Marine Ecology Enhancement Fund (MEEF) Completion Report

Funded project:	Impact of microplastics on the Chinese horseshoe crab *Tachypleus tridentatus* in Hong Kong western waters, Phase II
Reference number:	MEEF2018011A
Approved fund:	HK$ 349,000 (for July 2019 to 31 December 2020)
Name of organisation:	The Hong Kong Polytechnic University
Project Leader:	Dr James Kar-Hei Fang, Assistant Professor Department of Applied Biology and Chemical Technology
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I. Executive summary

Horseshoe crabs have existed on Earth for more than 400 million years and are referred to as "living fossils". The western waters of Hong Kong represent some of the few areas in the world where Chinese horseshoe crabs (*Tachypleus tridentatus*) live and breed. However, the same areas are subjected to microplastic pollution, an emerging environmental problem not only in Hong Kong but around the world. This project comprised two phases, aiming to evaluate the ecological risk of microplastics to *T. tridentatus*. Phase I comprised laboratory experiments to determine the ecophysiological responses of *T. tridentatus* to three common types of microplastics, while Phase II contained field surveys to investigate the current levels of microplastics in major nursery grounds of *T. tridentatus* in the western waters of Hong Kong.

Results of the Phase I experiments identified negative effects of all tested microplastics on *T. tridentatus* in different ecophysiological aspects, among which polyethylene terephthalate (PET) appeared to be more harmful that can lead to mortality. These findings are ecologically relevant, as about a third of the microplastics were identified to be PET in the sediment collected from local nursery grounds of *T. tridentatus* in Phase II. The majority of the identified microplastics existed as fragments and fibres. More than half of them fell in the size range of 31–250 µm. Among the five study sites, Shui Hau Wan on the southern coast of Lantau Island contained the highest number of microplastics in sediment and thus the highest risk to the local horseshoe crabs. Apart from the research work, a number of conservation education and publicity activities including guest seminars, exhibition and media coverage were organised for university students and the general public to promote conservation of horseshoe crabs in Hong Kong.

II. Brief description of the Project

There is growing evidence of plastic pollution in the nursery grounds of Chinese horseshoe crabs (*Tachypleus tridentatus*) in Hong Kong (Fig. 1a; Fok & Cheung 2015; Cheung et al. 2016; Tsang et al. 2017; Lo et al. 2018; Xu et al. 2020a, 2020b). The population densities of Chinese horseshoe crabs have decreased in some of these areas (Fig. 1b; Lee & Morton 2016). Microplastics can be one of the factors leading to this decline according to our findings in Phase I, which identified negative ecophysiological effects on juvenile *T. tridentatus* by three types of tested microplastics, among which PET appeared to be more harmful that was associated with mortality. In this connection, field surveys were carried out in Phase II to determine the numbers of PET and other microplastics in five major nursery grounds of *T. tridentatus* in the western waters of Hong Kong. Phase II has been

successfully completed. Research findings from Phase II, along with the conservation education and publicity activities, are summarised in this project completion report.

