Marine Ecology Enhancement Fund (MEEF)
Declaration

To: The Secretariat of the MEEF

Reference No.: MEEF2018011

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

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： 31 May 2020

Marine Ecology Enhancement Fund (MEEF) Completion Report

Funded project:	Impact of microplastics on the Chinese horseshoe crab *Tachypleus tridentatus* in Hong Kong western waters
Reference number:	MEEF2018011
Approved fund:	HK$ 499,000 (for July 2018 to March 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
Co-Investigator:	Dr Siu-Gin Cheung, Associate Professor Department of Chemistry, City University of Hong Kong
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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 area is subjected to microplastic pollution, an emerging environmental problem not only in Hong Kong but at a global level. This project aims to evaluate the ecological risk of microplastics to horseshoe crabs and to provide information for their conservation in Hong Kong and South China. This project comprises Phase I, a laboratory experiment to determine the ecophysiological responses of *T. tridentatus* to three types of common microplastics, and Phase II, a field survey to investigate the current pollution levels of microplastics at major nursery grounds of *T. tridentatus* in the western waters of Hong Kong. Phase I has been successfully completed and is reported here.

The experiment in Phase I focused on the juvenile stage of *T. tridentatus*, a critical life history stage of horseshoe crabs to determine their population success. All juveniles used for the experiment were artificially bred. The second-instar juveniles (after their second moult) were used in this study. Given the burrowing nature of horseshoe crabs in sand, microplastics that sink to the bottom may be associated with a higher health risk to *T. tridentatus*. Therefore, three types of sinking microplastics (higher densities than seawater) including poly(methyl methacrylate) (PMMA), nylon and poly(ethylene terephthalate) (PET) were used in the exposure experiment and were made from common domestic plastic waste using cryogenic grinding. Juveniles of *T. tridentatus* were exposed for 100 days to three treatments of PMMA, nylon and PET (< 100 µm) at an environmentally realistic concentration mixed in sand (0.1% w/w), along with a control treatment, under controlled laboratory conditions. Each treatment contained ten replicates of *T. tridentatus*. The sand substrate and microplastics were renewed every 20 days. Ecophysiological parameters of *T. tridentatus* in terms of growth, behaviour, moulting, mortality and ingestion of microplastics were regularly determined when exposed to PMMA, nylon and PET.

Over the 100-day experiment, the juveniles did not moult but showed reduced mean values of wet weight and prosomal width in the three treatments of microplastics. These decreases were statistically significant on wet weight exposed to PMMA and on prosomal width exposed to PMMA and nylon, compared to the control. Moreover, *T. tridentatus* exposed to nylon and PET was found to be less active showing significantly reduced locomotion, expressed as the rate of crawling on the sand substrate per h. This reduction of activity levels was not associated with the burrowing behaviour of *T. tridentatus*, as indicated by the proportion of the animal's body burrowed in sand, which however did not differ

significantly among all treatments. The highest mortality of *T. tridentatus* (70%) was observed in the PET treatment over the experimental period. The juveniles' survival probability was significantly lower when exposed to PET compared to the other treatments. Microplastics of PMMA, nylon and PET were found in the bodies of *T. tridentatus* in the corresponding treatments except the control, revealing ingestion of microplastics by Chinese horseshoe crabs.

Results of the Phase I experiment have identified negative effects of all tested microplastics on *T. tridentatus* in different ecophysiological aspects, among which PET appears to be the most harmful that can lead to mortality of the juveniles. Our findings are ecologically relevant and raising a great concern, given that PET has been found to be the most abundant high-density microplastics occurring in the major habitats of Chinese horseshoe crabs in Hong Kong. In this regard, the ongoing Phase II of this project will provide more details about the current amounts of microplastics in different size ranges at selected important nursery sites of *T. tridentatus* and evaluate the associated ecological risks of microplastics. Apart from the above research activities, we have organised five guest seminars and an open exhibition on horseshoe crabs, marine biodiversity and plastic pollution for university students and the general public, with the aim to arouse the public awareness of environmental protection and marine conservation.

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.

