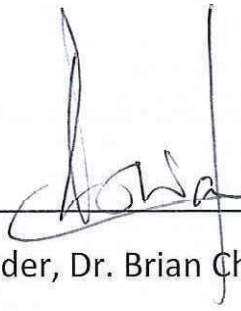
Declaration

Reference Number: MEEF2019010A

Project Title: Three-dimensional forensic scene investigation of marine vessel interaction in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises in the Hong Kong waters

Project Leader: Dr. Brian Chin Wing KOT

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. Brian Chin Wing KOT

Date: 17 Feb 2022

For Use by the Secretariat:
MEEF2019010A
(Reference Number)

MARINE ECOLOGY ENHANCEMENT FUND (MEEF)

Completion Report for Year 2020-2021

Report for the period ending 30 June 2021

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

Part A: Executive Summary

1. Executive Summary

Throughout 12 months, from 1 July 2020 to 30 June 2021, project entitled "Three-dimensional forensic scene investigation of marine vessel interaction in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises in the Hong Kong (HK) waters", has been ongoing progressively and its objectives have been 40-65% accomplished. Despite the social unrest during the project commencement, and subsequently the COVID-19 pandemic, all supporting staffs had been recruited to assist the workflow of this project. The realization of the 5 project objectives are illustrated as below:

a) To describe and compare the spatiotemporal patterns of vessel traffic between night and day and within the day in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises habitats in the HK waters;
b) To identify and document the vessel type and speed that pose interaction risk with cetaceans and reveal the unsuspected vessel interaction 'hot spots' by analyzing the AIS-cetacean population density data in the HK waters;

All the cetacean sighting data from 2014 to 2020 from line transect surveys by Agriculture, Fisheries and Conservation Department (AFCD: Monitoring of marine mammals in Hong Kong waters), Airport Authority Hong Kong (3RS: Expansion of Hong Kong International Airport into a Three-Runway System Construction Phase Annual EM&A Report), Hong Kong-Zhuhai-Macao Bridge Hong Kong Boundary Crossing Facilities (HKBCF), Hong Kong Link Road (HKLR), and Tun Mun-Chek Lap Kok Link (TMCLKL) were consolidated by our team. Data from different sources were normalized to survey effort for subsequent spatiotemporal analyses. Data from 2019 to 2020 were first processed to calculate the cetacean encounter probability using ArcGIS, which will be overlaid with vessel encounter probability calculated from vessel traffic density datasets (request submitted to Marine Department and data extraction is under process). Data of the remaining years will also be processed accordingly. On top of line transect survey data, land-based (theodolite) and passive acoustic monitoring survey data were also collected for further analysis.

c) To document and describe the types of suspected trauma-inflicting instrument using 3-D surface scanning technique, leading to injury induced by vessel interaction in stranded cetaceans;

After standardising the 3-D surface scanning (3DSS) protocol for marine vessels, our team had scanned 9 marine vessels in dockyards with long-range 3-D scanner Artec Ray to document the suspected trauma-inflicting structures of the vessels. In view of the COVID-19 pandemic and prohibition on group gathering, more scans will be conducted once the situation allows.

d) To combine the 3-D models obtained from virtopsy (internal) and 3-D surface scanning data (external) and perform matching analysis of the injury induced by vessel interaction and suspected injury-inflicting instrument using 3DSS, virtopsy and conventional necropsy findings;

As of 30 June 2021, 47 out of 51 HK stranded cetaceans (92%) were undergone virtopsy, including 6 Indo-Pacific humpbacked dolphins (SC), 33 Indo-Pacific finless porpoises (NP), and 8 of other species (OT). All the virtopsy findings were verified by subsequent necropsy, with supplemented information given for the analysis of human interaction related injury and death of stranded cetaceans. The remaining 4 stranded cetaceans (8%) were recognized to be non-transportable and inappropriate for virtopsy by cetacean stranding response team of the Ocean Park Conservation Foundation Hong Kong (OPCFHK). Onsite necropsy and sample collection were directly performed on these cetacean carcasses by the team of OPCFHK.

In these 47 cases, findings of 13 stranded cetaceans (10 NPs and 3 OTs) were sufficient to assign the cause of death with confirmed, probable or suspect category associated with human interactions (28%), which included evidence of fishery (e.g. fishing gear entanglement/digestion), and vessel interactions (e.g. sharp chopped wound). The rest 34 stranded cetaceans (6 SCs, 23 NPs and 5 OTs) were likely associated with different causes of death, e.g. respiratory diseases, drowning and natural death. Most virtopsy findings were consolidated and published bilingually on the project team stranded cetacean blog, allowing general public and international experts to obtain first-hand information and share their opinion on local stranding events.

For 3-D surface scanning, 52 cetacean carcasses and skeletal specimens (12 SCs, 35 NPs and 5 OTs) were scanned by the 2 handheld 3-D scanners. 9 vessels were scanned with the long-ranged 3-D scanner. 3-D models were reconstructed for matching analysis of the injury induced by vessel interaction and suspected injury-inflicting instrument using 3DSS, virtopsy and conventional necropsy findings.

e) To compile postmortem findings collected by original field notes, 3-D surface scanning, virtopsy and conventional necropsy, as well as the overlaid AIS-cetacean population density data, and transcribe into cetacean postmortem multimedia analysis platform for first time all-in-one real-databased models of the stranded cetacean for geometric comparison of patterned injuries with the presumed types of vessel interaction

A web-based database named "Cetacean Postmortem Multimedia Analysis Platform" was used to store all the data for post-processing and data analysis. Data compilation of 244 virtopsy cases (from March 2014 to 30 June 2021, included 52 SCs, 164 NPs and 28 OTs) and respective links between the web server and DICOM viewer were completed. Inclusion of the corresponding stranding information and other multimedia of retrospective cases are in progress.

Although the project happened during the COVID-19 pandemic with the unexpected surge of cetacean stranding, the project team had paid immediate efforts to convey precise conservative messages via outreach activities, such as maintaining social media engagement, conducting interviews with different print media and radio programs, holding talks, symposium and workshop, which jointly organized with the Hong Kong Science Museum, Hong Kong Maritime Museum and universities, to elevate the public awareness on cetacean stranding response program, immediate threats the local cetaceans facing, injury and death of local cetaceans caused by human interactions, and the greater scope: marine conservation and interest in science and technology.

Part B: The Project and Investigator(s)

2. Project Title

Three-dimensional forensic scene investigation of marine vessel interaction in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises in the Hong Kong waters

法證重組: 船隻撞擊對香港水域的中華白海豚及江豚造成的威脅

3. Project Period

From 1 July 2020 to 30 June 2021 (both dates inclusive)

4. Nature of the Project

- ☐ Marine Habitat & Resource Conservation & Enhancement
- ☑ Scientific Research & Studies
- ☐ Environmental Education & Eco-tourism

5. Brief description of the Purpose of the Project

The reported number of local cetacean stranding and mortality cases have increased dramatically in recent years in the HK waters, with marine vessel interaction believed as the most commonly identified threat. Accurate documentation and visualization of injury patterns are crucial to determine the mortality as a result of vessel interaction. This project will pay specific focus on three-dimensional (3-D) forensic scene investigation of marine vessel interaction, by conducting matching analysis on injury on all cetaceans stranded in the HK waters, using 3-D models obtained from virtopsy (internal) and 3DSS datasets (external) of the carcasses and suspected injury-inflicting instrument. Spatiotemporal models of vessel interaction risk based on the overlap between cetacean population density/habitat use and marine traffic will also be established, which can complement information provided by postmortem investigations and eye-witness reports. This project will achieve useful outcomes to facilitate related government officials from Agriculture, Fisheries and Conservation Department, Environmental Protection Department, and HK Marine Department to formulate conservation plan, marine vessel route diversion and speed control, and fellow stakeholders including relevant ferry service/shipping companies, academics, green groups and cetacean experts to implement effective management plan for injury prevention for vulnerable local resident cetaceans in our waters.

