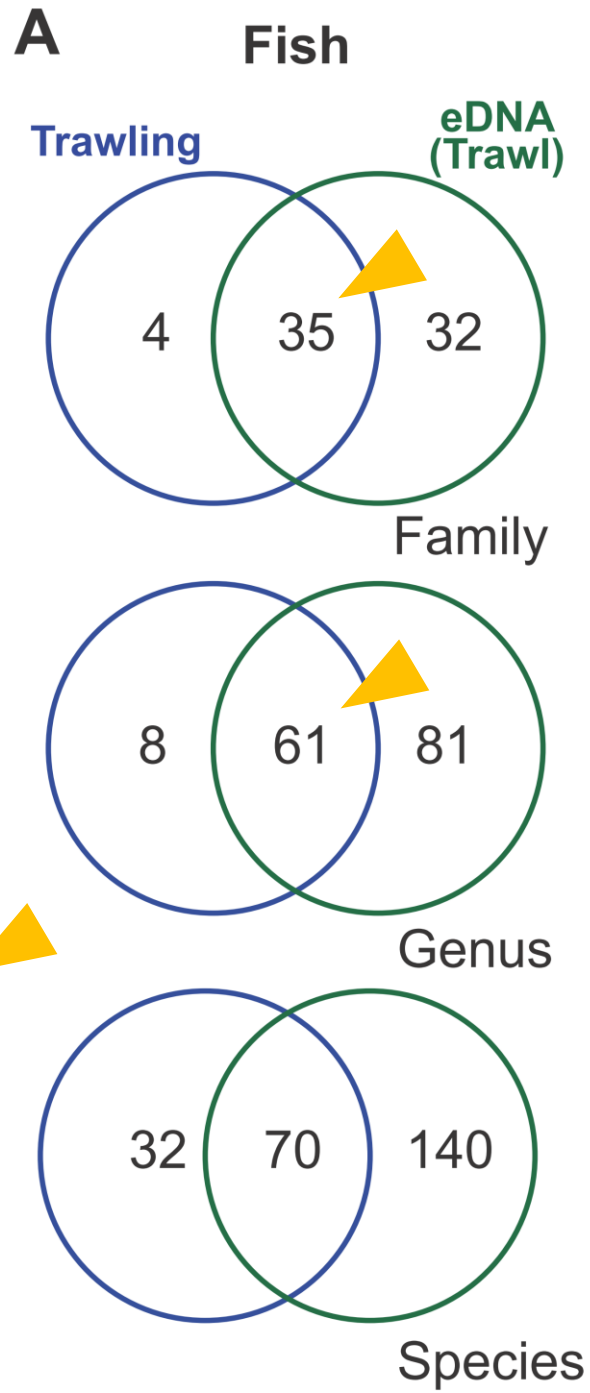
Comparison between trawling and eDNA methods

Fish community

- Trawling: 109 taxa, **102 species** from 39 families
- eDNA: 238 taxa, **210 species** from 67 families
- eDNA data covered most of the family (35/39) and genera (61/69) detected in trawling
- eDNA detected higher diversity and more fish per sample

In 2024, western waters surveys
Bottom trawling captured 85 fish species
eDNA approach detected 162 fish species