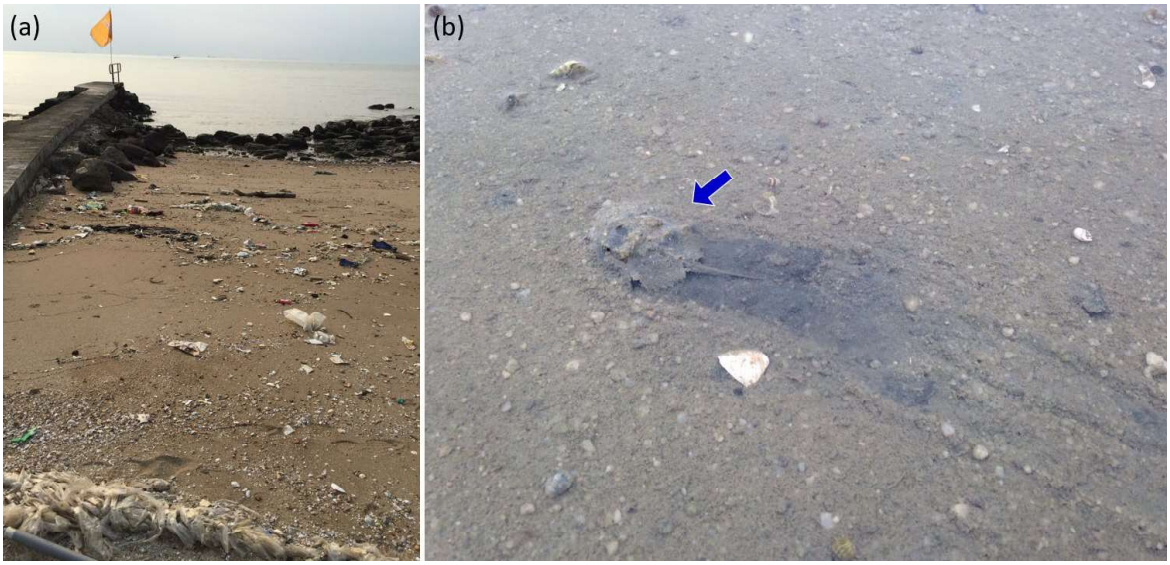


Fig. 1. (a) Plastic pollution in Yi O, one of the nursery grounds of Chinese horseshoe crabs (*Tachypleus tridentatus*) in Hong Kong. Plastic debris can be broken down into various sizes of microplastics, e.g. through ultraviolet degradation (Andrady 2017). (b) A juvenile *T. tridentatus* (arrowed, around 30 mm in prosomal width) found on a mudflat in Ha Pak Nai, another local nursery ground. The burrowing nature of horseshoe crabs (e.g. digging for food) makes them a high-risk group to microplastic pollution.

III. Completed activities against the proposed work schedule

Phase II of this project was in good progress according to the proposed schedule until November 2019, after which The Hong Kong Polytechnic University has been fully or partially closed unfortunately due to the social unrest and damage to the campus, followed by the outbreak of COVID-19. A six-month extension of the project period, without request for additional funding, has been approved (July–December 2020). All proposed tasks including sediment sampling, extraction of microplastics, their characterisation using Raman microspectrometry, data analysis and the seminar for university students have been completed (Table 1). Details of these activities are provided in Section IV.

Table 1. The approved extended schedule for Phase II (July 2019–December 2020).

Time (months, year)	Jul–Sep 2019	Oct–Dec 2019	Jan–Aug 2020	Aug–Nov 2020	Nov–Dec 2020
General preparation					
Field sampling of sediment					
Extraction of microplastics from sediment samples		The delay period due to the social unrest and COVID-19 outbreak			
Characterisation of microplastics using Raman microspectrometry					
Data analysis					
Seminar to university students					

IV. Results/descriptions on the completed activities with appropriate analysis, with the support of photos

Field surveys were carried out in summer 2019 to assess microplastics in five important nursery grounds of Chinese horseshoe crabs in Hong Kong western waters including, from north to south, Ha Pak Nai (HPN), Shan Tau (ST), Sham Wat (SW), Yi O (YO) and Shui Hau Wan (SHW; Fig. 2, 3). Nine sediment samples were collected per site along three line transects at 1.0 m above chart datum, 1.5 m above chart datum and below strandline. All collected samples were transported to the laboratory, dried and weighed to 500 g. The weighed samples were well mixed with a high-density solution of zinc chloride following Lo et al. (2018). Sediment and other higher-density particles deposited to the bottom, while the lower-density plastic particles floated to the surface. The top layer of the solution was collected and filtered through 5000 μm, 250 μm and 31 μm to retain and separate plastic particles into three size ranges including > 5000 μm, 250–5000 μm and 31–250 μm.

Fig. 2. Five sampling sites of microplastics in this study including mudflats in Ha Pak Nai, Shan Tau and Sham Wat, and sandy shores in Yi O and Shui Hau Wan. All selected sites are important nursery grounds of Chinese horseshoe crabs.

Fig. 3. (a) A field survey of microplastics in Ha Pa Nai, where (b) Chinese horseshoe crabs co-exist with (c) plastic debris. (d) Our team member is showing the sediment samples and a Chinese horseshoe crab to the filming crew of the Marine Ecology Enhancement Fund.