近年，鯨豚在香港水域擱淺及死亡的數字有顯著的上升，當中最常見的威脅相信便是船隻撞擊，判斷船隻撞擊為鯨豚死亡原因的關鍵則是準確地紀錄與重現其損傷的模式。

本項目會集中研究船隻撞擊鯨豚的法證重組。透過影像解剖及立體掃瞄，本項目可取得所有在香港擱淺鯨豚身上的傷口及有機會造成其損傷的船隻的立體模型，再加以配對及分析資料，從而重組船隻撞擊鯨豚的情況。其次，透過重疊鯨豚族群分佈及海上交通航道，本項目亦可建立鯨豚受到船隻撞擊風險的模型，藉此互補鯨豚的死後調查與生前目擊報告。

本項目的結果將會協助漁農自然護理署、環境保護署及香港海事處制定保育計劃、海上交通航道分流及速度管制。其他持份者包括船務公司、學者、環保團體及鯨豚專家亦可以藉此推行有效的管理計劃，防止在香港水域居住的易危鯨豚受到傷害。

6. Investigator(s) and Academic Department/Units Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. Brian Chin Wing KOT, Assistant Professor	Department of Infectious Diseases and Public Health; Department of Chemistry, City University of Hong Kong
Co-investigator	Prof. Sophie DENNISON, Adjunct Professor	Diagnostic Imaging, University of Wisconsin, Madison, Wisconsin, USA
Co-investigator	Dr. Paolo MARTELLI, Chief Veterinarian	Department of Zoological Operations and Conservation, Ocean Park Corporation
Co-investigator	Prof. Michael J. THALI, Director	Institute of Forensic Medicine, University of Zurich
Supporting body	Dr. Wai Chuen NG, Marine Conservation Officer	Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong SAR
Supporting body	Dr. Lindsay Porter	Southeast Asia Marine Mammal Research
Supporting body	Dr. Jonathan P. SPEELMAN	Peace Avenue Veterinary Clinic, City University of Hong Kong
Supporting body	Dr. Andreas SRTRUCK	NAVAMA

7. Timetable of Completed Activities against the Proposed Work Schedule

Activities	Original Period	Progress
Recruitment of support staff	July 2019	Completed
Training of support staff	July – September 2019	Completed
Compilation of retrospective cases and prospective data collection	July 2019 – June 2022	In progress
Stranded cetacean blog	July 2019 – June 2022	In progress
Press release/media tea reception	Winter 2019	Completed
HK SciFest 2020	Spring 2020	Cancelled due to the COVID-19 pandemic
Public seminar/Symposium I	Autumn 2020	Completed in June 2021
HK SciFest 2021	Spring 2021	Completed
Public seminar/Symposium II	Autumn 2021	On schedule
HK SciFest 2022	Spring 2022	Cancelled due to the COVID-19 pandemic
Data consolidation and write up publications	April – June 2022	In progress
Handbook of three-dimensional forensic scene investigation of marine vessel interaction in stranded cetaceans	April – June 2022	In progress

8. Project Expenditure

Project expenditure details are not disclosed due to confidentiality reason.

Part C: Completion Report on Year 2019-2020 Project Progress

9. Project Objectives

9.1 Objectives as per Original Application

a) To describe and compare the spatiotemporal patterns of vessel traffic between night and day and within the day in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises habitats in the HK waters;
b) To identify and document the vessel type and speed that pose interaction risk with cetaceans and reveal the unsuspected vessel interaction 'hot spots' by analyzing the AIS-cetacean population density data in the HK waters;
c) To document and describe the types of suspected trauma-inflicting instrument using 3-D surface scanning technique, leading to injury induced by vessel interaction in stranded cetaceans;
d) To combine the 3-D models obtained from virtopsy (internal) and 3-D surface scanning data (external) and perform matching analysis of the injury induced by vessel interaction and suspected injury-inflicting instrument using 3-D surface scanning technique, virtopsy and conventional necropsy findings;
e) To compile postmortem findings collected by original field notes, 3-D surface scanning, virtopsy and conventional necropsy, as well as the overlaid AIS-cetacean population density data, and transcribe into cetacean postmortem multimedia analysis platform for first time all-in-one real-databased models of the stranded cetacean for geometric comparison of patterned injuries with the presumed types of vessel interaction.

9.2 Revised Objectives

N/A

10. Research Activities

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

10.1 Areas addressed in relation to the project objectives that were carried out during this reporting period

A. Recruitment of the supporting staff

Due to the COVID-19 pandemic and the financial administrative procedure, 1 Research Assistant was recruited in November 2020 for a period of 6 months. The staff has been assisting P.I. to oversee project logistics arrangement, liaison and coordination among other team members, departments and collaborators, data collection and management (i.e. and data gathering and archiving) and publication preparation. Three more research personnel were recruited in January 2021 for a period of 6 months to catch up project progress and deliverables.

B. Training of the supporting staff

All the supporting staff underwent training by the P.I. in the first month of employment for PMCT/PMMRI imaging techniques, 3-D surface documentation techniques, and 3-D volume reconstruction and rendering processes, familiarization of highly specific cetacean anatomy and pathology, as well as the basic knowledge in vessel traffic and the cetacean habitats in HK waters. The staff was also advised the project should be executed according to timelines, with proper documentation maintained throughout the project. Following the initial training period, the supporting staff understood the stranding procedures, assisted all postmortem imaging procedures, stranded cetacean carcass logistics and liaison, and data gathering and archiving.

C. Stranded cetacean blog and media coverage

Two social media accounts named as "Aquatic Animal Virtopsy Lab" regarding this project have been developed, including Facebook (https://www.facebook.com/AAVLab2014) and Instagram (https://www.instagram.com/AAVLab2014), as a continuation of the good practice in the previous MEEF projects (reference number: MEEF2017014, MEEF2017014A and MEEF2019010). These social media acted as a world-first stranded cetacean social media platform: 1) to document stranding incidents (highlight vessel related injury and death) with virtopsy-driven stranding response effort in HK waters; 2) to allow clinicians, scientists, researchers, governmental officials and general public to archive first-hand information and share their views on HK local cetacean stranding cases; 3) to promote regional seminar and workshop to facilitate knowledge exchange on stranded cetacean postmortem investigation with vessel related injury and death; and 4) to deepen engagement with social networks around marine ecology, conservation and related research interests. Given the current social distancing recommendations and restrictions on both local and international travel, these social media platforms have facilitated dissemination of key information locally and internationally, and made conservation more accessible to people who do not have professional knowledge but want to know more about the local cetaceans, the threats they are facing, and how can they help with ocean health. Up to 30 June 2021, the blog has reached over 655,000 users with 3,451 followers and 195 posts were published.

Apart from the promotion of cetacean stranding blog, a press conference was organized on 12 June 2021 with 5 local media companies physically attended (Fig. 1). In the press conference, P.I. introduced the concept of this project and the preliminary findings. All the media published the news report on the same day.

Fig. 1. A press conference was organized by the P.I. and his team on 12 June 2021 in the Hong Kong Maritime Museum.

In total, twenty-four interviews on P.I. and his team's works and virtopsy findings were conducted by the various media and published from July 2020 to June 2021, as listed below:

A. TV and radio programs:

Media	Title	Date	URL Link
Radio Television Hong Kong 香港電台	《寰聽世界》: 寰宇百科 - 鯨魚	14th July 2020	https://bit.ly/3250qwl
	《文化快訊》: 海豚的吶喊	24th January 2021	https://bit.ly/2MmJ5JR
	《文化快訊》: 我們的海豚	31st January 2021	https://bit.ly/3lIz8nJ
	《綠。遊蹤》:【綠遊講場】海洋動物影像解剖 (一)	8th March 2021	https://bit.ly/3enzlLy
	《綠。遊蹤》:【綠遊講場】海洋動物影像解剖 (二)	15th March 2021	https://bit.ly/3t13FQy
	《綠。遊蹤》:【綠遊講場】海洋動物影像解剖 (三)	22nd March 2021	https://bit.ly/3cZbeQP
	《綠。遊蹤》:【綠遊講場】海洋動物影像解剖 (四)	29th March 2021	https://bit.ly/3uut59q

	《文化快訊》: 救救海豚	4th April 2021	https://bit.ly/31ZaTZa
TVB 無線電視	《Think Big天地》「Kids VIP」一從「影像解剖」認知海洋生物所受威脅	29th January 2021	NA (No upload is allowed due to the IP policy)
	《創科導航》: 海洋生物影像解剖平台	12th May 2021	NA (No upload is allowed due to the IP policy)
	城大團隊為各地擱淺鯨豚及海龜作影像解剖 冀有助保育	12th June 2021	https://bit.ly/3vs4vWY

B. Newspaper and online articles:

Media	Title	Date	URL Link
Apple Daily 蘋果日報	世界海洋日｜香港4年逾40條海豚死於人為因素 海龜成肚膠袋遭鬼網緊纏	8th June 2021	NA
	世界海洋日｜城大周末辦研討會TED talk講解海洋生態問題對鯨豚威脅	8th June 2021	NA
	城大影像解剖擱淺鯨豚覓死因 揭約2成受船隻撞擊致死	12th June 2021	NA
City University of Hong Kong 香港城市大學	World's first team to run post-mortem imaging routinely to determine the cetaceans' causes of death	11th March 2021	https://bit.ly/3lqnCgD
	城大於日內瓦發明展奪六金	24th March 2021	https://bit.ly/3smi2yz
HK01	疫情影響海上交通　鯨豚受船隻撞擊致死降　全年52隻擱淺仍高企	5th January 2021	http://bit.ly/3hVqqRd
	城大影像解剖研究鯨豚死因　推測船隻撞擊黑點　籲船隻減速	12th June 2021	https://bit.ly/3pXY3FW
HKCD 香港商報	城大於日內瓦發明展奪六金	24th March 2021	https://bit.ly/3fbPrrT
Ming Pao 明報	疑非法捕魚影響 鯨豚擱淺疫下反增	9th June 2021	https://bit.ly/3zuMCdd
	城大影像解剖尋鯨豚死因　成功率大增至七成以上	12th June 2021	https://bit.ly/3gzsSfT
Oriental Daily News 東方日報	南丫島石排灣疑現海豚屍體　屍身腐爛及發臭	20th April 2021	https://bit.ly/32yDPHS
	城大以影像技術解剖鯨豚　發現死因機會升至70%以上	12th June 2021	https://bit.ly/3xlZ25l
Phys.org	World's first team to run post-mortem imaging routinely to determine causes of whale and dolphin deaths	11th March 2021	http://bit.ly/394tLKA