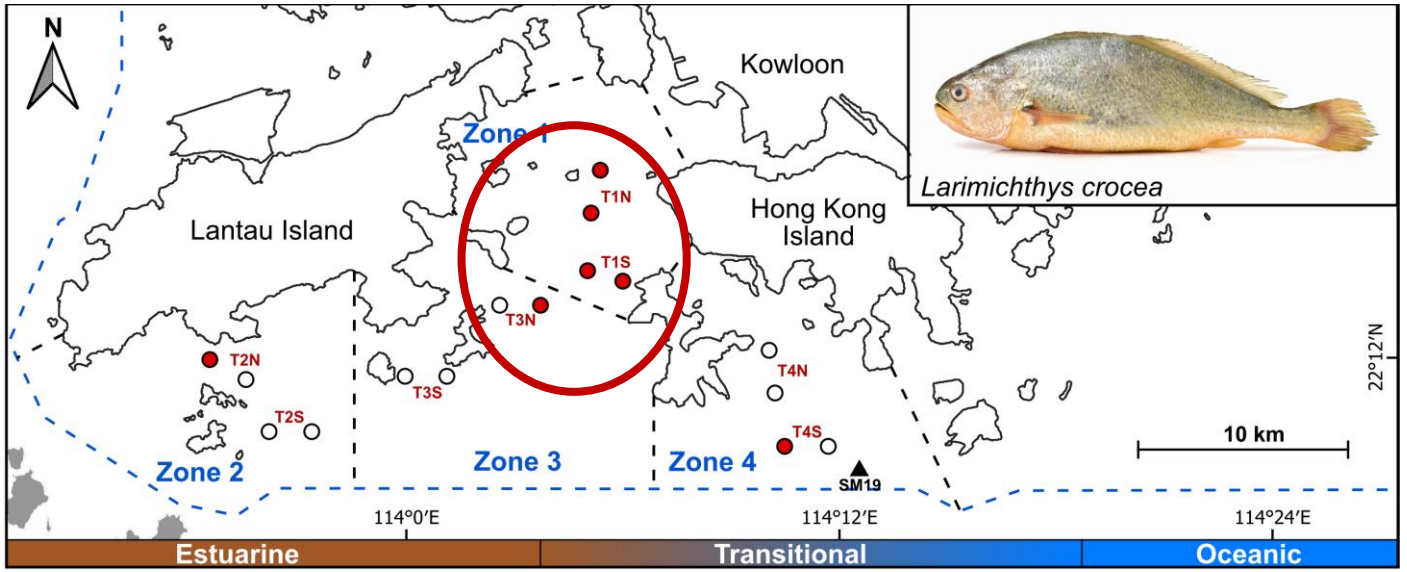


Comparison of eDNA and convectional surveys

eDNA as a sustainable tool for monitoring rare and threatened species

- Trawling captured **three threatened species**: two Vulnerable elasmobranchs (*Gymnura japonica* and *Telatrygon zugei*), Endangered Threadfin Porgy (*Evynnis cardinalis*).
- eDNA identified **nine threatened species**, including one marine mammals *N. phocaenoides*, two elasmobranchs, and Critically Endangered *Larimichthys crocea*, and Endangered *E. cardinalis*.

Large yellow croaker



ORIGINAL ARTICLE | Open Access

Bottom Trawling and Multi-Marker eDNA Metabarcoding Surveys Reveal Highly Diverse Vertebrate and Crustacean Communities: A Case Study in an Urbanized Subtropical Estuary

Jack Chi-Ho Ip, Hai-Xin Loke, Sam King Fung Yiu, Meihong Zhao, Yixuan Li, Yitao Lin, Chun-Ming How, Jiezhang Mo, Meng Yan, Jinping Cheng, Vincent Chi-Sing Lai, Leo Lai Chan ... See all authors