II. Brief description of the Project

Horseshoe crabs have existed on Earth for more than 400 million years but have undergone little morphological changes. They have thus gained the name "living fossils", playing a prominent role in marine ecological and evolutionary research. Given that horseshoe crabs only occur in limited areas in Asia-Pacific and North America, the western waters of Hong Kong should be considered globally ecologically significant as two of the four existing species of horseshoe crabs live and breed here (Kwan et al. 2016; Lee & Morton 2016). Some of us may not be aware of but horseshoe crabs have been integrated in our culture, with some places in Hong Kong named after horseshoe crabs (Hau Hok Wan, 鱟殼灣; and Hau Tei Square, 鱟地坊). However, our horseshoe crabs are under threat by pollution. Hong

Kong is positioned at the mouth of the Pearl River Delta and is known to be a pollution hotspot (e.g. Fang et al. 2008, 2009, 2010; Fok & Cheung 2015). For instance, plastic waste can be generated through local sources or carried by the freshwater inflow from the Pearl River network into Hong Kong western waters (e.g. Fang et al. 2010; Fok & Cheung 2015; Mak et al. 2020). These plastic debris can be broken down into microplastics through various environmental pathways (e.g. Andrady 2017). The increasing amount of microplastics in marine environments has raised a great ecological concern due to the potential ingestion of these plastics by marine animals and subsequent bioaccumulation and biomagnification along food chains (e.g. Wright et al. 2013; Sharma & Chatterjee 2017).

There is growing evidence of plastic pollution at the horseshoe crabs' nursery grounds 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), at some of which sites the population densities of Chinese horseshoe crabs (*Tachypleus tridentatus*) have decreased (Fig. 1b; Lee & Morton 2016). This decline in population may be due to increasing coastal development and pollution including microplastics in the environment. This project comprises Phase I, a laboratory experiment to determine the ecophysiological responses of *T. tridentatus* to three types of common microplastics, and Phase II, a field survey to investigate the current pollution levels of microplastics at major nursery grounds of *T. tridentatus* in the western waters of Hong Kong. Phase I has been successfully completed. Research findings from Phase I, along with the conservation education activities, are summarised in this project completion report.

III. Completed activities against the proposed work schedule

Phase I of this project aimed to investigate the ecophysiological responses of *T. tridentatus* to three types of microplastics under controlled laboratory conditions. The originally proposed schedule lasted 12 months (July 2018–June 2019; Table 1a). To generate microplastics for the experiment, we have acquired the Retsch Cryomill following the standard purchasing procedures at The Hong Kong Polytechnic University. The purchasing procedures took about six months after the project commencement and therefore we have applied for the first project extension for six months (July–December 2019; Table 1b), which has been approved. This project has been in satisfactory progress as planned until November 2019, after which the university campus has been fully or partially closed until recent unfortunately due to the social unrest and the more recent coronavirus outbreak. Due to the limited access to the laboratories, the second project extension for three months (January–March 2020; Table 1c) has been applied and approved. All proposed activities have been completed in March 2020.

Table 1a. Originally proposed schedule for Phase I (green cells; ended in June 2019)

Time (months, year)	Jul–Sep 2018	Oct–Dec 2018	Jan–Mar 2019	Apr–Jun 2019	Jul–Sep 2019	Oct–Dec 2019	Jan-Mar 2020
Major preparation	■						
Exposure experiment		■	■				
Sample processing		■	■				
Data analysis			■	■			
Report/paper writing				■			

Table 1b. First approved extended schedule for Phase I (blue cells; July–December 2019), due to the six-month delivery time of the Retsch Cryomill (orange cells).

Time (months, year)	Jul–Sep 2018	Oct–Dec 2018	Jan–Mar 2019	Apr–Jun 2019	Jul–Sep 2019	Oct–Dec 2019	Jan-Mar 2020
Major preparation							
Delay due to delivery of the Retsch Cryomill							
Exposure experiment							
Sample processing							
Data analysis							
Report writing							

Table 1c. Second approved extended schedule for Phase I (purple cells; January–March 2020), due to the social unrest at The Hong Kong Polytechnic University followed by the coronavirus epidemic (orange cells).

Time (months, year)	Jul–Sep 2018	Oct–Dec 2018	Jan–Mar 2019	Apr–Jun 2019	Jul–Sep 2019	Oct–Dec 2019	Jan-Mar 2020
Major preparation							
Delay due to delivery of the Retsch Cryomill							
Exposure experiment							
Sample processing							
Data analysis							
Delay due to the social unrest and coronavirus							
Report writing							

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

The sources of juvenile *T. tridentatus*, experimental design, ecophysiological measurements on *T. tridentatus*, statistical methods used to analyse the obtained data, and findings from the Phase I experiment are summarised below.