Larger-sized plastics (> 5000 μm) and microplastics of 250–5000 μm were identified using the point acquisition mode of a Renishaw inVia confocal Raman microscope (Wotton-under Edge, UK) equipped with a Leica 10× objective (Wetzlar, Germany) and a 785 nm edge laser (300 mW output power). Raman spectra were acquired for 10 s using 0.1–1% laser power in the wavenumber range of 676–1767 cm^{-1}. Baseline correction, smoothing and cosmic ray removal of the acquired spectra were performed with the Renishaw WiRE 5.2 software. The polymer types of microplastics were identified from these Raman spectra using the Renishaw Polymeric Materials Database.

The smaller microplastics of 31–250 μm were assessed in an automated mapping mode of the Renishaw inVia system using a 785 nm streamline laser. The whole circle coated with microplastics (8 mm in diameter) on each filter membrane was scanned at 10% laser power and a spatial resolution of 28.4 μm. Raman spectra were acquired at 5 s per pixel. The mapping process required approximately 14 h and generated more than 10,000 Raman spectra per sample, among which microplastics, if any, were identified using the Renishaw Polymeric Materials Database. Identified microplastics were colour-coded and illustrated in a two-dimensional panel (Fig. 4). Other settings and criteria remained the same as in the point acquisition mode.

The shapes and sizes of identified microplastics were visually verified under a stereomicroscope. Microplastics were categorised into five forms of shape including fragment, fibre, film, foam and pellet. The size of microplastics in all shapes was expressed as the longest dimension across the area, except for fibre of which the size was measured in

length along the central axis. The size measurements were performed on stereomicrographs of the microplastics using the software ImageJ (National Institutes of Health, MD).

