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MARINE ECOLOGY ENHANCEMENT FUND (MEEF)

Completion Report for Year 2021-2022

Report for the period ending 30 June 2022

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 2021 to 30 June 2022, 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 (HK) waters", has been ongoing progressively and its objectives have been 100% accomplished. Despite the social unrest during the project commencement, and the subsequent 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 2021 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 the project team. Data from different sources were normalized to survey effort for subsequent spatiotemporal analyses. Data from 2019 to 2021 were overlaid with marine vessel traffic data from Marine Department to calculate the cetacean encounter probability and reveal the interaction 'hot spots' using ArcGIS. Vessel type and speed that pose interaction risk with cetaceans were also investigated.

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, the project team had scanned 11 local marine vessels with the long-range 3-D scanner Artec Ray to document the suspected trauma-inflicting structures. Despite logistic difficulties and social distancing restrictions due to the COVID-19 pandemic, the project team will continue to acquire data from more vessel types for a comprehensive analysis.

d) To combine the 3-D models obtained from virtopsy (internal) and 3DSS 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;

During the reporting period, 21 out of 22 stranded cetaceans in HK waters (95%), including 1 Indo-Pacific humpbacked dolphin (SC) and 20 Indo-Pacific finless porpoises (NPs), had undergone virtopsy. 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. In these 21 cases, findings of 9 NPs were sufficient to assign the cause of death with confirmed, probable or suspect category associated with human interactions (43%), which included evidence of fishery (e.g., fishing gear entanglement or digestion) and vessel interactions (e.g., blunt force trauma). The other 12 stranded cetaceans (1 SC and 11 NPs) were associated with non-human causes of death and various biological health concerns (e.g., parasitic infection and pneumonia).

For 3DSS, a total of 66 cetacean carcasses (12 SCs, 48 NPs and 6 of other species (OTs)) were scanned using the 2 handheld 3-D scanners and 11 marine vessels were scanned using the long-ranged 3-D scanner throughout the project period. 3-D models of cetacean carcasses and marine vessels were reconstructed for matching analysis of the injury induced by vessel interaction and suspected injury-inflicting instrument using 3DSS, virtopsy and necropsy findings in a comprehensive manner.

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 265 virtopsy cases (from March 2014 to 30 June 2022, included 53 SCs, 184 NPs and 28 OTs) and respective links between the web server and DICOM viewer were completed.

Although the project happened during the COVID-19 pandemic, the project team had paid efforts to convey marine conservation messages via outreach activities, such as maintaining social media engagement, conducting interviews with different print media, holding symposium, public talks and workshops jointly organized with the Hong Kong Science Museum, to elevate the public awareness on cetacean strandings, immediate threats that cetaceans are facing, impacts of human interactions, and the greater scope: marine conservation and interest in science and technology.

As a wrap-up of the 3-year project, a stakeholder forum was organized to gather local stakeholders, including researchers, economics and policy groups, to review the current status of local cetaceans and marine traffic. Different parties engaged in sharing and discussion on the obstacles and strategic solutions to mitigate cetacean-vessel interactions. Potential collaborations were proposed, and a mitigation plan summarizing the discussion is to be published in late 2022.

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 2021 to 30 June 2022 (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	Completed
Stranded cetacean blog	July 2019 – June 2022	Completed
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	Completed in June 2022
HK SciFest 2022	Spring 2022	Cancelled due to the COVID-19 pandemic
Data consolidation and write up publications	April – June 2022	Completed
Handbook of three-dimensional forensic scene investigation of marine vessel interaction in stranded cetaceans	April – June 2022	Completed

8. Project Expenditure

Project expenditure is not disclosed due to confidentiality reasons.

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

Since July 2021, 3 Research Assistants were recruited for this project. 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, and publication preparation.