D. Public seminar and workshop

Eleven biodiversity workshops jointly organized with the Hong Kong Science Museum, entitled, "Virtopsy: Threats to Hong Kong's Cetaceans" were scheduled on 15 July, 29 August, 23 September, 4 November, 6 December 2020, 6 January, 6 February, 7 March, 7 April, 29 May and 6 June 2021 at the Nature Lab of the Biodiversity Gallery in the Hong Kong Science Museum (https://hk.science.museum/en_US/web/scm/pe/bdg/biodiversity_workshops.html). Each workshop day consisted of 2 biodiversity workshop sessions, which aimed to provide the general public an overview of cetacean stranding in HK waters and implementation of virtopsy into local stranding response program. However, due to the pandemic of COVID-19, all the workshops were cancelled by the Hong Kong Science Museum, for the concerns for the safety and health of the participants, partners and staff.

In view of the social distancing and health concern, the Hong Kong Science Museum decided to organize the HK SciFest 2021 virtually. The public seminar and workshop entitled, "STEM x SCM: Aquatic Animal Virtopsy Lab - Health Assessment of Marine Tetrapods" (http://bit.ly/HKSciFest2021H017) and "STEM x SCM: Aquatic Animal Virtopsy Lab - 45 Minutes with Cetacean Skeleton" (http://bit.ly/HKSciFest2021H020) respectively, were live broadcasted on 3 April 2021 (Fig. 2 and 3) – the annual large-scale event in HK to provide interesting scientific activities to elevate the public awareness and interest in science and technology, hosted by the Hong Kong Science Museum https://bit.ly/3gsfWco). Over 1100 participants attended the public seminar and workshop. These events were jointly organized among the HK Science Museum and CityU, and was aimed to promote the modern non-invasive imaging techniques, i.e. virtopsy and 3-D surface scanning, in assisting the investigation of injury and death caused by human interactions in stranded cetaceans, as well as the knowledge in cetacean skeletal specimen preparation. Acknowledgement of MEEF's support was included in all the presentation materials.

Fig. 2. Public seminar (Left) and workshop (Right) conducted in the HK SciFest 2021 on 3 April 2021.

Fig. 3. To facilitate the interaction of online workshop, the participants were encouraged to take a photo of their works and post them on our stranded cetacean blog as comments, after joining our workshop.

10.2 Research activities in relation to the project objectives that were carried out during this reporting period

A. Development of a web-based database

A web-based database named "Cetacean Postmortem Multimedia Analysis Platform" was used to store all the data for post-processing and data analysis. Data compilation of 244 virtopsy cases (from March 2014 to 30 June 2021, included 52 SCs, 164 NPs and 28 other cetacean species), and respective links

between the web server and DICOM viewer were completed. Inclusion of the corresponding stranding information and other multimedia of retrospective cases are in progress.

B. . Virtopsy cases performed

From 1 July 2020 to June 2021, 47 out of 51 stranded cetaceans (92%) in HK waters, included 6 SCs, 33 NPs and 8 OTs, were undergone virtopsy (Fig. 4). All the virtopsy findings were verified by subsequent necropsy, with supplemented information given for the analysis of human interaction related injury and death of stranded cetaceans. The remaining 4 stranded cetaceans (8%) were recognised to be non-transportable and inappropriate for virtopsy by the OPCFHK delegates. Onsite sample collections were directly performed on these cetacean carcasses by the stranding response team of OPCFHK.

Fig. 4. A finless porpoise stranded on 30 January and the reconstructed 3D images by virtopsy.

In the aforesaid 47 cases, findings of 13 stranded cetaceans (10 NPs and 3 OTs) were sufficient to assign the cause of death with confirmed, probable or suspect category associated with human interactions (28%), which included fishery and vessel interactions (Fig. 5). The rest 34 stranded cetaceans (6 SCs, 23 NPs and 5 OTs) were likely associated with different causes of death, with various biological health concerns recognised, e.g. parasitic infection and pneumonia. Most virtopsy findings were shared bilingually on our stranded cetacean blog.

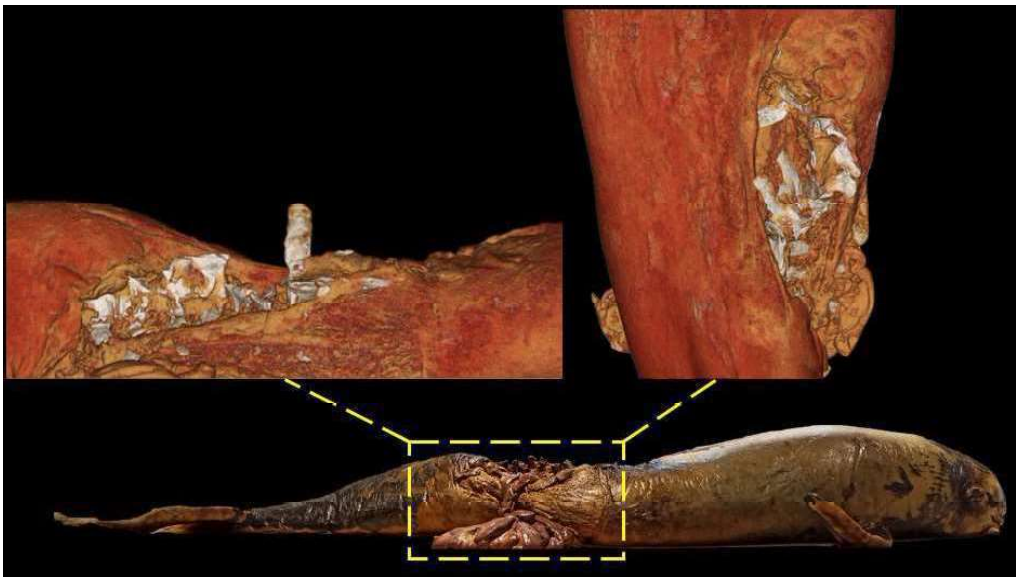


Fig. 5. A finless porpoise with open wound at left dorsal region with more than 6 fractured vertebrae, leading to vertebral misalignment and extrusion of abdominal viscera. This injury was likely caused by antemortem sharp trauma induced by vessel collision.

C. 3-D surface scanning (3DSS)

Two handheld 3-D scanners (Artec Eva and Artec Spider) and a long-range 3-D scanner (Artec Ray) were continuously used in the current project for the documentation of external injuries of cetacean carcasses (Fig. 6), and documentation of structures of the vessels, include hulls, keels, blunt and sharp skegs, propellers, boat strakes, antiventilation plates, trim tables and fins, with reference to the standardised techniques established by our team. By 30 June 2021, 52 cetacean carcasses and skeletal specimens (12 SCs, 35 NPs and 5 OTs) in HK were scanned with the 2 handheld scanners and reconstructed into 3-D models.

Fig. 6. A stranded finless porpoise was undergoing 3DSS by our team.

By 30 June 2021, 9 vessels were scanned with the long-ranged 3-D scanner and reconstructed into 3-D models. (Fig. 7 and 8)

Fig. 7. Vessels scanned with the long-range 3-D scanner in boatyard in Ap Lei Chau.

