Completion report

Project number: MEEF2020008

Non-invasive investigation of the microbiome of dolphins and porpoises in Hong Kong as a novel One Health approach to protect marine ecological health in Hong Kong.

Recipient organization: Hong Kong Wildlife Health Foundation Ltd

Reporting Period: 1 August 2020- 31 July 2021

Executive summary

The mammal microbiome consists of archaeal, bacterial, fungal and viral communities residing on the surface and within mammalian hosts. In humans and domestic animals, microbiome community composition and diversity are shown to be associated with the host's health status. Microbiome communities profoundly influence host health through their impacts on the immune system, digestion, development and even behaviour. There has been limited number of studies on the microbiome in wildlife, and even fewer in marine species. Human activities and their associated changes in the marine environment may disrupt such microbiota, causing negative impact to marine mammal hosts. In marine sciences, the marine microbiome has recently been highlighted as a key component of the "Blue economy", through which we will gain a better understanding of marine conservation issues. However, although there is an increasing amount of work in the area of microbiome study in wildlife, there's a lack of clear understanding and ability to transfer current knowledge into practical management/conservation tools. Microbiome research is therefore essential to better understand the interactions between microbiome, their hosts and the environment, in order to protect marine biodiversity. Evaluating these three components jointly is an example of a One Health approach. Our project uses this novel approach to investigate the microbiome of dolphins and porpoises in Hong Kong.

In view of the global pandemic and the majority of emerging diseases being zoonotic, priorities are often given to terrestrial species as they are more closely associated with humans within this populated city/territory. However, there is a pressing need to direct our focus towards marine species, as the microbiome and parasitic fauna of Hong Kong's marine wildlife is largely unexplored. Among all the known cetacean species worldwide, only two species occur all year-round in Hong Kong waters: the Indo-Pacific humpback dolphin (or Chinese white dolphin) (*Sousa chinensis*) and the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*). Both these species are listed as vulnerable on the IUCN red list and are facing multiple threats. Marine mammals are seen as ecosystem sentinels and their health can serve as an indicator of the environment's integrity. The rare existing studies on microbial communities in these two species cover singles stranded animals, or are reported in East Asian finless porpoises (*Neophocaena asiaeorientalis sunameri*), a cetacean species closely related to the species commonly found stranded in Hong Kong. However, to date, to the project team's knowledge, there has been no published paper available on microbiome studies of marine wildlife or resident cetaceans in Hong Kong.
Given the nature of cetaceans, it is extremely difficult or even unethical to restrain a live animal to collect samples. To overcome this challenge, a non-invasive technique was used. Researchers collected samples from stranded animals on the shores in Hong Kong. Thirty four Indo-Pacific finless porpoise and seven Indo-Pacific humpback dolphins were included in this project. Novel next generation sequencing (NGS) methods were used here to describe the bacterial communities of

cetaceans. Parasitology investigations, including immunodiagnostics, were carried out. Parasites such as Cryptosporidium and Giardia spp. were not identified in samples examined. This study confirms that molecular methods are the most reliable tools we have in order to evaluate the microbiome of cetaceans in Hong Kong, and that evaluation of this microbiome through non-invasive sampling of stranded animals is effective in identifying microbial communities. Twenty-six species of bacteria belonging to 21 genus were detected from gastro intestinal and respiratory samples collected. The results of our study suggest that microbiome in the two species of cetaceans studied here is species-specific. Therefore, as extrapolations from other species is unlikely to be reliable, this justifies the need for further studies in microbiome of local cetaceans. In particular, through ongoing health monitoring of health parameters of cetaceans, and of their microbiome, dysbiosis (changes of microbiome associated with disease), a better understanding of environmental factors affecting these changes may be gained. In turn, this will direct conservation actions to protect these species of protected cetaceans in Hong Kong. Long term monitoring of microbiome is an indirect and non-invasive method for environmental changes, in particular in relation to environmental bacterial pathogens which affect cetaceans and humans.

