Hong Kong Offshore LNG Terminal Project
Marine Conservation Enhancement Fund

Completion Report

PART A: The Project and Investigator

1. Funded Project Details

Project Number:	MCEF20114			
Project Title:	Feasibility study of detecting local seahorse and pipefish species in the coastal areas of Hong Kong by environmental DNA (eDNA)			
Name of Organisation:	The Chinese University of Hong Kong			
Reporting Period:	From:	1st January 2022	To:	30th September 2024
Date of Report Submission:	30 December 2024			

2. Information of the Principal Investigator

Name:	Apple Chui Pui Yi
Position Held:	Research Assistant Professor
Tel. No.	39438026
Email Address	applepychui@cuhk.edu.hk

3. Funded Project Schedule

Commencement Date		Completion Date	
Original	Actual	Original	Actual
1/1/2022	1/1/2022	31/12/2023	30/9/2024

(i) Executive summary of the Funded Project (1-2 pages)

The project aimed to investigate the feasibility of detecting local seahorse and pipefish species in the coastal areas of Hong Kong using environmental DNA (eDNA) methods, including quantitative PCR (qPCR) and metabarcoding. The project successfully implemented, validated, and employed eDNA assays to assess their potential for practical application. This executive summary outlines the main achievements, challenges encountered, and provides suggestions for future actions following the completion of the project.

The project successfully achieved several goals that demonstrated the promising potential of eDNA methods for monitoring local seahorses and pipefish in Hong Kong waters. The key accomplishments are as follows:

- Designed a qPCR assay specific for the yellow seahorse (*Hippocampus kuda*) of the West Pacific Ocean lineage.
- Tested and validated the developed qPCR assay alongside Nester's 16SFishSynShort using aquarium tank samples and seawater samples from field sites.
- Established a standardized sampling strategy for eDNA methods, demonstrating that the detection of target species can be achieved by collecting 5L of seawater at each sampling point along transects and precipitating the eDNA with multiple membranes.
- Conducted a comprehensive and systematic survey of seahorse and pipefish species in the coastal regions, covering western, southern, and eastern waters in Hong Kong during both wet and dry seasons, by underwater visual census (UVC) and eDNA techniques.

These accomplishments demonstrat the potential of eDNA for monitoring the biodiversity of rare and cryptic organisms. However, several challenges emerged during the project, highlighting certain limitations of qPCR and metabarcoding assays. Follow-up actions were taken to overcome these barriers. The main issues encountered included:

- The high turbidity of the western waters in Hong Kong caused rapid clogging of filter membranes, hindering the efficient capture of eDNA from seawater samples. To enhance sample collection effectiveness, the Smith-Root ANDe™ backpack eDNA sampler was used.
- Initial qPCR results from samples collected at the pier of Marine Science Laboratory were negative, despite the observation of *Hippocampus kuda*. After purifying the DNA samples using a PCR Inhibitor Removal Kit, subsequent results became positive.
- In the pilot study, 1 L samples collected at Yam O did not yield any positive eDNA signals, despite the presence of *H. kuda*. A follow-up water sampling was conducted to determine the minimum volume of water required for the detection of Syngnathidae eDNA.

Limitations in using eDNA methods for investigating Syngnathidae species in Hong Kong:

- The qPCR assay also amplifies DNA of *Hippocampus hilonis,* a seahorse species endemic to Hawaii, due to its shared cytochrome b sequence with *H. kuda*.
- In eDNA metabarcoding, only approximately 0.17% of the total reads belong to Syngnathidae, indicating a disproportionately small representation and suggesting that the primers might not be specific enough to exclude the amplification of non-target species.
- The use of short read sequences in the metabarcoding assay limited the ability to resolve some seahorses and pipefish at the species level.

Key outcomes in terms of Syngnathidae species monitoring and conservation:

- Successfully conducted the first comprehensive survey of seahorses and pipefish in the coastal areas of Hong Kong using eDNA methods.
- Assessed the biodiversity of local Syngnathidae species, including species richness, relative abundance, and species composition of seahorses and pipefish across various sites and seasons.
- Improved understanding of the distribution of local seahorse and pipefish populations, revealing their presence not only in eastern waters but also in estuaries and the central transition zone of Hong Kong’s waters.
- Organized educational outreach events to promote the conservation of seahorses and pipefish.

Based on the findings of our project, we provide the following recommendations that outline the key actions for future progress:

- Accurately curating the barcoding dataset and correcting mismatched or misidentified species sequences of local seahorses and pipefish in the online DNA database will enhance the effectiveness of the metabarcoding assay.
- A comprehensive database of local Syngnathidae species sequences is needed and should be developed by experienced taxonomists with strong identification skills.
- Optimizing and refining the 16SFishSynShort primer pair is crucial for improving the specificity and discriminatory power of the eDNA metabarcoding method.
- Future studies should implement the eDNA assay on a larger scale and over a longer time frame to enable more thorough monitoring of Syngnathidae species in Hong Kong and nearby regions.

Our study suggests that, with careful consideration of environmental factors and the implementation of appropriate sampling protocols, eDNA assays are sensitive tools for detecting Syngnathidae. They can effectively complement traditional survey methods for biodiversity assessments, particularly in turbid waters.