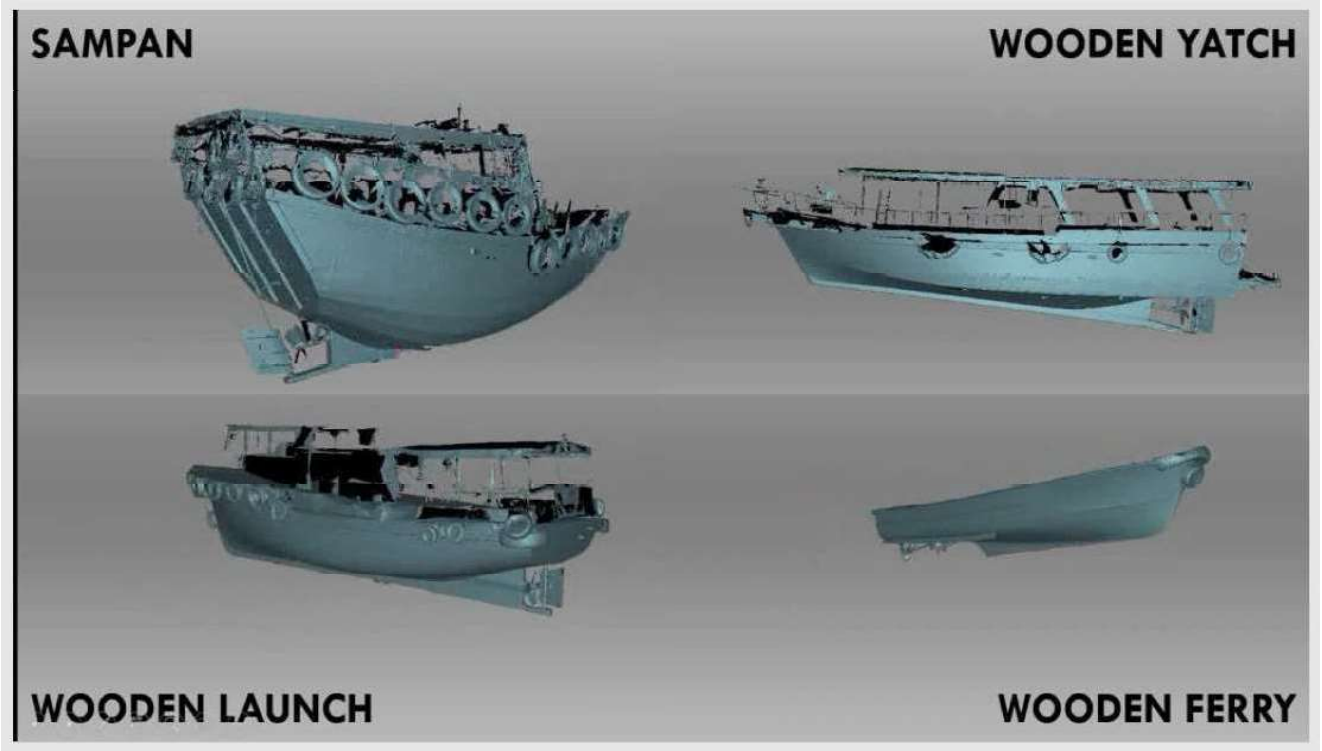


Fig. 8. The 3-D models of 4 scanned marine vessels after post-processing in Artec Studio.

D. Spatiotemporal patterns of vessel traffic and cetacean habitats in HK

Cetacean sighting datasets from line transect surveys for both SC and NP from 2019 to 2020 were obtained from long-term dolphin monitoring project conducted by AFCD and other Environmental Monitoring and Audit (EM&A) programs in West HK waters. The datasets were processed to calculate the dolphin per survey effort (DPSE) and cetacean encounter probability using ArcGIS Pro 2.9.1 (Fig. 9). The cetacean encounter probability will be overlaid with the vessel encounter probability, which will be calculated from the vessel traffic density datasets (request submitted to the Marine Department and data extraction is under process), to indicate the hotspots of high vessel-cetacean collision risk, the vessel types and speed involved. Retrospective data from 2014 to 2018, as well as data from 2021 onward, will be processed accordingly in the next step. Theodolite and Passive Acoustic Monitoring survey data will also be continuously collected from different EM&A programs for further analysis.

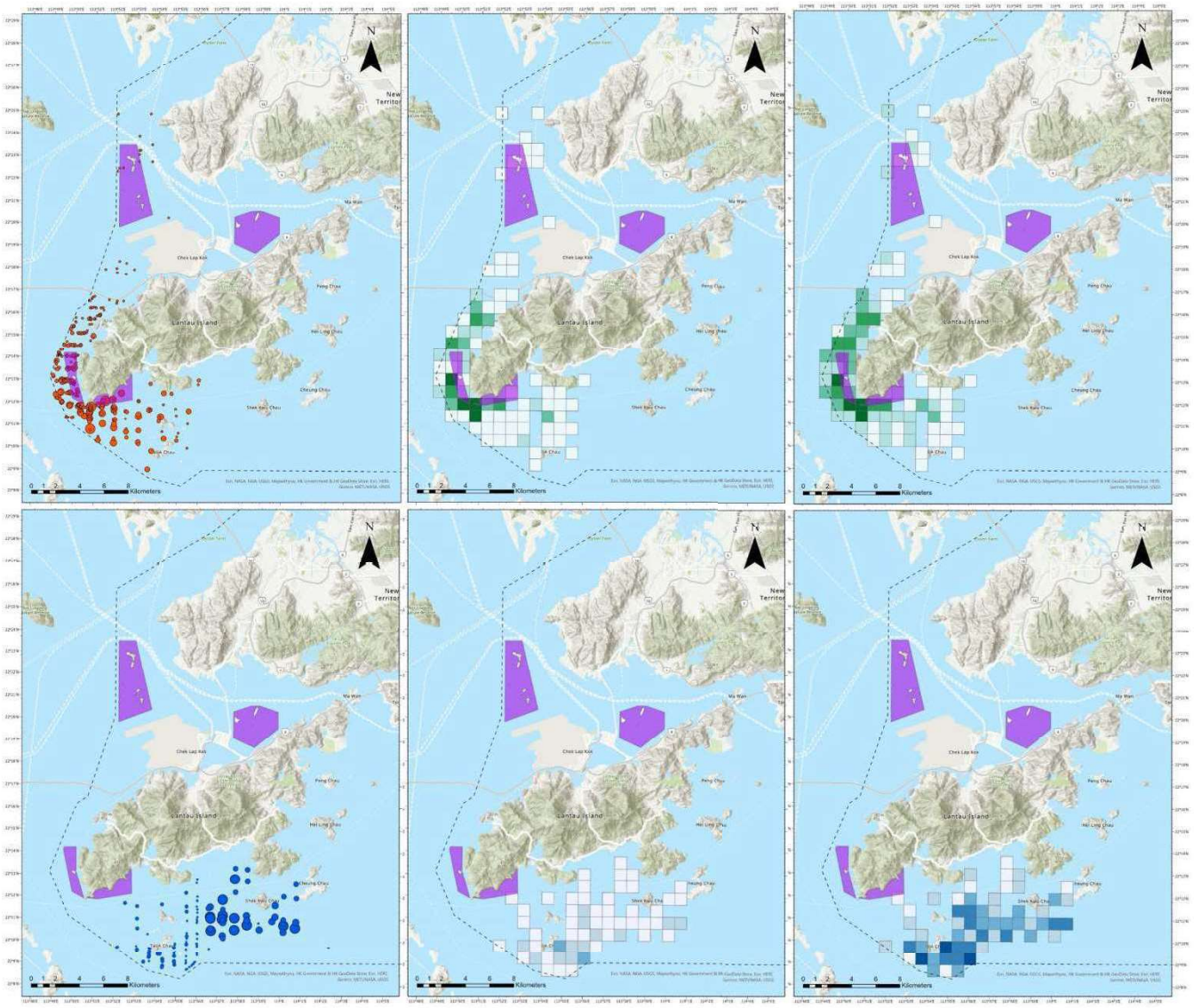


Fig. 9. Maps of Lantau waters showing distribution (Left, size of circles indicates increasing group sizes), dolphin per survey effort (Middle, shade of squares indicates increasing DPSE) and encounter probability (Right, shade of squares indicates increasing encounter rate) of Indo-Pacific humpbacked dolphins (Top) and Indo-Pacific finless porpoises (Bottom) in Lantau waters in 2020.

E. 2021 International Cetacean Symposium and TEDxCityUHongKong 2021

An international symposium was organized on 11 June 2021 with the theme of "Interaction", which is a follow up to its predecessor "Threats and Technology Afforded to Whales, Dolphins, and Porpoises in Hong Kong and Adjacent Waters" in 2019. Two scientific sessions were held in online and hybrid mode, with live online broadcast and physical attendance respectively (Fig. 10). The venue of the evening session was in the Hong Kong Maritime Museum. In this symposium, 19 speakers from 9 countries were invited. Invited experts shared their knowledge and experiences with the wider community on a range of

topics covered natural and human impacts on cetacean health in multi-disciplinary dimensions. In total our symposium attracted over 230 participants worldwide.