This project provides information for veterinarians involved in stranded cetaceans in Hong Kong and in the region. When dealing with rehabilitation of live-stranded cetaceans they are better equipped for therapeutic options and prognostic decisions. When participating in post mortem examinations, they have information which guides them in the investigation of causes of death and their trends.
Much has been described for chemical pollutants in local cetaceans, but this study highlights the likely interactions between not only chemical pollution of marine waters and cetaceans' health, but also with what is referred to as "pathogen pollution" as an additional element to consider in cetacean health and immunity, through alteration of microbial communities which compose the microbiome. Anthropogenic microbial pollution of seawater is a potential threat, as Hong Kong is a highly urbanised, densely populated territory. Within the dolphins and porpoise's coastal habitat, marine bacterial pollution may occur through human activities and discharge of material of human and domestic animal origin. Freshwater runoff, in some instances is due to flooding after extreme weather events may play an important role too. This project therefore emphasises the role of marine species as sentinels of the marine ecological health, establishing links between the threat marine pollution related to anthropogenic human activities and cetaceans' health.

This project emphasizes the role of cetaceans as sentinels of marine ecological health. This novel approach, within the One-Health concept which examines jointly human, marine species and ecological health is essential to enhance cetacean conservation in the Pearl River Estuary. Firstly, this provides insights on ecology and evolution of microbiome in relation to their hosts and their health. It has been shown in other species that both the microbial community and the parasites harboured by the host are central to the host's health status, in particular their immune system function and neonatal health. This is extremely important given that neonates and young of the year represent a large proportion of the mortalities observed in the declining population of Indo-Pacific humpback dolphins in Hong Kong. Secondly, our study provides a better understanding on anthropogenic threats, such as marine bacterial pollution in order to guide future decisions in relation to prioritisation of conservation actions. It is recommended that future microbiome investigations expand to skin and reproductive microbiome, and that we continue to examine thoroughly and systematically the reproductive system of these mammals of the sea. In the future, key microbial and parasitic species which are indicators of poor health may be identified, and summarized in a single quantitative index.

I. Project title and brief description of the Project

Project title:

Non-invasive investigation of the microbiome of dolphins and porpoises in Hong Kong as a novel One Health approach to protect marine ecological health in Hong Kong.

This project has for aim to provide a clear understanding the ecology and the threats posed by microbes and parasites both to the marine environment and to the health of local species of marine mammals. This project uses novel non-invasive methods to characterise the parasitic and bacterial microbiota of the two species of resident cetacean in Hong Kong: the Indo-Pacific humpback dolphin and the Indo-Pacific finless porpoise. This project emphasizes the role of marine species as sentinels of the marine ecological health. This is essential as links may be established between the marine environmental changes related to anthropogenic human activities and cetaceans' health. This novel approach, within the One-Health concept which examines jointly human, marine species and ecological health is essential to enhance cetacean conservation in the Pearl River estuary.

II. Completed activities against the proposed Work Schedule

The project was proposed to cover a period of 12 months. The project started in August 2020.

Timeline of activities as compared to timeline provided in original proposal.

Project activities	Proposed timeline	Actual date of execution
Research assistant recruitment	Aug 2020-Sep 2020	As planned
Preparation, reagents, equipment and consumables ordering	Aug 2020- Jul 2021	As planned
Sampling and stranding level A data collection	Aug 2020- July 2021	As planned
DNA extraction	Jan 2021 and May 2021	As planned and completed in May 2021
Parasitological examination	Aug 2020-Jul 2021	As planned
Bacterial cultures and antibiotic sensitivity patterns	Oct 2020- Jul 2021	As planned and completed in July 2021
PCR, amplification and Pyrosequencing (NGS)	Mar 2021-Jul 2021	As planned and completed in July 2021
Metagenomics library construction	June 2021	June 2021 as planned
Data archiving and data analysing	Aug 2020- Jul 2021	As planned
Presentation of the progress and Reporting	Feb 2021 and Jul 2021	Two reports: Feb 2021 and upon completion (completion report).