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, MEEF2019010 and

MEEF2019010A). 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, government 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 2022, the blog has reached over 700,000 users with over 3,600 followers and 233 posts were published. In total, 10 interviews on the P.I. and his team's work and findings (virtopsy and vessel traffic related to local cetaceans) were conducted by various media and published from July 2021 to June 2022, as listed below:

Media	Title	Date	URL Link
Asia Research News	Lessons from the dead Whale and dolphin post-mortem imaging could breathe new life into marine conservation.	8th March 2022	https://bit.ly/3703azP
Headline Daily 頭條日報	本港鯨豚擱淺去年回落至32條　10宗死因涉人類活動	18th February 2022	https://bit.ly/3v8BCm6
HK01	鯨豚擱淺去年回落至32條　三成涉人類活動 江豚被船撞至脊骨碎裂	18th February 2022	https://bit.ly/3t4eL8v
Oriental Daily News 東方日報	一群偽虎鯨突襲南丫島覓食　保育團體促船隻避開	15th February 2022	https://bit.ly/3BMSz6J
	去年鯨豚擱淺量下跌近4成　惟3成致死由人為因素所致	18th February 2022 19th February 2022	https://bit.ly/3IjSyts Printed copy
	南大嶼海岸公園　乏宣傳空談保育	27th June 2022	https://bit.ly/3PTRSyl
	人工魚礁放西部水域成效存疑	27th June 2022	https://bit.ly/3zs7R0f
South China Morning Post 南華早報	Chinese white dolphin deaths fall amid Covid-19 pandemic, probably because of halt to Hong Kong-Macau ferries, researcher says | South China Morning Post	15th March 2022	https://bit.ly/37AFbHZ
Sing Tao Daily 星島日報	本港鯨豚擱淺去年回落至32條　10宗死因涉人類活動	18th February 2022	https://bit.ly/352KUW5
University of Zurich	Institute of Forensic Medicine Yearly Report 2021	January 2022	https://bit.ly/3rsFMBJ (P.15)

D. Public seminar and workshop

Twelve biodiversity workshops jointly organized with the Hong Kong Science Museum, entitled, "Virtopsy: Threats to Hong Kong's Cetaceans" were scheduled on 14 July, 14 August, 12 September, 6 October, 3 November, 1 December 2021, 8 January, 20 February, 30 March, 3 April, 4 May and 15 June 2022 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, the 5 workshops scheduled from January to May 2022 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 cancel the HK SciFest 2022.

Fig. 1. A biodiversity workshop jointly organized at the Nature Lab of the Biodiversity Gallery in Hong Kong Science Museum, with Research Assistants trained to assist the delivery of workshops.

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 265 virtopsy cases (from March 2014 to 30 June 2022, included 53 SCs, 184 NPs and 28 of other species) and respective links between the web server and DICOM viewer were completed.

B. . Virtopsy cases performed

From 1 July 2021 to June 2022, 21 out of 22 stranded cetaceans in HK waters (95%), including 1 SC and 20 NPs, had undergone virtopsy. 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 1 NP (5%) was a mummified remain without skull and flippers, and was not retrieved for virtopsy by the OPCFHK delegates. In the aforesaid 21 cases, findings of 9 NPs were sufficient to assign the cause of death with confirmed, probable or suspect category associated with human interactions (43%), which included evidence of fishery (e.g., fishing gear entanglement or digestion) and vessel interactions (e.g., blunt force trauma) (Fig. 2). The other 12 stranded cetaceans (1 SC and 11 NPs) were associated with non-human causes of death and various biological health concerns (e.g., parasitic infection and pneumonia). Selective virtopsy findings were consolidated and shared bilingually on our stranded cetacean blog (Facebook page: https://www.facebook.com/AAVLab2014), allowing the public to learn about cetacean strandings and related conservation concepts.

Fig. 2. Photographs and 3-D model of a finless porpoise stranded in March 2022 and investigated by the project team. Blunt trauma wound at the dorsal head region (yellow arrow) exposing the fractured cranium was suggestive of vessel collision. The geometric dimensions of the wound were documented in the 3-D model, which allows matching analysis with suspected injury-inflicting tools.