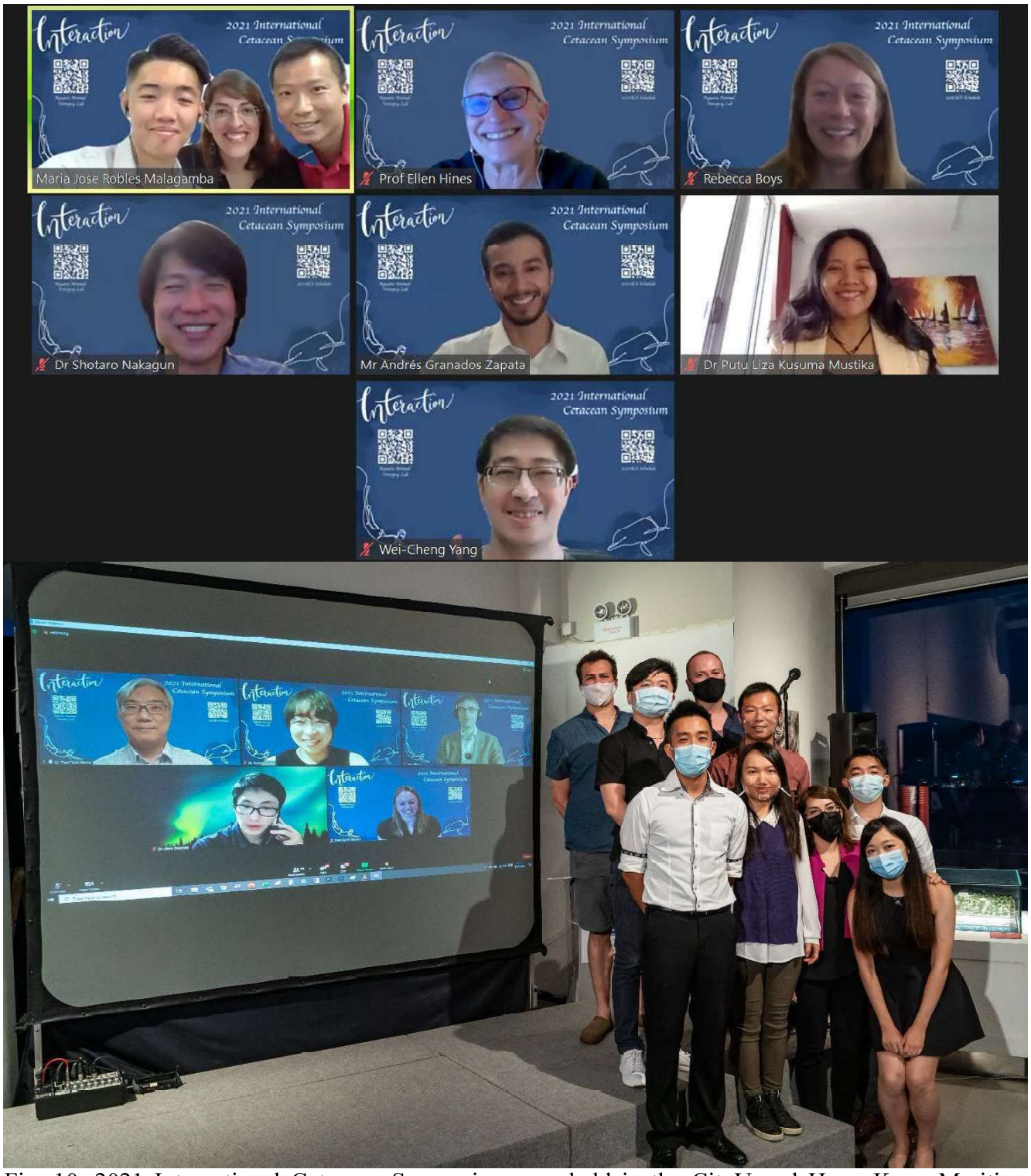


Fig. 10. 2021 International Cetacean Symposium was held in the CityU and Hong Kong Maritime Museum on 11 June 2021, with presented speakers in the morning session (Top) and evening session (Bottom).

Apart from the "2021 International Cetacean Symposium", this year we also successfully applied for a TEDx license to host "TEDxCityUHongKong" on 12 June 2021, with the theme of "Sink or Swim" (Fig. 11). Two TEDx talk sessions were held in a hybrid mode, with live online broadcast and physical attendance respectively. 16 speakers from 10 countries were invited. A total of 235 participants joined the TEDx event. All the videos of the TEDxCityUHongKong's talks have been uploaded to TEDx website and the corresponding YouTube channel. All the links could also be found at this link: https://bit.ly/3mAdl3W.

Fig. 11. The evening session of “TEDxCityUHongKong” was held in hybrid mode in Hong Kong Maritime Museum on 12 June 2021. Prof. Joost Schokkenbroek, the Museum Director was invited as one of the speakers.

11. Evaluation of the project effectiveness in achieving the proposed objectives addressed to date

Objectives *(as per 9.1/9.2 above)*	**Addressed** *(please tick)*	**Percentage achieved** *(please estimate)*
1. To describe and compare the spatiotemporal patterns of vessel traffic between night and day and within the day in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises habitats in the HK waters;	√	60
2. To identify and document the vessel type and speed that pose interaction risk with cetaceans and reveal the unsuspected vessel interaction 'hot spots' by analyzing the AIS-cetacean population density data in the HK waters;	√	40
3. To document and describe the types of suspected trauma-inflicting instrument using 3-D surface scanning technique, leading to injury induced by vessel interaction in stranded cetaceans	√	65
4. To combine the 3-D models obtained from virtopsy (internal) and 3-D surface scanning data (external) and perform matching analysis of the injury induced by vessel interaction and suspected injury-inflicting instrument using 3-D surface scanning technique, virtopsy and conventional necropsy findings;	√	60
5. To compile postmortem findings collected by original field notes, 3-D surface scanning, virtopsy and conventional necropsy, as well as the overlaid AIS-cetacean population density data, and transcribe into cetacean postmortem multimedia analysis platform for first time all-in-one real-databased models of the stranded cetacean for geometric comparison of patterned injuries with the presumed types of vessel interaction.	√	60

Part D: Research Output

12. Peer-reviewed journal publication(s) arising directly from this research project

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Attached to this report *(Yes or No)*	Acknowledged the support of MEEF *(Yes or No)*
Year of publication	Year of Acceptance	Under Review	Under Preparation				
Sept 2020				Brian C. W. Kot*, Derek K. P. Chan, Tabris Y. T. Chung, Henry C. L. Tsui	Image Rendering Techniques in Postmortem Computed Tomography: Evaluation of Biological Health and Profile in Stranded Cetaceans. Journal of Visualized Experiments (163), e61701, doi:10.3791/61701 (2020).	Yes (Appendix 6)	Yes
Oct 2020				Brian C. W. Kot*, Henry C.L. Tsui, Tabris Y.T. Chung, Amy P.Y. Lau	Postmortem Neuroimaging of Cetacean Brains Using Computed Tomography and Magnetic Resonance Imaging. Frontiers in Marine Science 7:544037. doi: 10.3389/fmars.2020.544037	Yes (Appendix 7)	Yes
Nov 2020				Henry C. L. Tsui, Brian C. W. Kot*, Tabris Y. T. Chung, Derek K. P. Chan	Virtopsy as a Revolutionary Tool for Cetacean Stranding Programs: Implementation and Management. Frontiers in Marine Science. 7:542015. doi: 10.3389/fmars.2020.542015	Yes (Appendix 8)	Yes

13. Recognized international conference(s) in which paper(s) related to this research project was / were delivered *(Please attach a copy of each conference abstract)*

Month/Year / Place	Title	Conference Name	Attached to this report *(Yes or No)*	Acknowledged the support of MEEF *(Yes or No)*
February 2021 / Virtual	Contribution of virtopsy to the assessment of cetacean biological health and profiles: Achievements, challenges and way Forward	The European College of Veterinary Pathologists Webinar	Yes (Appendix 2)	Yes
March 2021 / Virtual	Living in the clouds of a vain illusion? The rise of virtopsy and its applications: achievements, challenges and way forward	Regional marine mammal stranding workshop Keep swimming: Sharing marine mammal stranding expertise between East and West	Yes (Appendix 3)	Yes
May 2021 / Virtual	Virtopsy investigation of stranded cetaceans in Hong Kong waters (2017-2020)	2021 International Whaling Commission Scientific Committee Meeting	Yes (Appendix 4)	Yes
May 2021 / Virtual	Parasitic Infections in Stranded Cetaceans: A Virtopsy Approach	2021 International Association for Aquatic Animal Medicine Annual Conference	Yes (Appendix 5)	Yes

14. Other impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, Teaching enhancement, etc.)

Awards

P.I. and his team won a Gold Medal at the Inventions Geneva Evaluation Days 2021 (a virtual edition of the International Exhibition of Inventions of Geneva) with the invention entitled, “Aquatic Animal Postmortem Multimedia Analysis Platform”. With the support from this MEEF project, all postmortem multimedia are integrated and stored in the captioned well-developed Aquatic Animal Postmortem Multimedia Analysis Platform. This world first novel platform provides up-to-date results to governmental agencies, researchers and stakeholders to facilitate and support marine conservation and policy decisions, which allow the use of aquatic animals as sentinels of ecosystem health, working towards a “One Ocean-One Health” ideal. The competition was held in the Geneva, Switzerland from 10 to 14 March 2021. Relevant disclosure and acknowledgement of MEEF's support was be listed in the video submitted for the competition.

Part E: Summary and Way Forward

Throughout 12 months, from 1 July 2020 to 30 June 2021, the project entitled "Three-dimensional forensic scene investigation of marine vessel interaction in Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises in the Hong Kong waters", has been progressing and its objectives 45-60% accomplished. Despite the dynamic impacts of COVID-19 pandemic during the project time, various research tasks (e.g. spatiotemporal analysis on vessel-cetacean interaction, virtopsy and postmortem investigation on stranded cetaceans, 3DSS on stranded cetaceans and marine vessels) and outreach activities (e.g. 2 workshops and 1 public symposium and 1 TEDx event) targeted to fulfill the captioned 5 objectives in this project were conducted within the 12-month timeframe, with deliverables reported in various means.