III. Results/descriptions on the completed activities with appropriate analysis, with the support of videos, photos, social media platform etc… if any.

a. Research assistant recruitment

This was performed as planned, with part- time research assistants joining the project to be available on a 7/7 day basis to attend strandings at any time.

b. Preparation, reagents, equipment and consumables ordering

This went as planned.

c. Sampling and stranding data collection

A total of 41 resident cetaceans (seven Indo-Pacific humpback dolphin, *Sousa chinensis* and 34 Indo-pacific finless porpoise, *Neophocaena phocaenoides*) were included in the study during the project period.

d. Parasitological examination

When detected grossly during the post mortem procedure, parasites were collected and were stored in 70% ethanol. Thirty gastro-intestinal samples and 15 respiratory samples were examined microscopically for the presence of parasites using standard sedimentation and floatation techniques. Gastro intestinal samples were evaluated for the presence of Giardia and Cryptosporidium by antigen detection immunoassay. None of the samples from Indo-Pacific humpback dolphins or Indo-Pacific finless porpoises were positive.

e. Sampling for bacteriological analyses

For most of the cases we were able to obtained sample swabs from the animals directly in the field. This sampling settings were preferred as they are associated with the least human interaction with the carcass as possible. With this, the assumption was that this was minimising possible artefacts towards the composition of the microbiome. Additionally, we have been collecting blowhole, rectal and intestinal swab samples as well as parasites during the post-mortem examination.

f. PCR, amplification and Pyrosequencing (NGS)

DNA extraction

Swab samples were thawed and each swab head was placed into a separate lysis bead tube (Invitrogen, USA). A volume 360 µl of Buffer ATL was added to each bead tube and the samples were then homogenized using the TissueLyzer II. Following homogenization, 40 µl of Proteinase K was added to the contents of the bead tube and the tubes were incubated at 56°C for 30 minutes. Subsequently, 200 µl of the lysate was transferred from each bead tube into a separate sterile 2 mL microcentrifuge tube. The rest of the DNA extraction for these respiratory samples was competed as per the manufacturer's protocol in the QIAamp DNA Mini Kit (QIAGEN, Germany). For digestive samples, the DNA extraction was completed using the QIAamp PowerFecal Pro DNA Kit as per the manufacturer's protocol.

Amplification

A polymerase chain reaction (PCR) was performed to amplify the extracted DNA from samples. Each PCR reaction consisted of 10 µl DreamTaq Green PCR MasterMix (Thermo Fisher Scientific, USA), 8 µl of nuclease-free water, 0.5 µl of the forward primer, 0.5 µl of the reverse primer, and 1 µl of the DNA template. Running conditions were: 1 cycle of denaturation at 94 °C for 15 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s and elongation at 72 °C for 60 s, and a final cycle of elongation at 72 °C for 10 min.

g. Metagenomics library construction and sequencing

DNA libraries were constructed using the Nextera XT Library Preparation Kit (Illumina, UK). Libraries were normalized, pooled, and sequenced as paired-end reads using the Illumina MiSeq platform (Illumina, USA).

h. Data archiving and data analysing

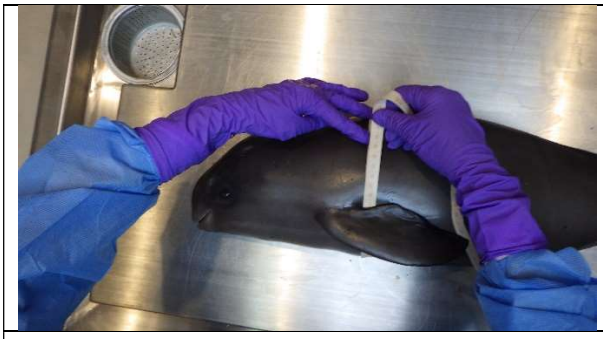
For bioinformatics analysing, the amplicon sequencing reads were denoised using filterAndTrim function and the ASVs were constructed using DADA2. Taxonomic assignment was performed using assignTaxonomy function based on the GTDB r202 database. The alpha (Shannon, Simpson) and beta diversity (Bray distance) was calculated using R package MicrobiotaProcess.