Sources of Chinese horseshoe crabs

We received four individuals of *T. tridentatus* from the bycatch of local fishermen, and successfully matched one mating pair in the laboratory (Fig. 2b). This mating pair laid ~1000 eggs. About ~30 of these eggs fertilised, from which ~20 developed into the first- or second-instar stage (after the first and second moults, respectively). The success rate of artificial breeding was ~2%, which should be considered high compared to the natural breeding/survival rate of horseshoe crabs in their first year of age (~0.001%; Ocean Park Conservation Foundation, Hong Kong, accessed on 27th May 2020, available at http://www.opcf.org.hk/en/species/horseshoe-crab). To secure enough individuals to carry out the Phase I experiment, juvenile Chinese horseshoe crabs were supplied by the Guangxi Marine Research Institute through a larger-scale artificial breeding programme. The experiment aimed to determine the ecophysiological effects of three types of microplastics on juvenile Chinese horseshoe crabs, a critical life history stage to determine population success (Fig. 2a).

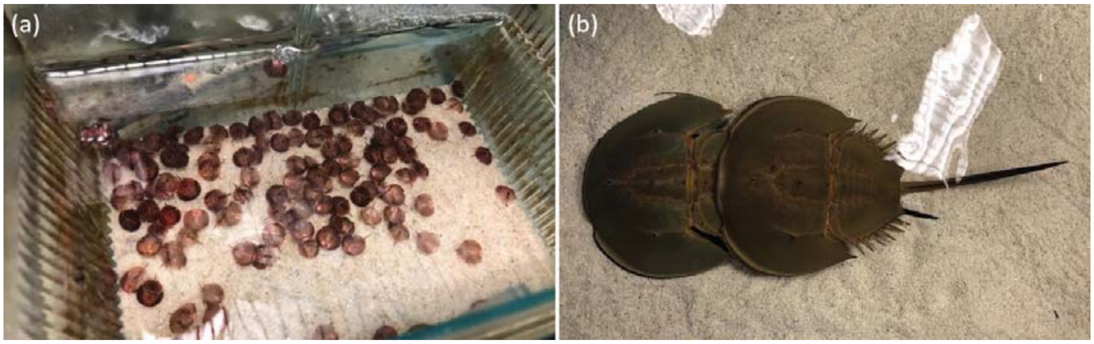

Fig. 2. (a) Juveniles and (b) a mating pair of *Tachypleus tridentatus* acclimated under laboratory conditions.

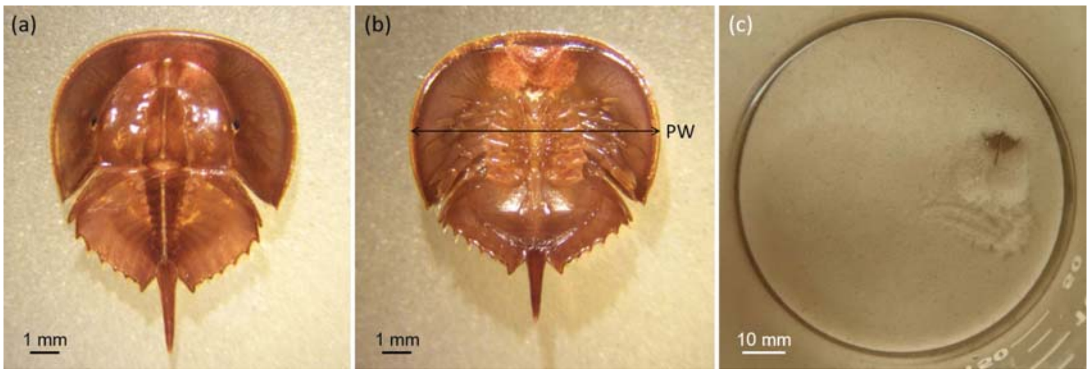

Fig. 3. (a) Dorsal view and (b) ventral view of a second-instar juvenile of *Tachypleus tridentatus*, from which wet weight and prosomal width (PW) were measured at each 20-day interval; (c) the crawling trail of juvenile *T. tridentatus* left on the sand substrate in 1 h. On average 54% of the top area of juvenile *T. tridentatus* borrowed in sand under control conditions.