The data collection and analyses on spatiotemporal patterns of vessel traffic and cetacean distribution are ongoing. Cetacean sighting data from different sources were consolidated and normalized for the first time, while the raw vessel traffic data is pending from Marine Department. Once the data is available, verified analyses will be performed to identify vessel-cetacean encounter hotspots and the type and speed of the vessels involved. Additional analyses on theodolite survey data at specific locations with high scientific value on dolphin research and conservation, e.g. Sha Chau and Lung Kwu Chau Marine Park, passive acoustic monitoring survey data for investigation on dolphin diel patterns and day and night vessel traffic activity, will be explored in the next phase of project period.

The virtopsy and 3DSS data collection are also ongoing. Virtopsy and 3DSS on stranded cetacean carcasses provide 3-D models of the injury victims, while the standardized protocol for 3DSS on marine vessels was established to provide 3-D models of the suspected injury-inflicting instruments. The results will be combined for matching analysis to simulate vessel-cetacean interactions in a 3-D forensic manner. Despite logistic difficulties and social distancing restrictions due to the COVID-19 pandemic, the team will continue to acquire data from more vessel types for a comprehensive analysis.

Data from postmortem investigation on stranded cetaceans has been archived and evaluated on the web-based "Cetacean Postmortem Multimedia Analysis Platform" database. Findings from this project had been shared to the public via different media and outreach activities. By identifying the hotspots and vessel types of high interaction risk and simulating the interaction in a 3-D forensic manner, decisive management plans will be suggested for injury prevention for vulnerable local resident cetaceans in our waters in the future.

Part F: Complete statement of accounts

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.*

Principal Investigator

Signature : 

Name of Project Leader : Brian Chin Wing Kot

Date : 2 July 2022

Official Chop : 

Appendix 1: List of project assets

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

Appendix 2. Abstract of conference proceedings

Oral presentation at the The European College of Veterinary Pathologists Webinar in February 2021

European College of Veterinary Pathologists Webinar
5 February 2021

Contribution of Virtopsy to the Assessment of Cetacean Biological Health and Profiles: Achievements, Challenges and Way Forward

Brian C.W. Kot,[1,2*] Henry C.L. Tsui,[2] Edgar K.C Leung[1]

[1]Department of Veterinary Clinical Sciences, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong SAR, China
[2]State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China;

- This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, and MEEF2019010A), the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

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- Agriculture, Fisheries and Conservation Department, HKSAR
- CityU Veterinary Medical Centre
- Student volunteers from local universities and OPCFHK
- Veterinary Hospital, Ocean Park Corporation
- Ocean Park Conservation Foundation Hong Kong
- Institute of Forensic Medicine, University of Zurich
- HK Science Museum
- State Key Laboratory of Marine Pollution, CityU
- Jockey Club College of Veterinary Medicine and Life Sciences, CityU
- Marine Ecology Enhancement Fund, (Ref no.: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A)

Appendix 3. Abstract of conference proceedings

Oral presentation at the Regional marine mammal stranding workshop Keep swimming: Sharing marine mammal stranding expertise between East and West in March 2021

Regional marine mammal stranding workshop
Keep swimming: Sharing marine mammal stranding expertise between East and West
11 March 2021

Living in the clouds of a vain illusion?
The rise of virtopsy and its applications:
achievements, challenges and way forward

Brian C.W. Kot

B.Sc. (Hons) Radiography, Ph.D. (Diagnostic Imaging), Cert. (RPC), Cert. (Virtopsy)

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- This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, and MEEF2019010A), the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

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- Marine Ecology Enhancement Fund, (Ref no.: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A)

Appendix 4. Abstract of conference proceedings

Oral presentation at the 2021 International Whaling Commission Scientific Committee Meeting in May 2021

Virtopsy investigations of stranded cetaceans in Hong Kong waters (2017-2020)

BRIAN C.W. KOT[1,2,3], HENRY C.L. TSUI[3], TABRIS Y.T. CHUNG[3], HEYSEN H.N. HO[3], MARIA JOSE ROBLES MALAGAMBA[2], JOHN Y.C. KWOK[2], EDGAR K.C. LEUNG[4], GABRIELLE Y.H. HO[4], AURORA S.Y. KWAN[2], JESSIE W.Y. YEONG[2,4]

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[4] *Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China*

With a rapidly expanding economy and population, Hong Kong (HK) has been experiencing an inevitable conflict between development and the environment. The constant growing need for space in HK drives land reclamation projects and the creation of artificial coastlines, thus removing the natural habitats for marine animals and has tremendously altered the ecological and biological health status of marine environment, which is essential for the survival of the endangered local cetaceans and their preys.

As the consequence, a significant reduction in the populations of both endangered residential cetacean species, namely the Indo-Pacific humpback dolphin *Sousa chinensis* (SC), and the Indo-Pacific finless porpoise *Neophocaena phocaenoides* (NP), is expected. Over the past decade, the annual abundance estimates of SC in HK waters were constantly below 100 and reached a historical low of 32 in 2018 (Hung, 2019). Huang et al. (2012) estimated that a loss of 74% of the local SC population is anticipated within three generations, while the rate of decline may accelerate with rapid development in the region. For the more seasonal NP population in HK, abundance estimate remains less certain, however a recent study estimated there to be 176 NPs in 2019 during the peak seasons (winter/spring; Jefferson & Moore, 2020). As the local cetacean species in HK are subjected to various direct and indirect anthropogenic threats (e.g., vessel collision, fishery interaction, pollution), unless decisive and effective conservation measures are implemented to halt the declining trend, the long-term survival of these 2 species in HK waters is unlikely (Karczmarski et al., 2017). This has also been reflected by an increasing trend in stranding numbers in HK waters over the past decade and reaching historical highs of 55 and 52 in 2019 and 2020.

In HK, vast resources have been unequally allocated to the monitoring of local cetaceans for the assessment of abundance and population dynamics through vessel or other forms of cetacean surveys. While these facilitated the development of spatial and population modelling tools to assess the population health (e.g., Huang et al., 2012; Karczmarski et al., 2017; Hung, 2019; Jefferson & Moore, 2020), direct studies focusing on the biological health of local cetaceans remain scarce. To date, there is limited understanding on the aforementioned anthropogenic threats in HK and the responses from local cetaceans to these threats. Currently available analyses of the postmortem (PM) investigations conducted on SC and NP stranded in HK waters are outdated and scattered (Parsons & Jefferson, 2000; Jefferson et al., 2002; 2006; Mauroo, 2017). These past records suggested that cetaceans in HK waters are prone to respiratory diseases and parasitic infections, whereas human-related mortality typically involves vessel collisions, as well as entanglement or entrapment by fishery interactions.

A pioneering virtopsy project was initiated in March 2014 to advance the cetacean stranding response programme in HK (Kot et al., 2015; Kot et al., 2016; Tsui et al., 2020). Applying postmortem imaging techniques on stranded cetaceans, the role of virtopsy has become more pivotal as veterinarians and other personnel involved in the stranding response became more aware of its strengths (Tsui & Kot, 2015). Over the past years, Kot and his team have developed standardised virtopsy protocols, as well as techniques to

diagnose pathologies and causes of deaths (CODs) in stranded cetaceans (Kot et al., 2016; Chan et al., 2017; Yuen et al., 2017; Kot et al., 2018a; 2018b; 2019; 2020a; 2020b; 2020c). Recently, pitfalls encountered during the Cetacean Virtopsy Stranding Response Programme and relevant practical management measurements were reviewed in hope to facilitate the implementation of virtopsy in stranding response worldwide (Tsui et al., 2020). To address the critical knowledge gap of cetacean biological health assessment through PM examination, this paper summarises and discusses the findings from virtopsy investigations of stranded cetaceans in HK waters from July 2017 to November 2020.

ACKNOWLEDGEMENTS

We would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong Special Administrative Region Government for the continuous support in this project. Sincere appreciation is also extended to veterinarians, staff, students, and volunteers from the Aquatic Animal Virtopsy Lab, City University of Hong Kong, the Ocean Park Conservation Foundation Hong Kong, and the Ocean Park Hong Kong. Special gratitude is owed to technicians in the CityU Veterinary Medical Centre and the Hong Kong Veterinary Imaging Centre for operating the CT and MR units in the present study. This project is funded by the Marine Ecology Enhancement Fund (Grant Nos: MEEF2017014, MEEF2017014A, MEEF2019010, and MEEF2019010A) of the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Any opinions, findings, conclusions, or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or Trustee.