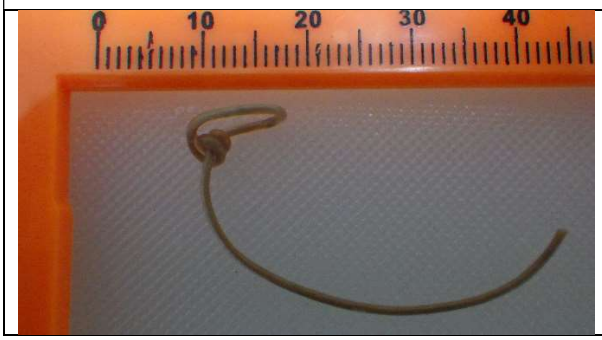
i. Presentation of the progress and Reporting

In the initial proposal, based on the number of strandings over the previous years, we were anticipating approximately 20-30 animals in decomposition code 1-4. The number of such animals which stranded during the project period has been much higher than expected (almost double), in particular for Indo-Pacific finless porpoises. This signifies that the number of animal subjects available for sampling was higher, which is of course alarming for their conservation but which allows us a larger sample population size. However, compared to previous years, we noted that many of these carcasses (>80%) unfortunately are severely decomposed (code 4 decomposition) and the samples material being in a degraded condition. Parasitological examinations have been performed and parasitic fauna characterization proceeding as planned despite this, as parasitological analyses are affected to a lesser degree by decomposition stage compared to material submitted for bacteriological analysis. Parasites belonging to the genus *Crassicauda*, *Halocercus* and *Globicitis* are present in resident cetaceans examined during the reporting period. Two more parasite types are identified as nematodes in the genus *Stenurus* and *Pseudostenurus*.

Due to higher number of stranded animals, available resources and logistics have been challenged for our team. In other instances, several animals stranded within a short timeframe (in one case there were three animals on the same day). This necessitated more than one team, which stretched our team resources. Additionally, some animals were found at night, and had to be attended to after dark. We acquired additional equipment such as headlights, and backpacks and this allowed us to carry out our fieldwork safely and effectively. In some instances, cetaceans were found either stranded either in remote areas.

Photos of project fieldwork:





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

The proposed project objectives originally set were as follow:

1. Characterise the microbiome present in resident cetaceans in Hong Kong and determine whether differences are seen in environmental or host characteristics: species, sex and age category
2. Determine antibiotic resistance patterns of major pathogenic bacteria identified and correlate this to marine environmental microbial pollution and bacterial resistance of anthropogenic origin to demonstrate the role of cetaceans as sentinels of Hong Kong marine ecological health
3. Make recommendations to prevent and minimize occurrence of these microbial agents and the associated antimicrobial resistance, in order to protect the marine environment and the health of the Hong Kong populations of endangered Chinese white dolphin and finless porpoise.
4. Make recommendations for treatment modalities of rehabilitated cetaceans and prevention of zoonoses in humans

The project has achieved all the above objectives, as well as shedding light on usefulness of the One Health approach of Hong Kong cetaceans' conservation. This project used novel non-invasive methods to characterise the parasitic and bacterial microbiota of the two species of resident cetacean in Hong Kong: the Indo-Pacific humpback dolphin and the Indo-Pacific finless porpoise. There are practical applications in areas such as animal health and disease monitoring (for zoonotic/emerging diseases) and potential applications for ecosystem health.

The project has demonstrated that stranded cetaceans present a unique opportunity to maximise health information obtained from these species, not only related to histology and gross pathology but also to microbiome analyses. In domestic animals, microbiome is investigated in live animals. In the cases of cetaceans, this type of information is usually available in very small sample size (the rare animals which strand alive, i.e. stranding code 1), or not available at all due to the logistic challenges and ethics of handling live animals.

Characterisation of parasitic fauna of stranded cetaceans was achieved. Parasites belonging to the genus *Crassicauda*, *Halocercus* and *Globicitis* are present in resident cetaceans examined during the reporting period. Two more parasite types are identified as nematodes in the genus *Stenurus* and *Pseudostenurus*. Among endogenous host differences (host related, i.e. species, sex, age category), findings show an over-representation of female *Neophocaena phocaenoides* for *Crassicauda*. Infestation.

Twenty six species of bacteria belonging to 21 genus were detected from gastro-intestinal and respiratory samples collected. *Erysipelothrix rhusiopathiae* was identified by culture in one of the blowhole samples. Consistent with bacteria culturing results, we were able to identify *Erysipelothrix* genus in our sequencing results. This is in line with other studies that have reported this genus of bacteria in *Tursiops truncatus* and *Delphinus delphis*. We also detected the *Peptostreptococcus* genus in both species of cetaceans, which matches what was found in a study on the microbiome of *Tursiops truncatus*. Being a commensal bacterium that is associated with different mammalian hosts, it is interesting that species from the *Peptostreptococcus* genus were also found in our study.