C. 3-D surface scanning

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. 3), and structures of marine vessels, with reference to the standardised techniques established by the project team. As of 30 June 2022, 66 cetacean carcasses (12 SCs, 48 NPs and 6 OTs) and 11 marine vessels in HK were scanned and reconstructed into 3-D models (Fig. 4).

Fig. 3. 3-D surface scanning of a stranded finless porpoise using the scanner Artec Eva.

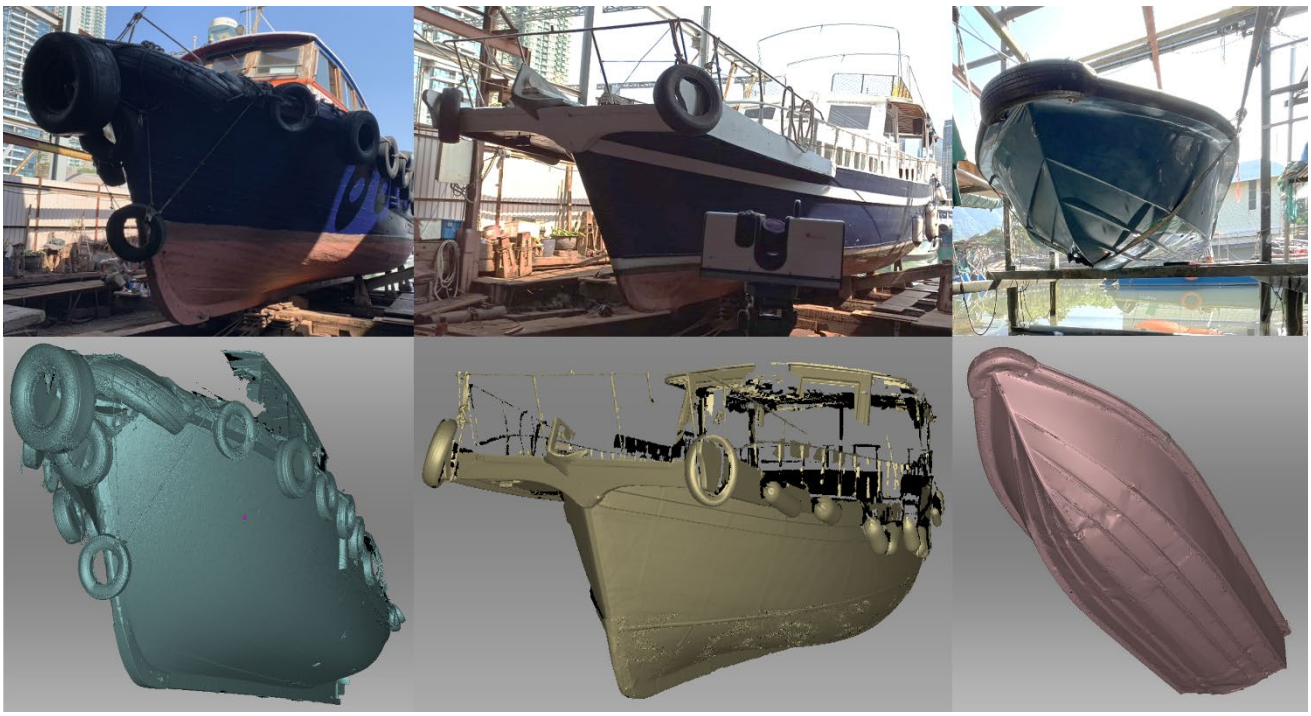


Fig. 4. Examples of marine vessels scanned using the scanning Artec Ray and the corresponding 3-D models showing details of the underwater parts, which allows matching analysis with cetacean carcasses with suspected vessel-inflicted injuries.