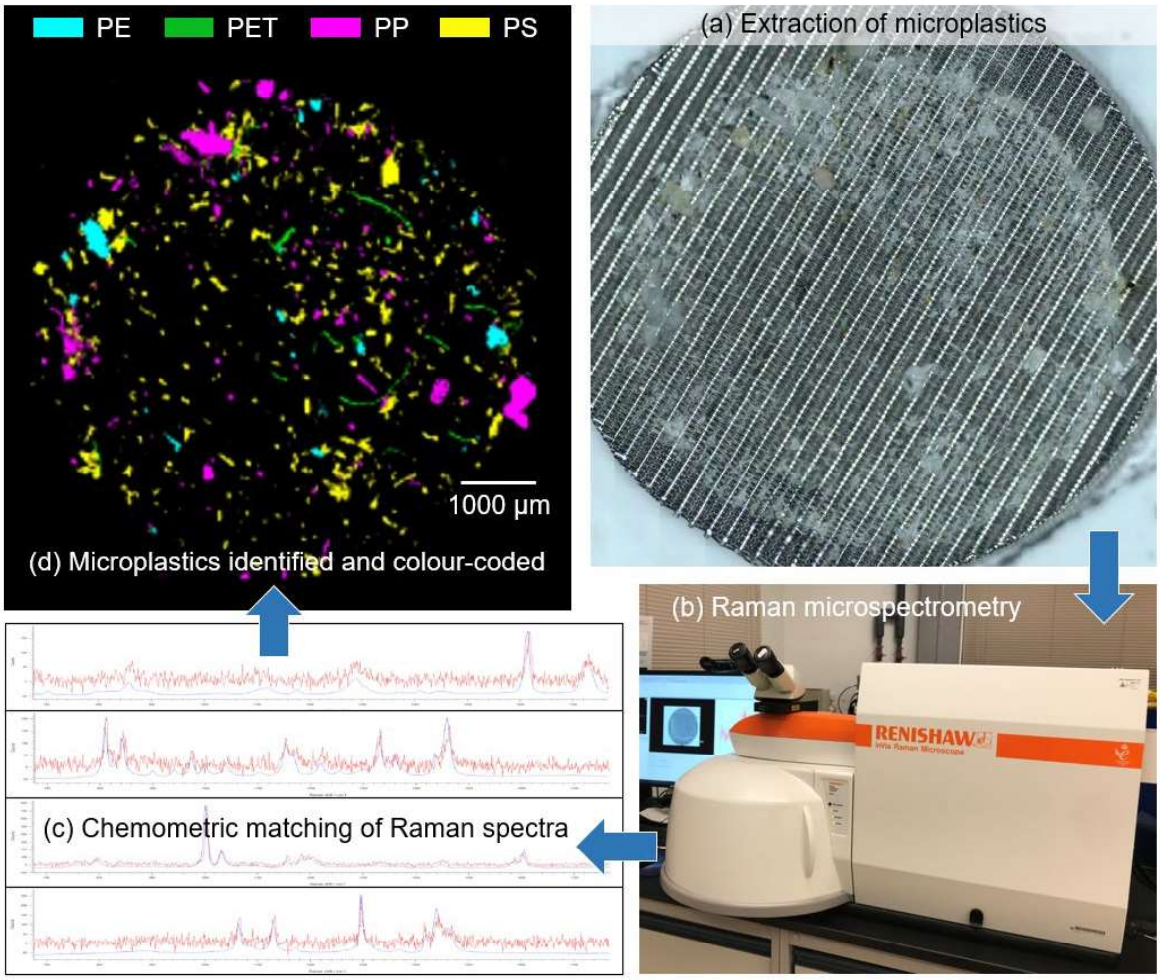


Fig. 4. (a) Microplastics extracted on a stainless-steel filter membrane are identified using (b) a Renishaw inVia confocal Raman microscope by (c) matching their Raman spectra with reference plastic polymer spectra. Results are presented as (d) a two-dimensional and colour-coded image of Raman spectra. Microplastics of polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP) and polystyrene (PS) found in this sample are presented as aqua, green, pink and yellow, respectively.

All sediment samples collected from the five sites were successfully analysed. The numbers of plastic debris of various sizes at 1.0 m and 1.5 m above chart datum were determined to be 35–125 items kg^{-1} on average among the five sites. Higher abundance of plastic debris occurred below strandline, where 319, 315, 343, 227 and 1,441 items kg^{-1} were found at HPN, ST, SW, YO and SHW, respectively (Fig. 5). Overall, the grant mean value at SHW was 3.0–3.8 times higher than those at the other sites.

About a third of these plastic particles were identified to be polyethylene terephthalate (PET; 33.9%), followed by polystyrene (PS; 32.1%), polypropylene (PP; 21.3%), polyethylene (PE; 12.7%) and polymethyl methacrylate (PMMA; 0.07%; Fig. 6a). More than half of the particles fell in the size range of 31–250 µm (54.2%), while the larger size classes at 250–5,000 µm and > 5,000 µm accounted for 44.4% and 1.33%, respectively (Fig. 6b). The plastic debris existed as different shapes and were dominated by fragments (77.4%), followed by fibres (15.1%), films (7.42%) and foams (0.06%; Fig. 6c).