This project gives us a better understanding of the microbial and parasitic communities hosted by marine mammals in the Pearl River estuary and of the potential threat to the health of local species of marine mammals. The final findings, if deemed worthy of further publication, will be submitted to a scientific journal for publication.

VI. Summary and way forward

Summary

The findings of the study, namely the bacterial communities identified and the parasites identified will be useful to the scientific community in Hong Kong as well in the PRE and internationally across the range of the Chinese white dolphin and the Indo-Pacific finless porpoise. Mainly two groups are the target of this information: veterinary teams and professionals and scientists (biologists, ecologists) from academia, governmental and non-governmental bodies

Veterinarians working with strandings, treatment and rehabilitation of Indo-Pacific humpback dolphins and Indo-Pacific finless porpoises may use these findings whilst treating live stranded cetaceans. The resistance noted among bacteria identified in local cetaceans during the project period suggests that antimicrobial resistance is frequently present. This information is essential for sampling procedures and therapeutic agents selection by veterinarians treating live stranded Indo Pacific Humpback dolphins and Indo-Pacific finless porpoises during the rehabilitation process. This information will act as an immediate and direct benefit for stranded dolphins and porpoises in the Pearl River Estuary region.
Erysipelothrix rhusiopathiae was detected here both through microbial cultures and microbiome analysis. This Gram- positive bacteria may infect humans. Sub sections of the population such as veterinarians and other professionals handling cetaceans, through direct contact or skin trauma are at higher risk of infection and may suffer from the severe forms of the disease, such as septicemia and endocarditis. This serves as a strong reminder that anyone should use adequate personal protective equipment whenever handling stranded cetaceans or participating in post mortem procedures.
Professionals as well as institutes involved in the conservation planning and decision making of conservation measures adopted for these species benefit from the new information derived from this project. Here, we contributed to further documenting the baseline health data in these two species of resident cetaceans. This information is unfortunately still in its infancy. Since the early 1990's, a vast number of publications are available in the fields of life history traits, demographics, abundance and distribution, as well as contaminant load. However, apart from a few recent selected studies, long term, continuous and systematic monitoring of infectious agents allowing conclusions to be made with regards to health trends is still crucially needed.

Overall, not only the present project describes microbial and parasitic communities, but it highlights that microbiome data and detailed parasitic load status should be included in the long-term monitoring of cetacean, as one of the health indices to guide marine mammal conservation measures and environmental protection actions.

Way Forward and recommendations

The major difficulty encountered in this study was that we collecting and handled a high proporition of samples that were highly decomposed animals. Although our investigators made every effort to attend to the stranded cetaceans at the earliest time possible, i.e. in the field, the animals were already decomposing. The decomposition of samples is an inherent limitation of sampling from stranded animals. Despite this limitation, we have taken the initiative to store the samples in ice during transport to minimize decomposition and note down the varying stages of decomposition so that this information can be used for further analysis. With decomposed samples, there was also a challenge in extracting a sufficient amount of DNA for 16s rRNA sequencing. To remedy this situation, PCR amplification was performed to increase the DNA amount for sequencing.

Microbiome characteristics of other systems (respiratory, integument, oral cavity, reproductive) should be investigated further, taking into account the fact that only less decomposed animals should be included.
Some comments may be made in regards to the stranding programme. Although an endangered animal's 'health status and cause of death deeply concerns most scientists involved in this programme, the programme could benefit from a higher degree of logistical and financial support. This would allow systematic early retrieval of the carcass, and it would allow at least an experienced veterinarian or a senior researcher with cetacean experience to be present in the field to participate in the first evaluation of the carcass, and collect samples.
Given that those animals have been rarer among stranded animals in recent years, future studies are likely to benefit from over multiple years. Continuous and systematic health monitoring over a minimal duration of 10 years, allowing trends to be detected is crucially needed. In particular, parallel investigations of a. parasitic and microbial communities of local endangered cetaceans and b. of environmental pollution indicators (chemical and bacterial) are needed. In particular, the monitoring of parasitic load for a full range of parasitic infestations and of antimicrobial resistance in cetaceans, sentinels of the marine environment may be used as indicators and warning systems in relation to harmful anthropogenic activities or extreme weather events (such as runoff water and floods). This is a highly valuable method of investigating the threats to marine species posed by anthropogenic activities from a One Health perspective. This will necessitate a dedicated, multidisciplinary research team to be formed.