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

Cetacean sighting datasets from line transect surveys for both SC and NP from 2014 to 2021 were obtained from long-term dolphin monitoring project conducted by AFCD and other Environmental Monitoring and Audit (EM&A) programs in West HK waters (Fig. 5A). These datasets were normalised with survey effort to obtain the dolphin per survey effort (DPSE) (Fig. 5B) and cetacean encounter probability using ArcGIS Pro 2.9.1. Marine vessel transit density from 2019 to 2021 obtained from Marine Department (Fig. 5C) were converted to vessel encounter probability. The cetacean encounter probability was overlaid with the vessel encounter probability to generate the cetacean-vessel encounter probability (Fig. 5D), with 'hot spots' of high collision risk for Indo-Pacific humpbacked dolphins and Indo-Pacific finless porpoises noted outside the Southwest Lantau Marine Park waters and in the Tai A Chau waters respectively (Fig 6). With the establishment of South Lantau Marine Park in June 2022, including Soko islands (e.g. Tai A Chau and Siu A Chau), the aim of this marine park implementation, i.e. to help better conserve the Indo-Pacific humpback dolphins and Indo-Pacific finless porpoises, could be evaluated therein in term of the impact of vessel traffic. To investigate the impact of vessel traffic, the transit density data was categorized by vessel type and speed (Fig. 7). For 2021, with the halt of high speed ferry operation between Hong Kong and Macau since early February 2020, most of the remaining marine traffic was under the safety threshold of 14 knots.

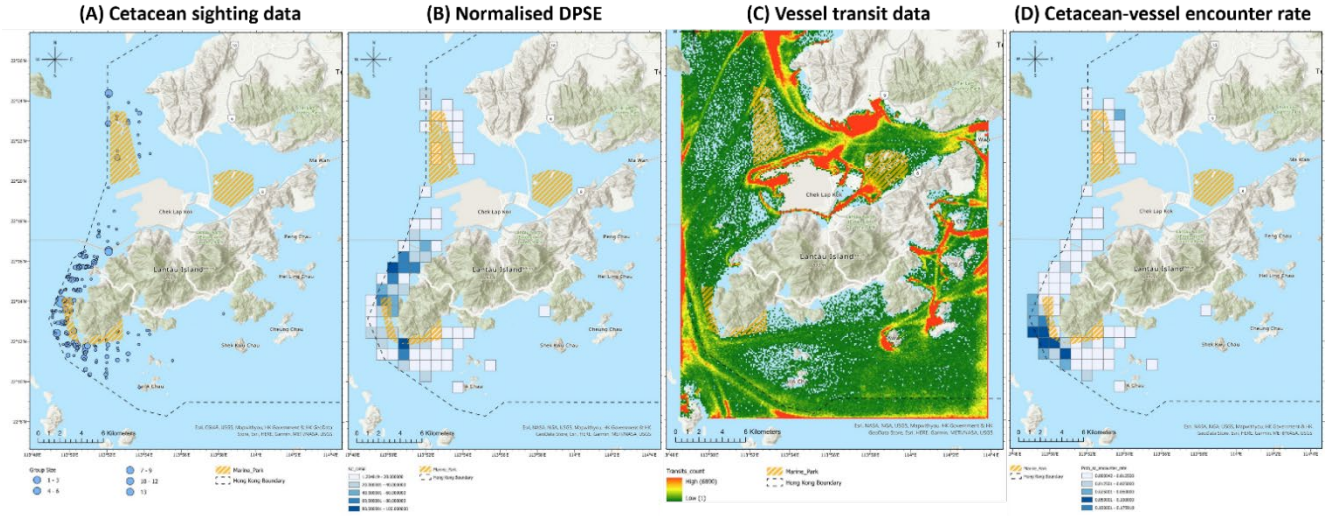


Fig. 5. Maps of Lantau waters showing **(A)** Indo-Pacific humpbacked dolphin sighting locations, **(B)** normalized dolphin per survey effort (DPSE), **(C)** vessel transit data, and **(D)** cetacean-vessel encounter 'hot spots' (deeper shade of squares: higher encounter probability) in 2021.