First published: 04 November 2024 | https://doi.org/10.1002/edn3.70031

Ongoing Biodiversity Projects

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Southern & Western Waters

1. LNG-MCEF: Mangrove habitat eDNA
2. MEEF: Chinese Bahaba eDNA
3. ECF: SLMP's AR & habitat restoration

Eastern Waters

4. GRF – Coral-Zooxanthellae health
5. ECS – Coral and coral reef eDNA

Hong Kong Coastline

6. ECF: Intertidal biodiversity & connectivity

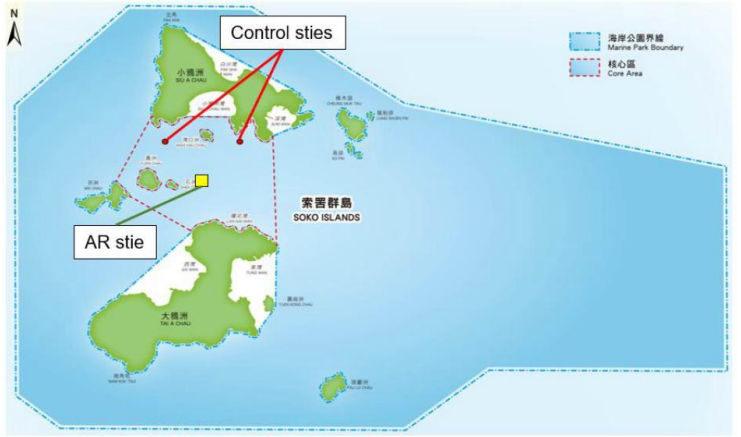
Fishery Enhancement

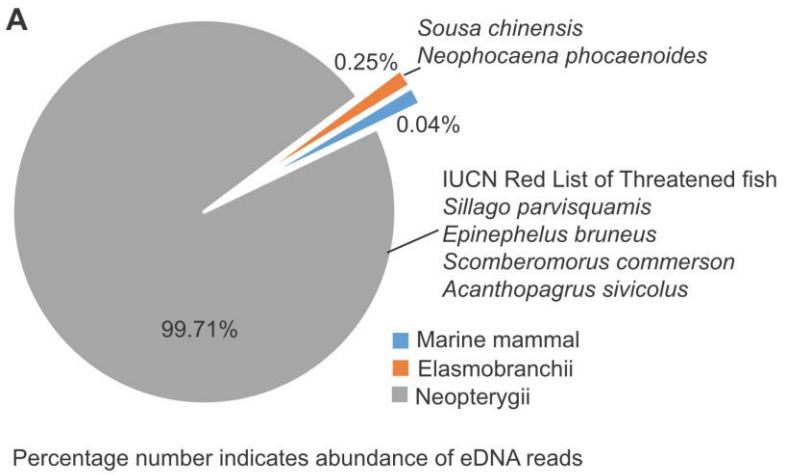
7. LNG-FEF: Upcycling fruit waste as adductive aquafeed

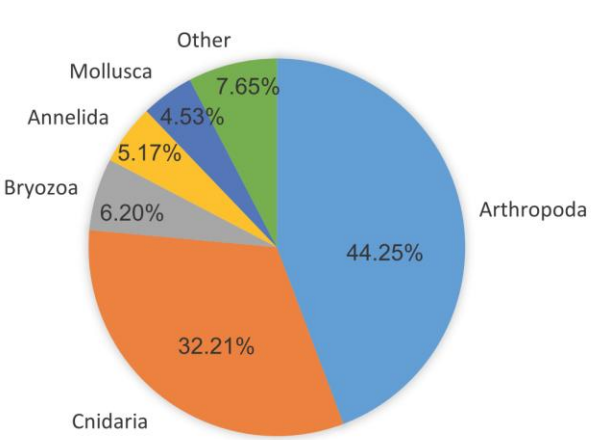
Ongoing projects -eDNA surveys

Monitoring Artificial reef communities in South Lantau Marine Park

- Monitoring the species communities' changes before and after the deployment of AR in SLMP
- Evaluate the AR performance using eDNA and convectional approaches
- Detected over **150 fish species** and **two marine mammals** in premonitoring surveys





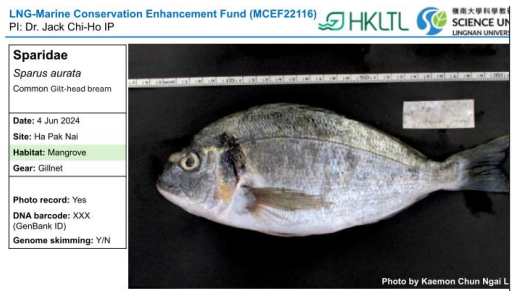


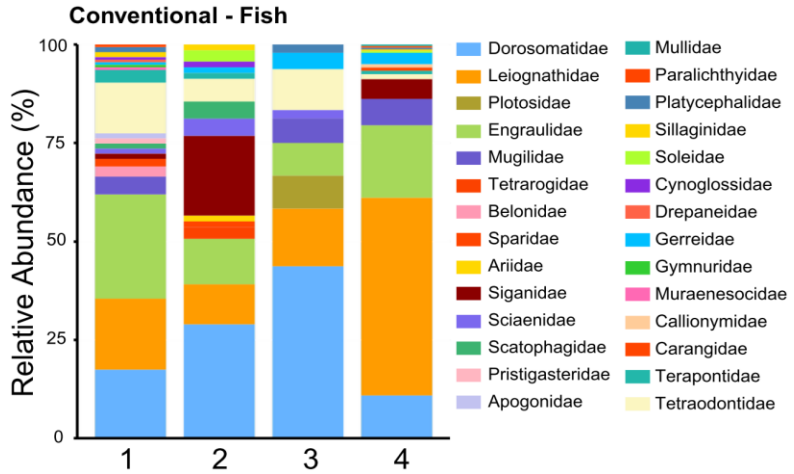
ECF, HKSAR project 2024-2026
PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Prof. Jian-wen Qiu (HKBU)