Dissemination of the findings of this project will be achieved through the following channels:
A paper was presented at the 14th European Wildlife Association/ 69th Wildlife Disease Association Joint Conference:
Nathalie France Mauroo, Hein Min Tun, Frederick Chi-Ching Leung, Katie Wing In So, Mickey Hiu Chin Tsang, Yorkie Yu Ki Wong, Key Ho Man Cheung, Dengwei Zhang, Hogan Kok-Fung Wai. (2021) Non-invasive investigations of the microbiome and antimicrobial resistance patterns in cetaceans: a one health approach to ecological health in Hong Kong, Proceedings of the 69th Wildlife Disease Association / 14th European Wildlife Disease Association - Joint Virtual Conference, Cuenca, Spain, 31st Aug 2021- 2 Sep 2021
(See appendix)
Further dissemination of summary of the findings to peers in scientific community, to managers and stakeholders involved in cetacean conservation within Hong Kong and the Pearl River Delta area are from at least two sources: a. the project summary made available on HKWHF's website and b. the findings, if worthy of publication, will be submitted to a scientific journal and presented at a scientific conference, and this will thereby promote locally and internationally the dissemination of this new knowledge. Acknowledgements of MEEF's support received toward the completion of this project has been and will be duly made through all these channels.

Acknowledgments
The authors thank the Agriculture, Fisheries and Conservation Department, the Government of the Hong Kong Special Administrative Region, for providing access to the animal files and material, the Ocean Park Corporation veterinarians and Clinical Laboratory staff, as well as the Ocean Park Conservation Foundation scientific officers who were involved in the postmortem examinations. HKWHF is grateful for the efforts and dedication shown by research assistants for this project. Partial support from the Marine Ecology and Fisheries Enhancement Fund trustees LTD for this work is gratefully acknowledged.

VII. Audited statement of account

Audited statement of accounts is not disclosed due to confidentiality reasons.

VIII. List of all project assets with photos

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

IX. Staff attendance record in accordance with the attendance monitoring plan (see Section 5.17)

Staff attendance record is not disclosed due to confidentiality reasons.

X. If applicable, **recruitment record for all project staff employed under the project** enclosed as an appendix to the completion report in accordance with the recruitment plan (see Section 5.17).

Recruitment record is not disclosed due to confidentiality reasons.

Declaration

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 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."

Prepared by:

Project leader, Dr Nathalie Mauroo, DVM, PhD

Signature

Date: 8 February 2023

69th WDA
14th EWDA

Joint conference

CUENCA 2021
August 31 to September 2

The Organizing Committee certifies that:

Nathalie France Mauroo, Hein Min Tun, Frederick Chi-Ching Leung, Katie Wing In So, Mickey Hiu Chin Tsang, Yorkie Yu Ki Wong, Key Ho Man Cheung, Dengwei Zhang, Hogan Kok-Fung Wai

Have presented the poster

Non-invasive investigations of the microbiome and antimicrobial resistance patterns in cetaceans: a one health approach to ecological health in Hong Kong

Cuenca, Spain, 31 August - 2 September 2021

José Francisco Ruiz Fons
Organizing Committee

Christian Gortázar
Scientific Committee

Non-invasive investigations of the microbiome and antimicrobial resistance patterns in cetaceans: a one health approach to ecological health in Hong Kong

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BACKGROUND

- Among all the known cetacean species worldwide, only two species occur all year-round in Hong Kong waters: the Indo-Pacific humpback dolphin (or Chinese white dolphin) (*Sousa chinensis*) (Fig. 1) and the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) (Fig. 2).