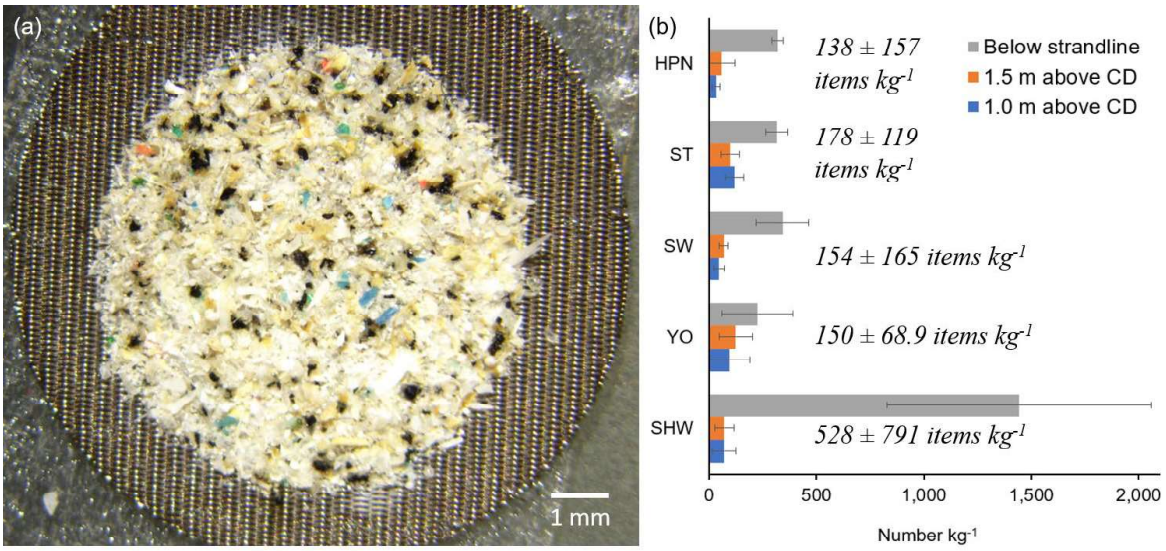


Fig. 5. (a) Sediment particles mixed with microplastics collected below strandline at SHW. (b) The numbers of microplastics in sediment sampled at 1.0 m and 1.5 m above chart datum (CD) and below strandline from the five sites (mean ± standard deviation). The site abbreviations and locations are provided in Fig. 2.

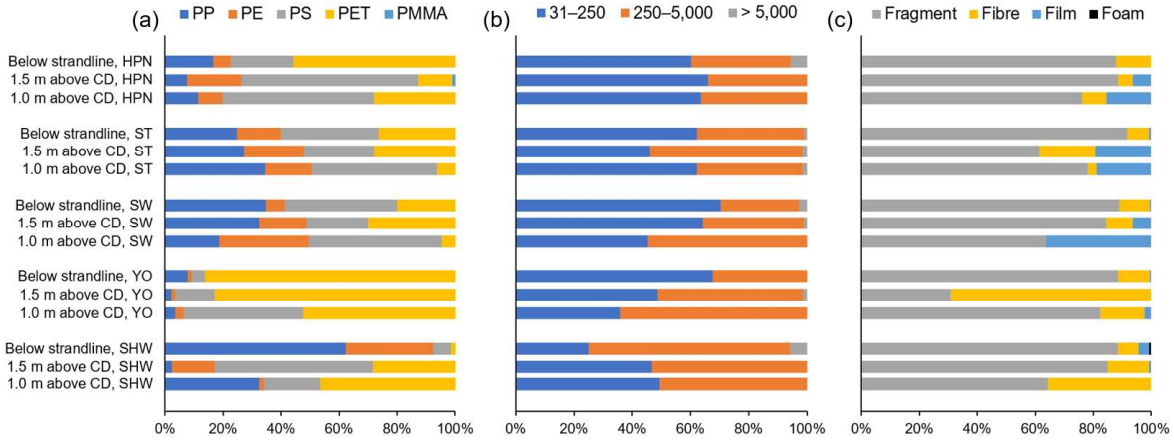


Fig. 6. Proportions of the (a) polymer types, including polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA), (b) size ranges (μm) and (c) shapes of the identified plastic debris at 1.0 m and 1.5 m above chart datum (CD) and below strandline from the five sites. The site abbreviations and locations are provided in Fig. 2.

Cited references

Andrady AL (2017) The plastic in microplastics: a review. Marine Pollution Bulletin 119, 12–22

Cheung PK, Cheung LTO, Fok L (2016) Seasonal variation in the abundance of marine plastic debris in the estuary of a subtropical macro-scale drainage basin in South China. Science of the Total Environment 562, 658–665

Fok L & Cheung PK (2015) Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution. Marine Pollution Bulletin 99, 112–118

Lee CNW, Morton B (2016) Changes in the distributions of juvenile horseshoe crabs (Arthropoda: Chelicerata) (2004–2014) related to environmental perturbations at Pak Nai and Ha Pak Nai, Deep Bay, Hong Kong SAR, China. Marine Pollution Bulletin 108, 134–146

Lo HS, Xu X, Wong CY, Cheung SG (2018) Comparisons of microplastics pollution between mudflats and sandy beaches in Hong Kong. Environmental Pollution 236, 208–217

Tsang YY, Mak CW, Liebich C, Lam SW, Sze ETP, Chan KM (2017) Microplastic pollution in the marine waters and sediments of Hong Kong. Marine Pollution Bulletin 115, 20–28

Xu XY, Wong CY, Tam NFY, Liu HM, Cheung SG (2020a) Barnacles as potential bioindicator of microplastic pollution in Hong Kong. *Marine Pollution Bulletin* 154, 111081

Xu XY, Wong CY, Tam NFY, Liu HM, Cheung SG (2020b) Microplastics in invertebrates on soft shores in Hong Kong: influence of habitat, taxa and feeding mode. *Science of the Total Environment* 715, 136999