Experimental design

The second-instar juveniles (after their second moult; Fig. 3a, b) were used in this study. Given the burrowing nature of horseshoe crabs (Fig. 3c), microplastics that sink to the bottom (higher density than seawater; 1.03 g cm^{-3}) pose a higher health risk to the animals. In this regard, three types of high-density microplastics including poly(methyl methacrylate) (PMMA; 1.18 g cm^{-3}), nylon (1.14 g cm^{-3}) and poly(ethylene terephthalate) (PET; 1.38 g cm^{-3}) were used in the exposure experiment and were made from common domestic plastic waste using a cryogenic grinder (Fig. 4). The microplastics of PMMA, nylon and PET were sieved through 100 μm before use.

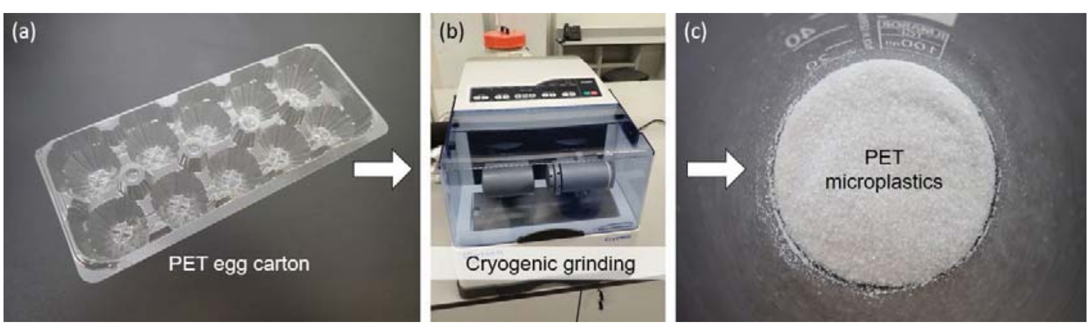

Fig. 4. (a) Plastic egg cartons represent a common domestic waste of poly(ethylene terephthalate) (PET) and (b) were cryogenically ground at –196 °C using a Retsch CryoMill (Haan, Germany) to produce (c) PET microplastics for the experiment. Microplastics of poly(methyl methacrylate) and nylon were similarly made from acrylic sheets and cable ties, respectively.

Juveniles of *T. tridentatus* were exposed to three treatments of microplastics including PMMA, nylon and PET, along with a control treatment. Each treatment had its own circulation system, which comprised ten beakers (0.25 L), one replicate of *T. tridentatus* per beaker, and a reservoir containing ceramic rings and a cotton filter (150 L). Artificial seawater made to a salinity of 15 ‰ (Instant Ocean, Blacksburg VA) was circulated through the beakers and reservoir at 25 °C. *T. tridentatus* was daily fed with brine shrimp larvae (*Artemia* sp.) and provided with appropriate sand substrate following Kwan et al. (2015), in which microplastics were added at an environmentally realistic concentration of 0.1% w/w (Carson et al. 2011). The exposure experiment lasted 100 days, during which the sand substrate and microplastics were renewed every 20 days. Ecophysiological parameters of *T. tridentatus* in terms of growth, behaviour, moulting and mortality were measured or recorded at the 20-day intervals. Ingestion of microplastics by *T. tridentatus* was determined at the end of the experiment.

Ecophysiological measurements

The conditions of *T. tridentatus* were closely monitored during the experiment. Two growth parameters including wet weight and prosomal width (Fig. 3b) were measured at each 20-day interval. Top-view photographs were regularly taken on each individual juvenile, at 1 h after renewal of the sand substrate on days 40, 60, 80 and 100. These photographs were processed using the ImageJ software (Schneider et al. 2012) to assess two behavioural parameters including locomotion of *T. tridentatus*, which was expressed as a rate of the crawling trail area made by each individual on sand during the 1 h period (Fig. 3c), and the extent of burrowing by each individual, which was set to be a proportion of the animal's top area burrowed in sand (Fig. 3c). Times of moulting and mortality of *T. tridentatus*, if any, were recorded.