Fig. 1. Stranded Indo-Pacific humpback dolphin

Fig. 2. Adult Indo-Pacific finless porpoise

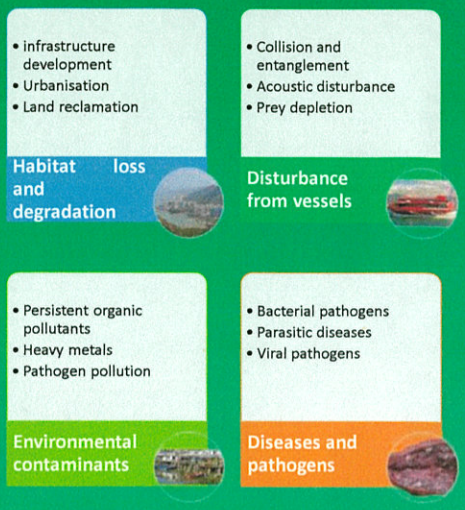

Fig. 3. Threats impacting resident cetaceans in Hong Kong

METHODS AND GOALS

- Both these species of cetaceans are listed as vulnerable on the IUCN red list and are facing multiple threats[1,2] (Fig. 3). Among those threats, anthropogenic microbial pollution and bacterial pathogens are being investigated here.

- Hong Kong is a highly urbanised, densely populated territory. Within the dolphins and porpoise's habitat, marine bacterial pollution occurs through human activities such as sewage and waste discharge of material of human and domestic animal origin or from freshwater runoff. This increases during extreme weather events.

- It has been shown in other species that both the microbial community and the parasites harboured by the host are central to the host's health status, in particular their immune system function and neonatal health. However, information on microbial ecology is extremely important given that neonates and young of the year represent a large proportion of the mortalities observed in the declining population of Indo-Pacific humpback dolphins in Hong Kong.

Fig. 4. Field blowhole sampling of an Indo-Pacific finless porpoise neonate

- Non-invasive collection methods were used to investigate the ecology of the microbiome of local cetaceans. Blowhole and intestinal material was obtained non-invasively, and was collected from stranded dolphins and porpoises, using neonatal nylon flocked swab (516C, Copan, Brescia, Italy) or nylon-flocked swab in modified liquid Amies medium; eSwab™ (480CE, Copan, Brescia, Italy) (Fig. 4). Seventy one samples belonging to 16 Indo-Pacific humpback dolphins and 34 Indo-Pacific finless porpoises were obtained and were frozen at -20 deg C until processing.

- Next generation sequencing (NGS) is used in this project to describe the ecology of bacterial communities of cetaceans in Hong Kong, in relation to parameters such as species, age category, sex, and geographical stranding location.

CONCLUSIONS

- **This project emphasizes the role of marine species as sentinels of the marine ecological health. This novel approach, within the One-Health concept which examines jointly human, marine species and ecological health (Fig. 5) is essential to enhance cetacean conservation in the Pearl River Estuary.**

- **Firstly, this provides insights on ecology and evolution of microbiome in relation to their hosts and their health.**

- **Secondly, our study provides a better understanding on anthropogenic threats, such as marine bacterial pollution and antimicrobial resistance, in order to guide future decisions in relation to prioritisation of conservation actions. This will be extremely beneficial for these cetacean species declining fast in a heavily and increasingly urbanized part of the world.**

- **Future interdisciplinary studies investigating the interactions between odontocetes' microbiome and environmental parameters such as microbial pollution load in Hong Kong marine and estuarine waters will allow evaluation of anthropogenic threats in order to promote ecologically sound conservation measures.**

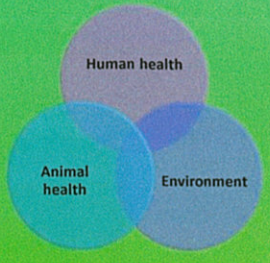

Fig. 5. The One health approach

Acknowledgments

The authors thank the Agriculture, Fisheries and Conservation Department, the Government of the Hong Kong Special Administrative Region, for providing access to the animal files and material, the Ocean Park Corporation veterinarians and Clinical Laboratory staff, as well as the Ocean Park Conservation Foundation scientific officers who were involved in the postmortem examinations. Partial support from the Marine Ecology and Fisheries Enhancement Fund trustees LTD for this work is gratefully acknowledged.

Marine Ecology & Fisheries Enhancement Funds Trustee Limited
海藻海洋生態及漁業提升基金信託有限公司

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69th Wildlife Disease Association and European Wildlife Disease Association joint virtual conference
31 August-2 September 2021