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

Phase I of this project indicated the ecophysiological impacts of microplastics on juvenile *T. tridentatus* and identified PET as the most harmful among three tested types. In Phase II, we carried out field surveys to assess the numbers of microplastics including PET in the nursery grounds of horseshoe crabs. These findings provide important information about the current pollution status of microplastics and their ecological risks to marine life in the western waters, and can be shared with relevant Governmental Departments including AFCD and EPD, as well as other environmental conservation groups such as the Ocean Park Conservation Foundation and The Nature Conservancy. To promote marine biodiversity and conservation of horseshoe crabs in Hong Kong, a relevant guest seminar has been arranged in Phase II for students at The Hong Kong Polytechnic University in October 2019, after which all normal teaching sessions and seminars unfortunately have been suspended due to the social unrest followed by the COVID-19 epidemic. Nevertheless, similar seminars have been held in Phase I and the outcomes are very satisfactory. The invited speakers and presentation topics are summarised in Table 2. We have carried out pre- and post-seminar questionnaire surveys, in which the average score of students has sharply increased from 22.6% to 92.3%, suggesting the effectiveness of our work to increase the environmental awareness among students. The questionnaire used in the surveys and the results have been provided in the Phase I progress report. It should also be noted that this project has attracted attention of the public media. Our work has been reported by the Oriental Daily, i-Cable News, HK01 News and Hong Kong Economic Times. This media coverage is certainly of great benefit to the promotion of horseshoe crab conservation that also increases the publicity of the Marine Ecology Enhancement Scheme (Fig. 7).

Table 2. Guest seminars arranged for university students

Date	Speaker	Content
18 September 2018	Dr Siu-Gin Cheung (City University of Hong Kong)	On conservation of horseshoe crabs in Hong Kong and other places
19 September 2018	Dr Jacky Kwok (Agriculture, Fisheries and Conservation Department, Hong Kong)	On marine biodiversity and the impact of plastic pollution on marine life
29 September 2018	Dr Patrick Yeung (World Wide Fund, Hong Kong)	On marine plastic pollution in Hong Kong
1 April 2019	Dr Patrick Yeung (World Wide Fund, Hong Kong)	On marine plastic pollution including microplastics
30 October 2019	Dr Jacky Kwok (Agriculture, Fisheries and Conservation Department, Hong Kong)	On marine biodiversity and ecology in Hong Kong