After the experiment, the amounts of microplastics ingested by *T. tridentatus* among treatments were determined using Raman microspectrometry. Each sample of *T. tridentatus* was digested in a solution containing 10% potassium hydroxide, 30% hydrogen peroxide and 14% ethylenediaminetetraacetic acid at 40 ºC for 48 h, after which remained solids including microplastics were filtered on a stainless-steel filter paper (pore size = 31 µm). Microplastics of PMMA, nylon or PET on the filter papers were identified and counted using a Renishaw inVia confocal Raman microspectrometer with a 785 nm excitation laser source (Wotton-under Edge, England), by matching the Raman spectra between samples and polymer standards provided in the Renishaw Polymeric Materials Database.

Statistical analyses

The impacts of microplastics on growth and behaviour of *T. tridentatus* were compared among the four treatments using repeated measures analysis of variance (RM-ANOVA). Growth rates were expressed as % changes over time in wet weight and prosomal width of *T. tridentatus*, of which behaviour was determined in terms of locomotion and the extent of burrowing. The datasets of wet weight, prosomal width, locomotion and burrowing behaviour did not meet the assumptions for RM-ANOVA and were aligned-rank transformed (ART) prior to the analysis. The ART procedure is an emerging approach to analyse non-parametric datasets with a lower chance of committing Type I errors compared to the conventional rank transformation (Wobbrock et al. 2011). The RM-ANOVA and post hoc tests, if any, were performed using R software as documented in the *ARTool* package. The overall relationship between wet weight and prosomal width of *T. tridentatus* was examined by a linear regression analysis.

The survival probability of *T. tridentatus* was compared among the control, PMMA, nylon and PET treatments using the log-rank test based on the Kaplan-Meier method, which is a non-parametric method designed to assess the survival curves over a period of observation, and is popular among oncology studies to detect clinically adverse outcomes. Analysis for % survival was performed across time (day 20, 40, 60, 80 and 100) and animal status (binary enumeration: live = 0 and dead =1), followed by pairwise comparisons among the four treatments. The log-rank test was carried out using R software as documented in the *Survival* and *Survminer* packages.

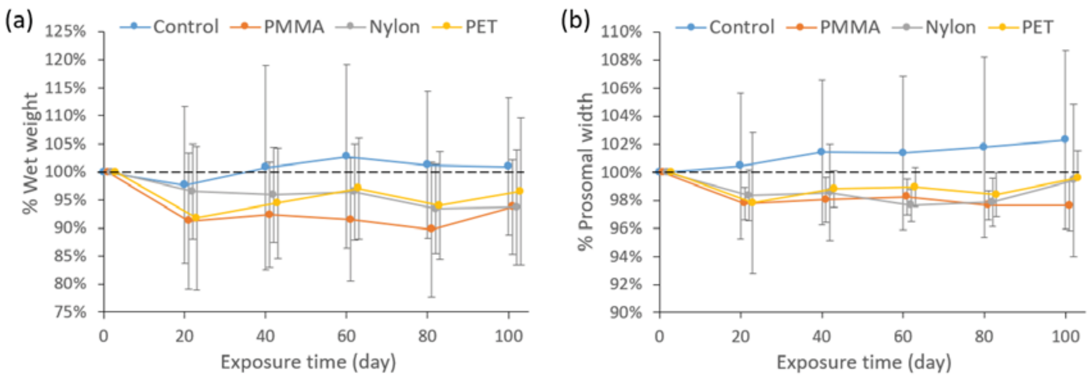

Fig. 5. Relative changes in (a) wet weight and (b) prosomal width of *Tachypleus tridentatus* over the 100-day exposure to poly(methyl methacrylate) (PMMA), nylon and poly(ethylene terephthalate) (PET) compared to the control (mean ± standard deviation). The replicate numbers of live *T. tridentatus* started at 10 but decreased towards day 100 due to mortality (see Fig. 8b). The dash lines indicate the 100% levels of wet weight and prosomal width on day 0. The % wet weight of *T. tridentatus* was significantly lower in the PMMA treatment, while significantly lower values of % prosomal width were found in the PMMA and nylon treatments (repeated measures analysis of variance; $p < 0.001$).