Appendix 5. Abstract of conference proceedings

Oral presentation at the 2021 International Association for Aquatic Animal Medicine Annual Conference in May 2021

Parasitic Infections in Stranded Cetaceans: A Virtopsy Approach

Brian C.W. Kot[1,2], Edgar K.C. Leung[1*+], Henry C.L. Tsui[2], Tabris Y.T. Chung[2], Heysen H.N. Ho[2], María J. Robles Malagamba[1], John Y.C. Kwok[1], Gabrielle Y.H. Ho[1]

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Abstract

Parasites in marine mammals have been documented for decades, and recent taxonomic and systematic revisions of different genera have advanced our understanding of these parasites, especially their pathogenicity, evolution, and impacts on marine mammal biology. A review in 2016 revealed that a total of 176 species of helminths have been documented in cetaceans.[1] Infections from these parasites can have direct or indirect effects on the biological and population health of cetaceans. Apparently benign parasites can become opportunistic in weakened or immunocompromised animals, especially in endangered species or populations that are already challenged by other environmental or anthropogenic stressors.[2-5]

In human clinical radiology, advanced imaging techniques like computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography-computed tomography, can be helpful in solving the differential diagnosis involving parasitic infections.[6] These imaging modalities, particularly CT and MRI, have been pioneered and applied by Kot and his team in postmortem investigation of stranded cetaceans and sea turtles.[7] The postmortem use of such techniques (PMCT and PMMRI), also known as virtopsy, has demonstrated to be useful in assessing the biological and pathological profiles of stranded cetaceans.[8] However, to the best of our knowledge, there is an absence of available literature on the applicability of virtopsy in diagnosing parasitic infections in stranded cetaceans. Thus, this study aimed to evaluate the applicability of virtopsy, notably PMCT, on investigating parasitic infections in stranded cetaceans, with comparison to conventional necropsy whenever possible.

Cetacean stranding cases from Hong Kong and adjacent waters between 2014 and 2020 which underwent both PMCT and conventional necropsy, and reported with parasitic infection, were retrospectively analysed. PMCT was effective in identifying the sites of the parasitic infections, which commonly included cranial sinuses, lung, liver, mammary glands, and abdominal muscles. PMCT also revealed a broad variation in the radiological appearance of the parasitic infections, especially for the lesions around the mammary glands and nearby muscles, which was correlated to findings during conventional necropsy and may related to the progress, chronicity, or severity of the parasitic infections. Furthermore, we aim to develop and implement a grading system using PMCT to describe the chronicity and severity of these parasitic lesions for better pathological assessment. Related morphological and molecular identification studies are currently undergoing to provide extra information on the epidemiology and life history of these parasites affecting the cetacean populations in Hong Kong and adjacent waters.[9]

Acknowledgements
We would like to thank the Agriculture, Fisheries and Conservation Department of the HKSAR Government for the continuous support in this project. Sincere appreciation is also extended to the personnel from the Aquatic Animal Virtopsy Lab, City University of Hong Kong, Ocean Park Conservation Foundation Hong Kong, and Ocean Park Hong Kong. Special gratitude is owed to technicians in CityU Veterinary Medical Centre for operating the CT and MR units in the present study. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, and MEEF2019010A) and the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Any opinions,

findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

Image Rendering Techniques in Postmortem Computed Tomography: Evaluation of Biological Health and Profile in Stranded Cetaceans

Brian C. W. Kot[1,2], **Derek K. P. Chan**[3], **Tabris Y. T. Chung**[1], **Henry C. L. Tsui**[1]

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Abstract

With 6 years of experience in implementing virtopsy routinely into the Hong Kong cetacean stranding response program, standardized virtopsy procedures, postmortem computed tomography (PMCT) acquisition, postprocessing, and evaluation were successfully established. In this pioneer cetacean virtopsy stranding response program, PMCT was performed on 193 stranded cetaceans, providing postmortem findings to aid necropsy and shed light on the biological health and profile of the animals. This study aimed to assess 8 image rendering techniques in PMCT, including multiplanar reconstruction, curved planar reformation, maximum intensity projection, minimum intensity projection, direct volume rendering, segmentation, transfer function, and perspective volume rendering. Illustrated with practical examples, these techniques were able to identify most of the PM findings in stranded cetaceans and served as a tool to investigate their biological health and profile. This study could guide radiologists, clinicians and veterinarians through the often difficult and complicated realm of PMCT image rendering and reviewing.

Introduction

Virtopsy, also known as postmortem (PM) imaging, is the examination of a carcass with advanced cross-sectional imaging modalities, including postmortem computed tomography (PMCT), postmortem magnetic resonance imaging (PMMRI), and ultrasonography[1]. In humans, PMCT is useful in investigating traumatic cases of skeletal alterations[2,3], foreign bodies, gaseous findings[4,5,6], and pathologies of the vascular system[7,8,9]. Since 2014, virtopsy has been routinely implemented in the Hong Kong cetacean stranding response program[1]. PMCT and PMMRI are able to depict patho-morphological findings on carcasses that are too decomposed to be evaluated by conventional necropsy. The non-invasive radiological assessment is objective and digitally storable, allowing

Preparation and scanning of carcasses is critical for subsequent postprocessing and visualization of virtopsy data. Operation of a CT machine, an ionizing radiological unit, must be performed by a certificated radiological technician or clinician in compliance with the law. Although the scanned subjects were carcasses, the radiation dose should be kept to as low as reasonably achievable. The control of scanning parameters, especially slice thickness, would highly influence the accuracy of the reconstructed coronal and sagittal planes. Moreover, reduction in CT slice thickness permits more precise diagnosis. For instance, acquiring PMCT images at 3 mm thickness may neglect a 1×1×1 mm parasitic granuloma, commonly observed in the mammary glands of stranded cetaceans. To avoid missing any finding and improve the resolution of 2D and 3D rendering, a standardized scanning protocol was used. The slice thickness was controlled at 1 mm, and down to 0.625 mm whenever possible, which is the minimum slice thickness available for the CT machine used.

A proper postprocessing visualization and manipulation of virtopsy datasets requires clear understanding of the principles and pitfalls of the common rendering techniques used for cetacean PM investigation, e.g., the identification of strength and weakness between the techniques[21]. The choice of rendering techniques depends on the anatomical structures and the underlying pathologies to be illustrated, there is no single technique that can comprehensively recognize all the PM findings. Knowing the pros and cons and choosing the appropriate rendering techniques can boost image quality and interpretability of virtopsy datasets, which aid to obtain a correct diagnosis. Carefully reviewing virtopsy datasets and correlating them with other techniques can avoid potential rendering and segmentation error[18]. Still, the final judgement and diagnosis should be made by veterinary radiologists or radiological clinicians who are certificated and experienced to report virtopsy findings.

Disclosures

The authors have nothing to disclose.

Acknowledgments

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong Special Administrative Region Government for the continuous support in this project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, City University of Hong Kong, Ocean Park Conservation Foundation Hong Kong and Ocean Park Hong Kong for paying great effort on the stranding response in this project. Special gratitude is owed to technicians in CityU Veterinary Medical Centre and Hong Kong Veterinary Imaging Centre for operating the CT and MRI units for the present study. Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee. This project was funded by the Hong Kong Research Grants Council (Grant number: UGC/FDS17/M07/14), and the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010 and MEEF2019010A), Marine Ecology Enhancement Fund, Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Special thanks to Dr. María José Robles Malagamba for English editing of this manuscript.

References

1. Tsui, H.C.L., Kot, B.C.W., Chung, T.Y.T., Chan, D.K.P. Virtopsy as a revolutionary tool for cetacean stranding

Appendix 7. Peer-reviewed journal publication(s) arising directly from this research project

frontiers in Marine Science

ORIGINAL RESEARCH
published: 05 October 2020
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Postmortem Neuroimaging of Cetacean Brains Using Computed Tomography and Magnetic Resonance Imaging

Brian C. W. Kot[1,2], Henry C. L. Tsui[1], Tabris Y. T. Chung[1] and Amy P. Y. Lau[3]*

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Postmortem computed tomography (PMCT) and postmortem magnetic resonance (PMMR) imaging (PMMRI) have been applied to provide vital or additional information for conventional necropsy, along the pioneering virtopsy-driven cetacean stranding response program in Hong Kong waters. It is common for stranded carcasses to become badly degraded and susceptible to rapid cerebral autolysis and putrefaction. Necropsy on decomposed brains with limited sample analysis often defy a specific diagnosis. Studies on PMMR neuroimaging have focused on neuroanatomy and brain morphology in freshly deceased or preserved specimens. Moreover, the literature is devoid of any reference on the potential value of PMMRI examination of decomposed cetacean brains. To that end, this project evaluated the benefits of PMMR neuroimaging *in situ* in decomposed carcasses in comparison to PMCT. A total of 18 cetacean carcasses were studied by PMCT and PMMRI examinations. Anatomical brain structures and visible brain pathologies were evaluated and scored using Likert-scale rating. Intracranial gas accumulation was clearly depicted in all cases by all radiological techniques. Other features were more clearly depictable in PMMRI than in PMCT images. Results of this study indicated that superiority of PMMRI compared to PMCT increased with advanced putrefaction of the brain. The preservation of structural integrity was presented by PMMRI due to its superior capability to evaluate soft tissue. Brain PMMRI should be incorporated in postmortem investigation of decomposed stranded cetaceans.