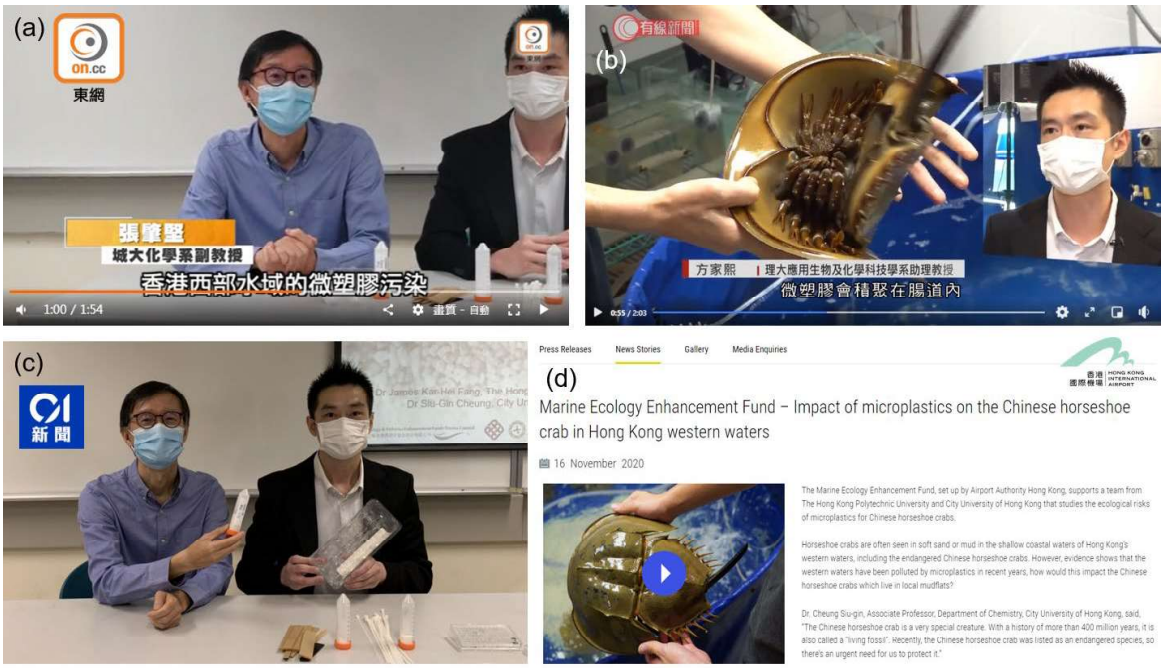


Fig. 7. Our project broadcasted as a feature story by (a) the Oriental Daily, (b) i-Cable News, (c) HK01 News and (d) on the Hong Kong International Airport's website.

VI. Summary and way forward

Phase I of this project demonstrated the negative effects of microplastics on juvenile Chinese horseshoe crabs including reduced locomotion and increased mortality under laboratory conditions, while PET was found to be more harmful. In Phase II, we provided the most updated information about the pollution levels of microplastics in the western waters where horseshoe crabs live and breed. In general, the more harmful PET was more abundant than the other polymer types in these nursery grounds. Our findings have raised the alarm about the ecological impacts of microplastics on horseshoe crabs and other marine life. The next goal is to investigate how the wild population of horseshoe crabs responds to microplastics. Our preliminary findings suggest that the sampling areas with a higher amount of microplastics could be associated with lower abundance of horseshoe crabs (Fang JKH, unpubl. data). A series of field surveys will be performed to determine the correlation between the numbers of horseshoe crabs and microplastics in their nursery grounds. This will also help us to establish a longer-term database of microplastics in the western waters, and this database will be accessible by relevant environmental conservation groups to make our work more applicable. Another goal is about remediation and conservation education. For instance, clean-up activities can be organised for tertiary or secondary students to remove plastic waste from local mudflats and sandy bays including the nursery grounds of horseshoe crabs in the western waters of Hong Kong.

VII. Audited statement of account (enclosed as an appendix to the completion report) in the suggested format as provided in Appendix 2 to this Guidance Note

The audited statement of account has been submitted by The Hong Kong Polytechnic University's Finance Office in March 2021.

VIII. A list of all project assets (as defined in Section 5.14) with photos (see Appendix 4) enclosed as an appendix to the completion report

List of project assets is not disclosed due to confidentiality reason.

IX. Staff recruitment record

Staff recruitment record is not disclosed due to confidentiality reason.

X. Disclaimer

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.

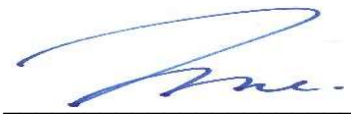
XI. Declaration

I hereby irrevocably declare, warrant and undertake to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that I myself, and the Organisation:-

1. do not deal with, and are not in any way associated with, any country or organisation or activity which is or may potentially be relevant to, or targeted by, sanctions administered by the United Nations Security Council, the European Union, Her Majesty's Treasury-United Kingdom, the United States Department of the Treasury's Office of Foreign Assets Control, or the Hong Kong Monetary Authority, or any sanctions law applicable;

2. have not used any money obtained from the Marine Ecology Enhancement Fund or the related Top-up Fund (and any derived surplus), in any unlawful manner, whether involving bribery, money-laundering, terrorism or infringement of any international or local law; and

3. have used the funds received (and any derived surplus) solely for the studies or projects which further the MEEF Objectives and have not distributed any portion of such funds (including any derived surplus) to members of the Recipient Organisation or the public.



Signed by Dr James Kar-Hei Fang, Project Leader
Assistant Professor
Department of Applied Biology and Chemical Technology
The Hong Kong Polytechnic University

Marine Ecology Enhancement Fund (MEEF)
Declaration

To: The Secretariat of the MEEF

Reference No.: MEEF2018011A

Project Title: Impact of microplastics on the Chinese horseshoe crab *Tachypleus tridentatus* in Hong Kong western waters, Phase II

Name of Project Leader: Dr James Kar-Hei Fang

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.

Signature: 

Project Leader, Dr James Kar-Hei Fang

Date: 9 May 2021