Results

The second-instar *T. tridentatus* did not moult in all treatments during the 100-day experimental period, from which the mean % changes in wet weight and prosomal width indicated positive growth of *T. tridentatus* in the control treatment, but negative growth in the three treatments of microplastics. Significantly greater effects were found on wet weight exposed to PMMA (RM-ANOVA; $p < 0.001$; Fig. 5a), and on prosomal width exposed to PMMA and nylon (RM-ANOVA; $p < 0.001$; Fig. 5b). Nevertheless, as revealed in the regression analysis, prosomal width of *T. tridentatus* can be a fair predictor of the body wet weight across all treatments and timepoints (adjusted $r^2 = 0.50$, $p < 0.001$; Fig. 6).

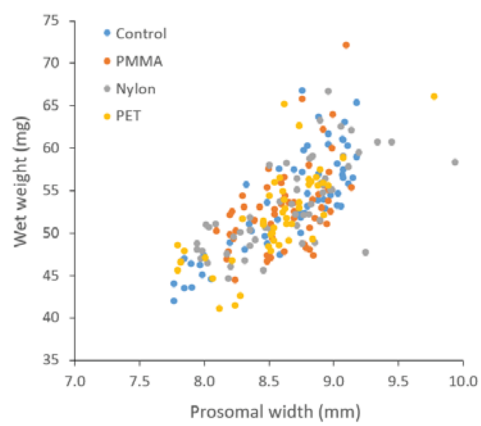

Fig. 6. Linear relationship between wet weight and prosomal width of *Tachypleus tridentatus* across the four treatments reported in Fig. 5. Measurements were taken on live *T. tridentatus* at all time points, leading to 216 pairs of data. The regression model was found to be *wet weight (mg) = 9.91 × prosomal width (mm) – 32.5* (adjusted $r^2 = 0.50$, $p < 0.001$).

T. tridentatus became significantly less active when exposed to nylon and PET, as indicated by locomotion of which mean rates were reduced by 57% and 62%, respectively, compared to the control over the 100-day experiment (RM-ANOVA; $p < 0.001$; Fig. 7a). However, the horseshoe crabs in all treatments did not show any significant difference in their burrowing behaviour (RM-ANOVA; $p = 0.69$; Fig. 7b). Although the extent of burrowing in the PET treatment on day 100 was reduced by >80% on average compared to the control, this change was not statistically detected, partly due to the reduced statistical power under high mortality of *T. tridentatus* when exposed to PET, a treatment in which only three individuals remained by day 100. As such, the survival probability of *T. tridentatus* in the PET treatment was tested to be significantly lower than the other treatments over the experimental period (log-rank test; $p < 0.05$; Fig. 8). Samples of *T. tridentatus* were dissected after the experiment and confirmed to have ingested microplastics in the PMMA, nylon and PET treatments. These microplastics were not found in the control *T. tridentatus* (Table 2).

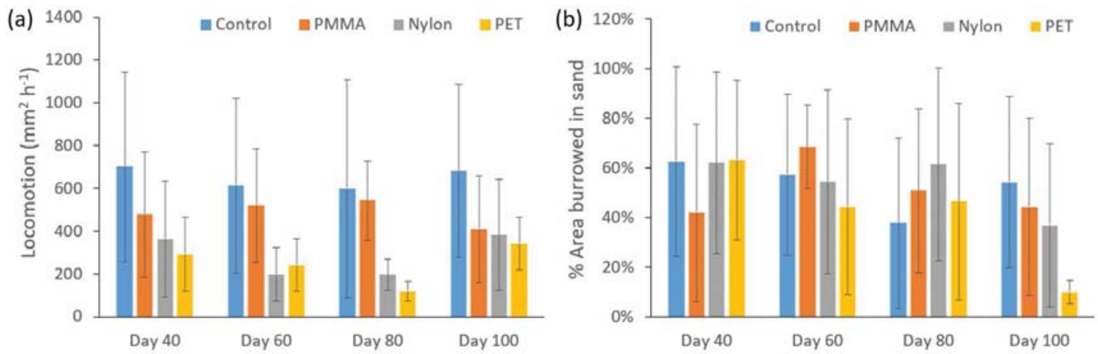

Fig. 7. The behavioural effects of poly(methyl methacrylate) (PMMA), nylon and poly(ethylene terephthalate) (PET) on *Tachypleus tridentatus* in terms of (a) locomotion and (b) the extent of burrowing, compared to the control (mean ± standard deviation). The replicate numbers of live *T. tridentatus* started at 10 but decreased towards day 100 due to mortality (see Fig. 8b). Significantly reduced rates of locomotion was determined when exposed to nylon and PET (repeated measures analysis of variance; $p < 0.001$). The burrowing behaviour of *T. tridentatus* was not significantly different among all treatments (repeated measures analysis of variance; $p = 0.69$).