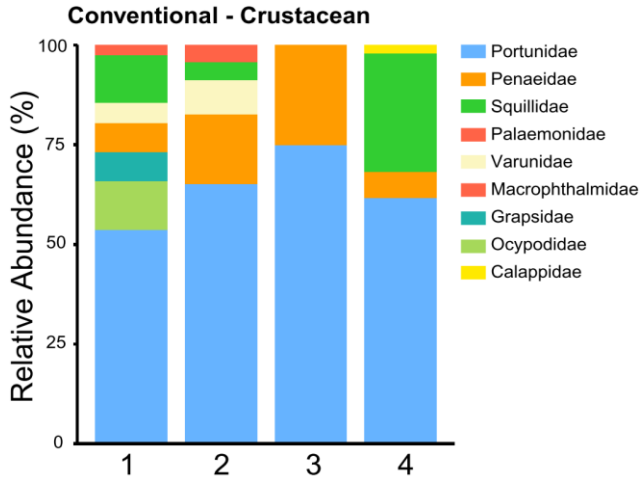
Ongoing projects -eDNA surveys

Monitoring mangrove communities using eDNA and gill netting surveys

- Survey the biodiversity of fish and crustaceans in mangrove habitat
- Conventional surveys captured **626 individuals from 76 species**
- Submitted for sequencing







LNG-MCEF project 2024-2025

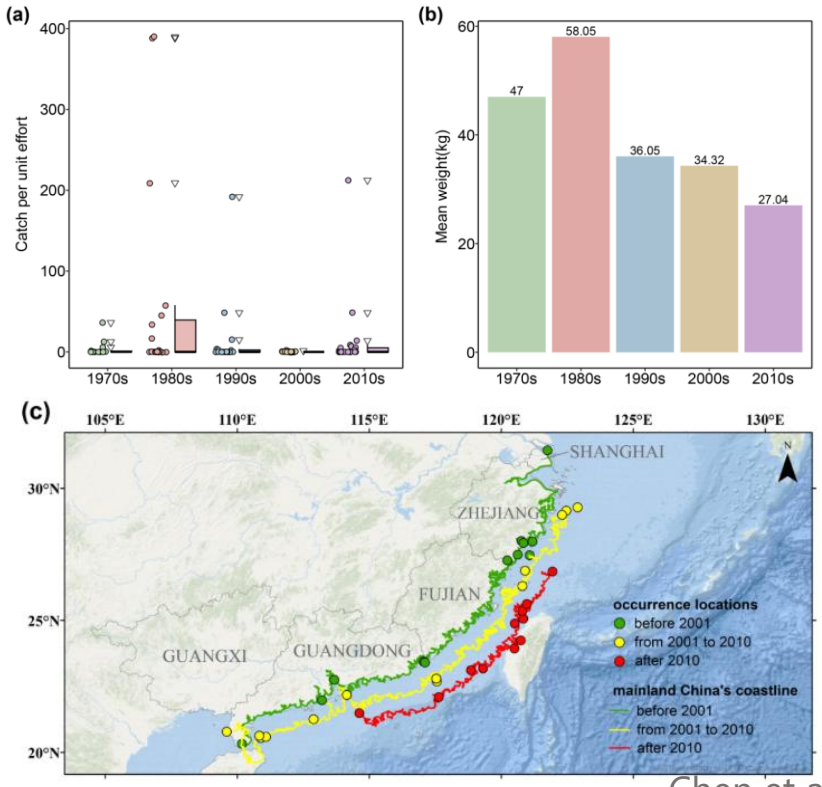
PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Prof. Jian-wen Qiu (HKBU), Dr. Meng Yan (SKLMP)

Ongoing projects -eDNA surveys

Monitoring Chinese bahaba 黃唇魚 in Hong Kong western waters

Endemic to China 原产于中国, also called Huang Chun Yu (黄唇鱼), Tai O Fish (大澳鱼)

National Second-class protection 国家二级保护 (1988) → First-Class 国家一级保护 (2021); Listed as Critically Endangered (濒临灭绝) in IUCN