Keywords: postmortem, magnetic resonance imaging, computed tomography, cetacean, decomposed brain

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INTRODUCTION

Virtopsy using postmortem computed tomography (PMCT) and postmortem magnetic resonance (PMMR) imaging (PMMRI) has been implemented in the pioneering virtopsy-driven cetacean stranding response program in Hong Kong (HK) waters, to provide vital or additional information for conventional necropsy (Kot et al., 2016, 2018, 2019, 2020; Chan et al., 2017; Yuen et al., 2017). Stranded cetacean carcasses found in HK waters are often badly degraded (Jefferson et al., 2002) as high temperature and humidity accelerate

(Cartocci et al., 2020). However, gas accumulation may degrade image quality on PMMRI to a greater extent than on PMCT, since the PMMR signal intensity can be decreased, especially during the T1w sequence image acquisition, due to changes in the soft tissue chemical properties (Lundström et al., 2012). This might explain why brain structures could not be clearly depicted, and in some of our subjects, were barely identifiable with T1w PMMR images.

One limitation of the present study was that carcass temperature was not recorded, forbidding the investigation on the influence of changes in subject temperature on the PMMR image quality. Temperature is not considered to be an influencing factor on image quality of PMCT as it is on that of PMMR. On the contrary, image contrast in PMMRI is temperature-dependent, as T1 and T2 relaxation times are greatly affected by the temperature of the subject. Previous studies in humans demonstrated that different, and particularly low corpse temperatures may alter image quality in PMMRI (Thali et al., 2003a; Zech et al., 2015). In the present study, carcasses were kept out of the freezer for 24 h prior to the PMMRI examinations, and the PMMRI examination room temperatures ranged from 16 to 22°C. At these temperatures, the image quality was appeared optimal with sufficient grey value contrast. However, in cases with lower corpse temperatures, image quality and recognisability of brain structures could be significantly affected (Flach et al., 2016). Rectal temperature measurements of cetacean carcasses before and after PMMRI are suggested to facilitate a retrospective evaluation of the correlation between body temperature and PMMR image quality in both research and clinical setting in future studies.

Another limitation of the present study was the lack of variety on choices of PMMRI sequence for comparative evaluation of cetacean neuroimaging. PMMRI sequences such as fluid-attenuated inversion recovery, diffusion-weighted image and tensor image sequences were excluded due to the time constraints in image acquisition. These advanced PMMRI techniques have not yet been fully explored. Yen et al. (2006) described the potential application of diffusion tensor imaging of traumatic fibre tract rupture in human with a brain stem lesion. Scheurer et al. (2011) examined 20 human cases with diffusion-weighted and tensor imaging to estimate the postmortem interval, and suggested that the apparent diffusion coefficient could be an indicator for the assessment of the postmortem interval. Further study on the application of various PMMRI sequences for cetacean neuroimaging is suggested to investigate their potential values and implications.

To conclude, results of the present study demonstrated that PMMRI of decomposed brains offered a more distinct soft tissue contrast and allowed detailed morphological assessment of decomposed brains compared to PMCT. Brain PMMRI should be incorporated in postmortem investigation of decomposed cetacean carcasses.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available due to restrictions from the Agriculture, Fisheries and Conservation Department of Hong Kong Special Administrative Region. The raw data supporting the conclusions of this article can be made available by contacting Dr. Ng Wai-Chuen (waichuen_ng@afcd.gov.hk), upon reasonable request.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because research activities coincided with the Local Cetacean Stranding Response Programme in Hong Kong, sanctioned by the Agriculture, Fisheries and Conservation Department of Hong Kong Special Administrative Region.

AUTHOR CONTRIBUTIONS

BK, HT, and AL wrote the first draft of the manuscript. All authors contributed to the conception and design of the study, analysed the data, and wrote sections of the manuscript. All authors also contributed to the field work and the data collection, contributed to the manuscript revision, and read and approved the submitted version.

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Appendix 8. Peer-reviewed journal publication(s) arising directly from this research project

frontiers in Marine Science

ORIGINAL RESEARCH
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Virtopsy as a Revolutionary Tool for Cetacean Stranding Programs: Implementation and Management

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On top of conventional necropsy, virtopsy (postmortem computed tomography and postmortem magnetic resonance imaging) has been integrated into the Cetacean Stranding Response Programme in Hong Kong since March 2014. To date, 177 out of 240 local stranded cetaceans have been examined by virtopsy. This integration has modernised the characterisation and documentation of cetacean biological health and profiles, and causes of death. During this 6-year period, critical pitfalls regarding logistics, carcass recovery, handling, and preservation have been identified. A strategic management scheme is crucial for the successful incorporation of virtopsy into this pioneer programme. This study explains the workflow of the Cetacean Virtopsy Stranding Response Programme in Hong Kong waters. Difficulties encountered are highlighted and practical solutions to address management issues are proposed to consolidate the stranding response network.

Keywords: pitfalls, management, cetacean, stranding, virtopsy

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INTRODUCTION

Postmortem (PM) investigation generally involves an invasive "body-opening" autopsy in humans (or necropsy, same procedure in animals). Virtopsy, also known as PM imaging, is the examination of a carcass with modern imaging modalities, including the use of postmortem computed tomography (PMCT) and postmortem magnetic resonance imaging (PMMRI) (Kot et al., 2016) prior to conventional autopsy. Indeed, virtopsy can supplement or even partially replace an autopsy (Dirnhofer et al., 2006). Whole-body PMCT and PMMRI are non-invasive techniques that create volumetric image datasets, while two- and three-dimensional (3D) volumetric reconstruction and rendering are performed with advanced visualisation technology (e.g., multiplanar reconstruction and direct volume rendering). These results allow objective visualisation and recapitulation of PM findings prior to conventional necropsy (Kot et al., 2020a,b).

In human forensic medicine, the correlation between findings of whole-body virtopsy and conventional autopsy was first established in 2003 (Thali et al., 2003). PMCT allows excellent identification of osseous lesions, foreign bodies, pathological gas formation, and organ trauma with higher precision over conventional autopsy (Dirnhofer et al., 2006; Aghayev et al., 2007; Thali et al., 2007). PMMRI has proved to be superior in demonstrating soft tissue injury, neurological and non-neurological organ trauma as well as non-traumatic pathology (Thali et al., 2007). Examination with

efficient workflow of PM examination is warranted (Ebert et al., 2015). The planning should also make a reference to human and veterinary forensic settings. Guidelines for facilities and operation of hospital and forensic mortuaries (National Pathology Accreditation Advisory Council, 2013) should be considered and implemented.

A tentative workflow is proposed as follows: Upon receiving a stranding report, response personnel will reach the site by designated transportation. Basic measurements and photos of the carcass and any associated wounds or entanglements will be taken. The carcass will be cleaned and packed in a natural prone position without bending it, into a radiolucent cadaver bag without any metal parts. Cooling measures will be applied when deemed necessary. The carcass will be transferred on a stretcher to the virtopsy facility. Radiological clinician who is certificated and experienced to report virtopsy findings will be notified to setup the scanning modules in advance so that the PMCT scan can be performed instantly upon arrival. After PMCT data acquisition, the carcass bag will be opened to allow 3DSS of the carcass directly on the CT couch. Without altering the carcass position between PMCT and 3DSS, external and internal findings can be directly correlated for proper interpretation of the deceased animal's biological health and profiles. A typical 3DSS of a stranded cetacean takes about 30 min, preliminary PMCT interpretation will be conducted in the meantime, followed by PMMRI scanning whenever applicable. After the virtopsy session, the carcass will be transferred to the necropsy room for conventional necropsy and tissue sampling with reference to the virtopsy findings. Instead of performing random sectioning of organs, any abnormalities and pathologies observed in the virtopsy will be followed. If refrigeration is necessary, the carcass will be kept in the morgue with a natural prone position and sufficient spacing. Data of the field report, virtopsy, and necropsy reports will be analysed as a whole, and consensus on the assignation of cause of death and biological health concerns will be reached by all of the involved parties, with an addressed degree of confidence (i.e., confirmed, probable or suspected).

A one-stop examination facility could streamline the logistics and minimise carcass decomposition, producing more meaningful biological health data of stranded cetaceans with less resources. Administration complexity could be reduced, quality control and sharing of the PM examination data among different collaborators could be enhanced, and the overall management of the virtopsy integrated stranding response programme could be advanced. This study is a valuable reference for other stranding response programmes worldwide that are interested in integrating virtopsy or other modern diagnostic modalities into their routine workflow.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available due to restrictions from the Agriculture, Fisheries and Conservation Department of Hong Kong Special Administrative Region. The raw data supporting the conclusions of this article can be made available by contacting Dr. Ng Wai-Chuen (waichuen_ng@afcd.gov.hk), upon reasonable request.

ETHICS STATEMENT

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AUTHOR CONTRIBUTIONS

BK, HT, and DC wrote the first draft of the manuscript. All authors contributed to the conception and design of the study, analysed the data, wrote sections of the manuscript, contributed to field work, data collection, and manuscript revision, read and approved the submitted version.

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