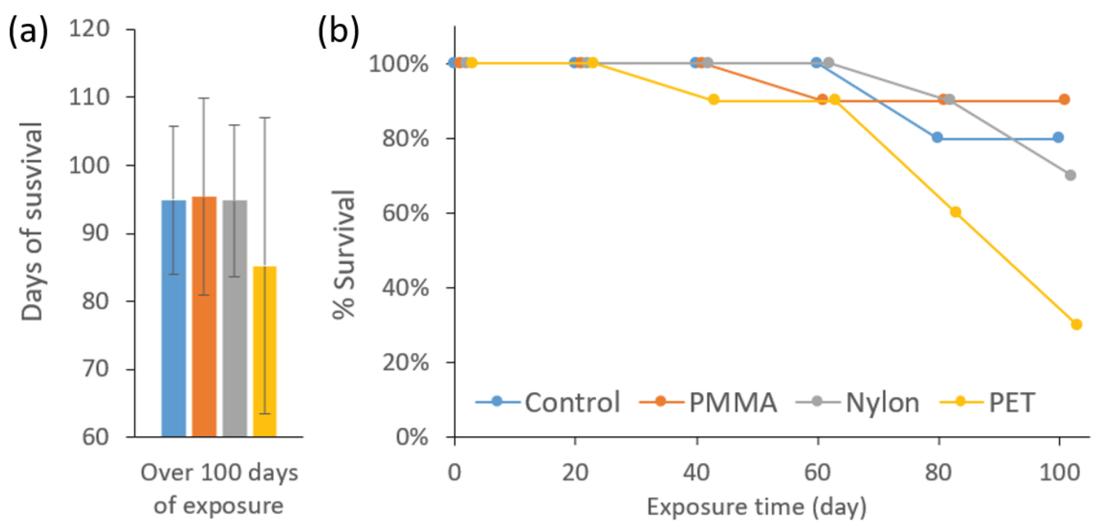

Fig. 8. (a) Mean days of survival of *Tachypleus tridentatus* in the treatments of control, poly(methyl methacrylate) (PMMA), nylon and poly(ethylene terephthalate) (PET) over 100 days (mean ± standard deviation; 10 replicates per treatment), and (b) the % change in number of live *T. tridentatus* in each treatment at each 20-day interval. *T. tridentatus* in the PET treatment showed a significantly lower probability of survival than in other treatments (log-rank test based on the Kaplan-Meier method; $p < 0.05$).

Table 2. Numbers of microplastics ingested by *Tachypleus tridentatus* per individual in the control treatment and three treatments exposed to poly(methyl methacrylate) (PMMA), nylon and poly(ethylene terephthalate) (PET). Analysis of microplastics was performed on all live and dead individuals over the 100-day experimental period (mean ± standard deviation; 10 replicates per treatment).

Treatment	Ingested microplastics (number individual^{-1})		
	PMMA	Nylon	PET
Control treatment (8 live, 2 dead)	0	0	0
PMMA treatment (9 live, 1 dead)	2.5 ± 3.0	0	0
Nylon treatment (7 live, 3 dead)	0	7.8 ± 8.9	0
PET treatment (3 live, 7 dead)	0	0	1.1 ± 1.2

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

The experiment in Phase I has indicated the ecophysiological impacts of microplastics on juvenile *T. tridentatus* and identified PET as the most harmful among three tested types. Exposure to PET can lead to mortality of the juveniles. These findings lead to a great ecological concern, as PET has been found to be the most abundant high-density microplastics in the western waters of Hong Kong where Chinese horseshoe crabs live and breed (Lo et al. 2018). The knowledge obtained from Phase I has laid a good foundation for the implementation of Phase II, which is a field survey aiming to determine the current pollution levels of microplastics in local major nursery grounds of *T. tridentatus* and to evaluate the ecological risks of microplastics to these horseshoe crabs, providing important information for their conservation in Hong Kong and South China. Apart from these research activities, conversation education activities including guest seminars and an open exhibition have been organised for university students and the general public.