Chen et al. 2025

2025 – 61Kg Bahaba CNY3.5M

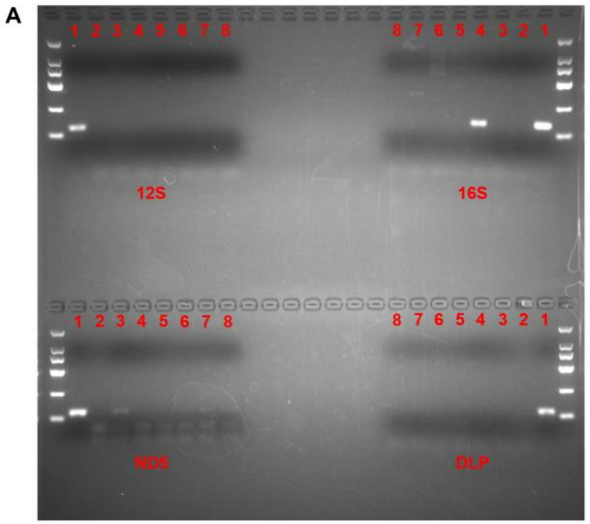
MEEF, HKSAR project 2024-2026

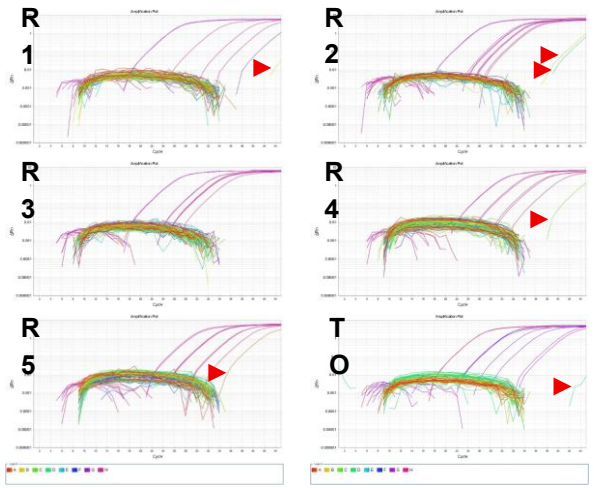
PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Dr. Junjie Wang (SCNU)

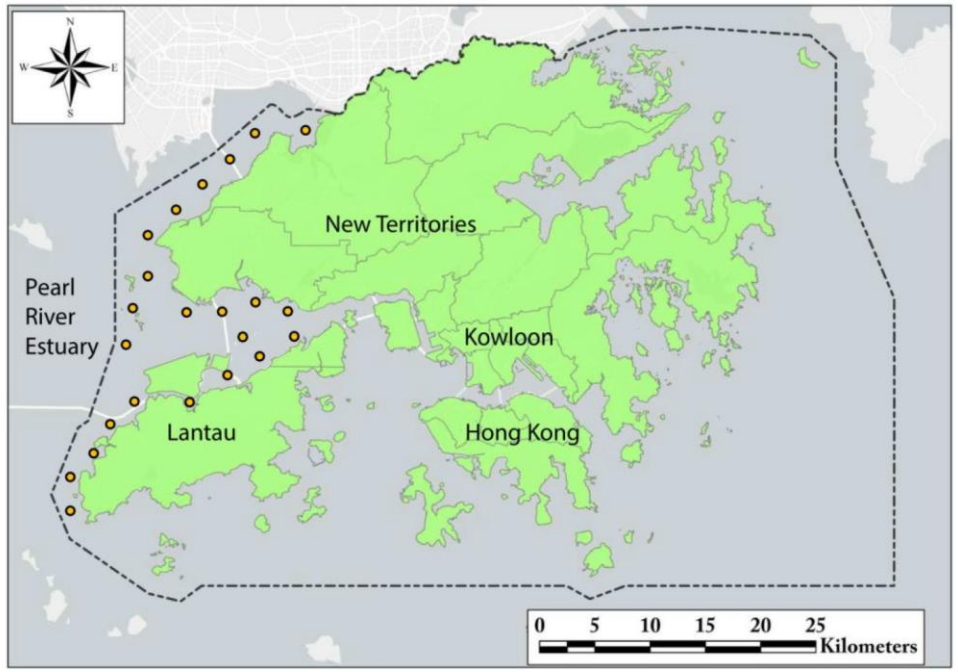
Ongoing projects -eDNA surveys

Can we find Chinese bahaba (黃唇鱼) in Hong Kong western waters?