Guest seminars

Five guest seminars relevant to this project were arranged for university students at The Hong Kong Polytechnic University and City University of Hong Kong in 2018–2019. The invited speakers and presentation topics are summarised in Table 3. We have carried out pre- and post-seminar questionnaire surveys and the outcomes are encouraging (Fig. 9). The average score of students in the pre- and post-seminar questionnaires has sharply increased from 22.6% to 92.3%, suggesting the effectiveness of this project to increase the environmental awareness among students (Fig. 10). The questionnaire used in the surveys has been submitted as an appendix in the first progress report in December 2018.

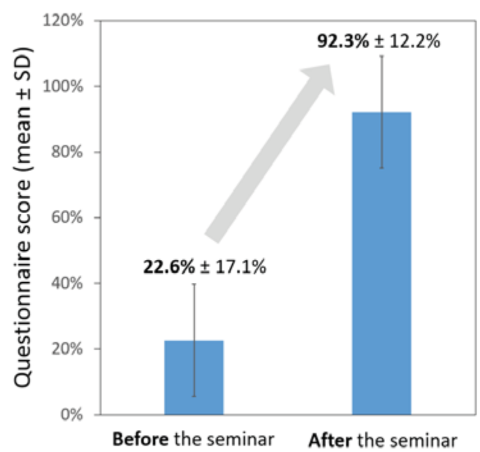

Fig. 9. Students' knowledge of the biology and ecology of horseshoe crabs has significantly improved after the guest seminar held on 18 September 2018.

Table 3. Guest seminars arranged for university students in 2018–2019

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

*After which all normal teaching sessions and seminars have been suspended due to the social unrest in November 2019 followed by the more recent coronavirus epidemic

Fig. 10. One of the guest seminars on marine plastic pollution given by Dr Patrick Yeung at City University of Hong Kong on 1 April 2019.

Open exhibition

We have invited the Campus Sustainability Office at The Hong Kong Polytechnic University to co-organise an open exhibition on the ecology and conservation of horseshoe crabs in Hong Kong (Fig. 11). The exhibition event was held for two weeks in the Hung Hom main campus on 18 February–1 March 2019 and was open to all university students and the public. Part of the display materials were provided by the Agriculture, Fisheries and Conservation Department. Free souvenirs and leaflets were given to all visitors. This exhibition event is considered successful, as many students have shown great interests in our project and in the current status of Chinese horseshoe crabs in Hong Kong waters.

Fig. 11. Open exhibition of the ecology and conservation of horseshoe crabs in Hong Kong on 18 February–1 March 2019. Miss Queenie Tsz-Wan Tse, the former Research Assistant of this Project (the lady first on the right), is introducing the purposes and significances of our project to the visitors.

VI. Summary and way forward

Phase I of this project has been successfully completed, in which we have used a controlled laboratory experiment to evaluate the ecophysiological responses of juvenile Chinese horseshoe crabs to three types of microplastics including PMMA, nylon and PET. Our findings indicated negative impacts of all tested microplastics, while PET was found to be the most harmful that can reduce locomotion and induce mortality of the horseshoe crabs.

The mechanisms behind this toxicity are yet to be explored. Given the high abundance of PET microplastics in Hong Kong waters including the nursery grounds of Chinese horseshoe crabs, our findings from Phase I have raised the alarm about the potential ecological impacts of microplastics on the wild population. The ongoing Phase II of this project will follow up on these results of Phase I and provide the most updated information about the pollution levels of microplastics at five major nursery grounds of Chinese horseshoe crabs in Hong Kong, allowing us to evaluate the associated ecological risks. Presently there is growing evidence of microplastics, e.g. in our western waters where Chinese horseshoe crabs live and breed (e.g. Xu et al. 2020a, 2020b). A longer-term monitoring programme of microplastics at these nursery sites will be of high importance in the conservation efforts of local Chinese horseshoe crabs.

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

Project expenditure details are not disclosed due to confidentiality reason.

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

Details of project assets are not disclosed due to confidentiality reason.

IX. Staff attendance record and recruitment record

Details of staff attendance record and recruitment record are 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
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Department of Applied Biology and Chemical Technology
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