Developed species-specific qPCR assays







MEEF, HKSAR project 2024-2026

PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Dr. Junjie Wang (SCNU)

Ongoing projects -eDNA surveys

Marine Ecology & Fisheries Enhancement Funds Trustee Limited
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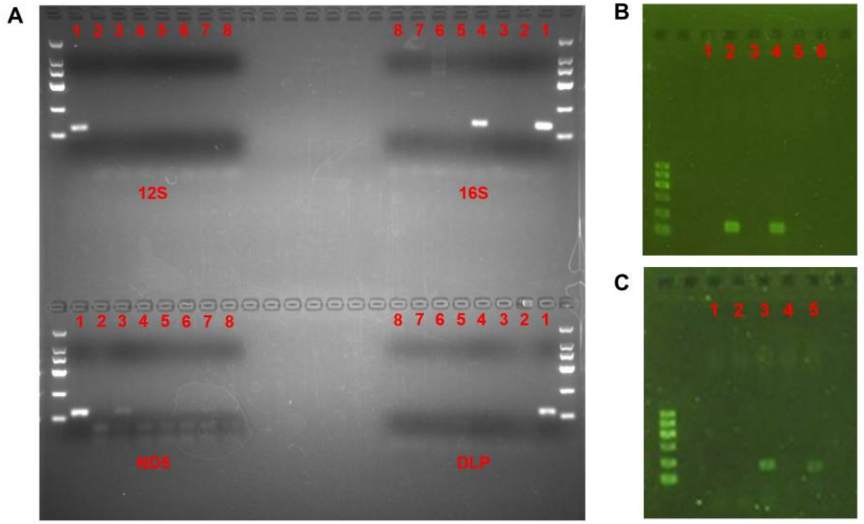
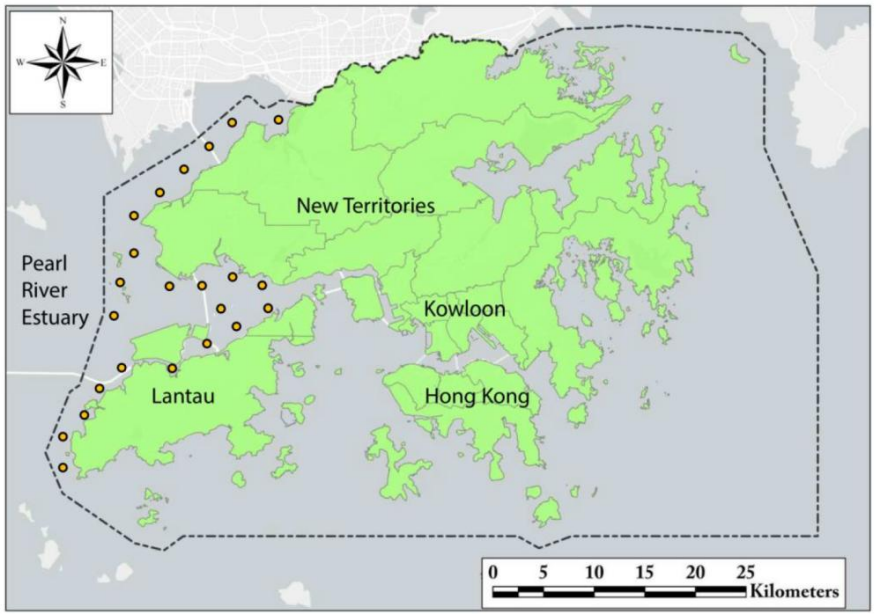
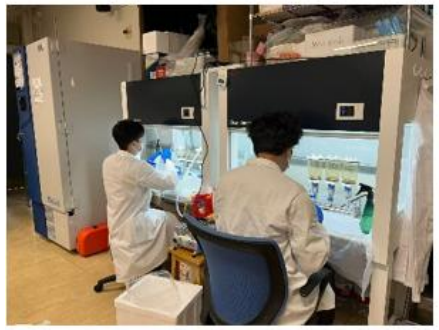
Monitoring Chinese bahaba 黃唇魚 in Hong Kong western waters

- Conduct eDNA surveys using a species-specific qPCR approach to trace any Chinese Bahaba in western Hong Kong waters.







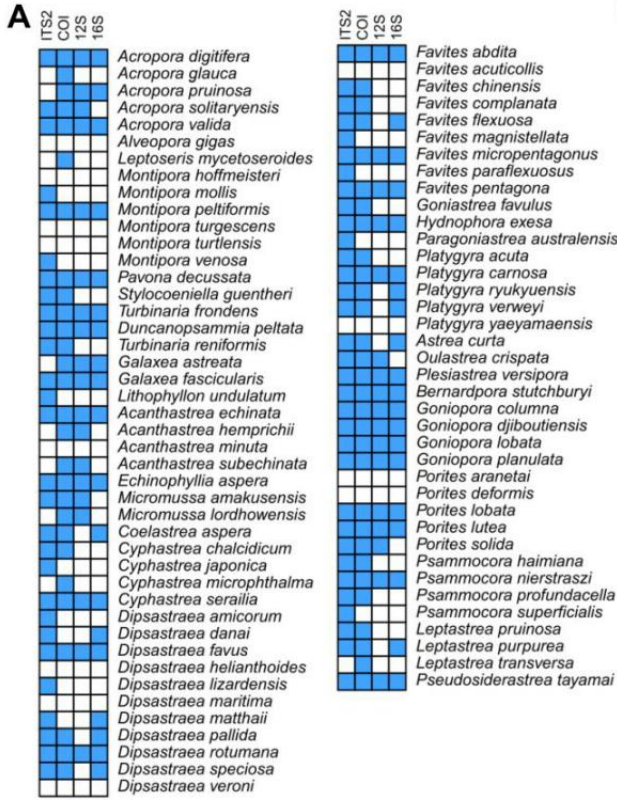
MEEF, HKSAR project 2024-2026

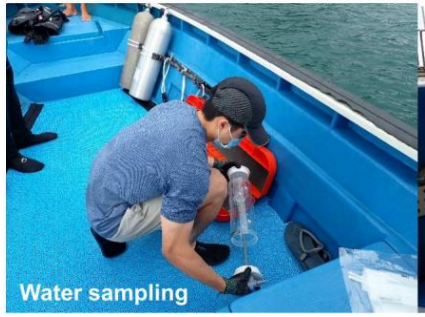
PI: Dr. Jack Chi-Ho Ip (LU), Co-I: Dr. Junjie Wang (SCNU)

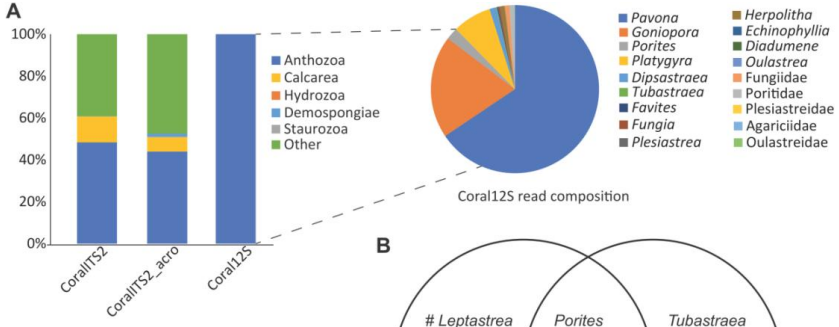
Ongoing projects -eDNA surveys

Monitoring coral communities using eDNA and SCUBA

- Enhance scleractinian DNA reference database
- developing new scleractinian-specific primers
- Assess the performance of eDNA method by comparing eDNA results with visual data
- establish a comprehensive baseline of spatial and temporal variation in scleractinian assemblages in **33 reef check sites**



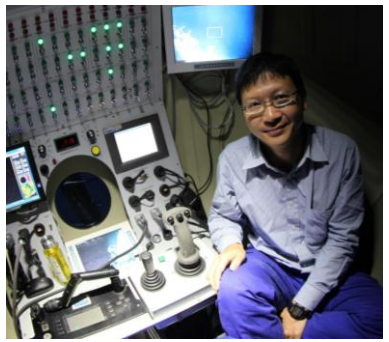




ECS, UGC, HKSAR project 2025-2027
PI: Dr. Jack Chi-Ho Ip (LU)

Acknowledgements

Collaborators



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Dr. Tommy Hui
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Hong Kong Offshore LNG Terminal Project

HKLTL