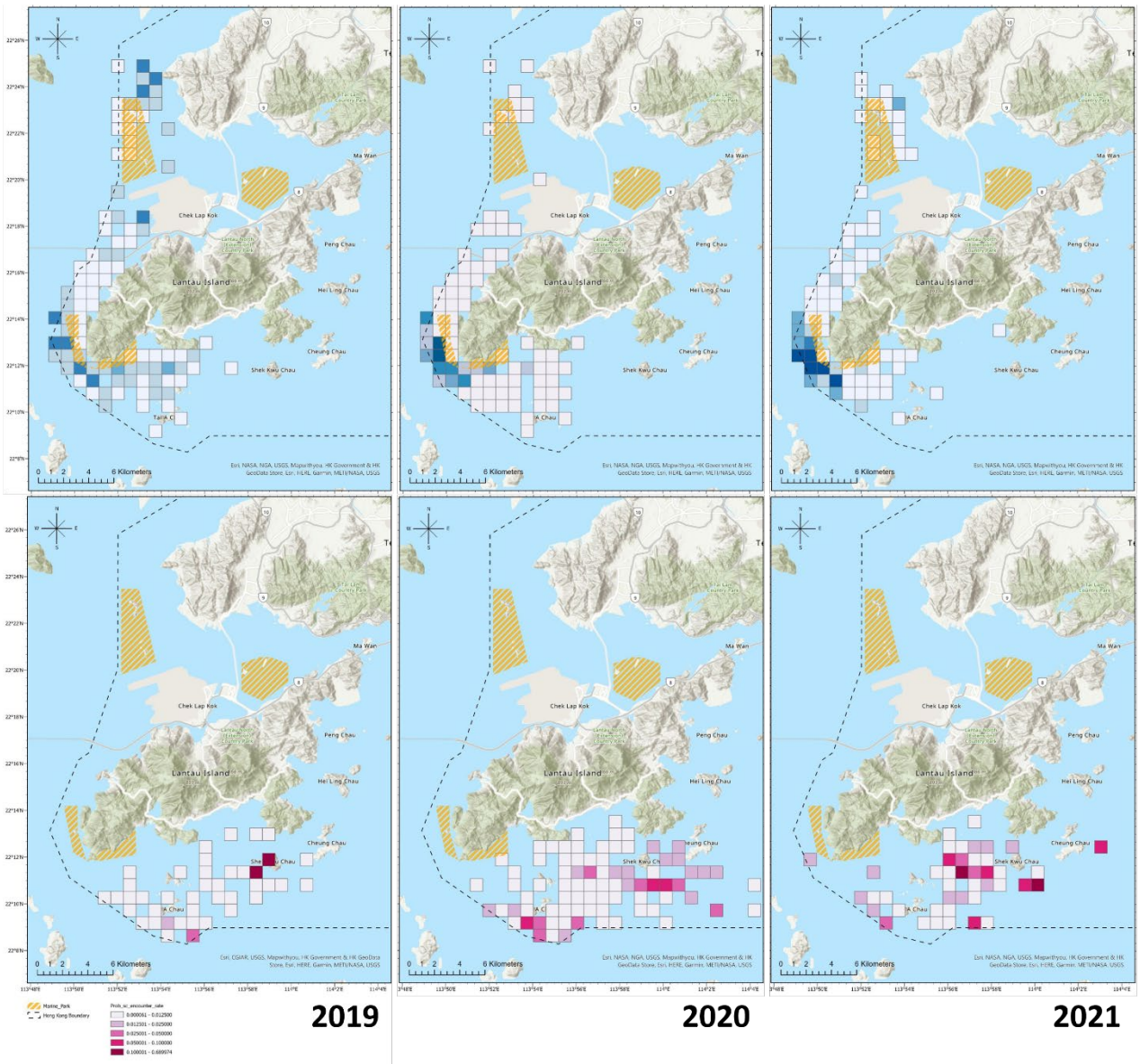


Fig. 6. Maps of Lantau waters showing cetacean-vessel encounter probability and collision 'hot spots' in **(left)** 2019, **(middle)** 2020, and **(right)** 2021 (deeper shade of squares: higher encounter probability). 'Hot spots' for **(upper)** Indo-Pacific humpbacked dolphins and **(lower)** Indo-Pacific finless porpoises are noted outside the Southwest Lantau Marine Park waters and in the Tai A Chau waters respectively.

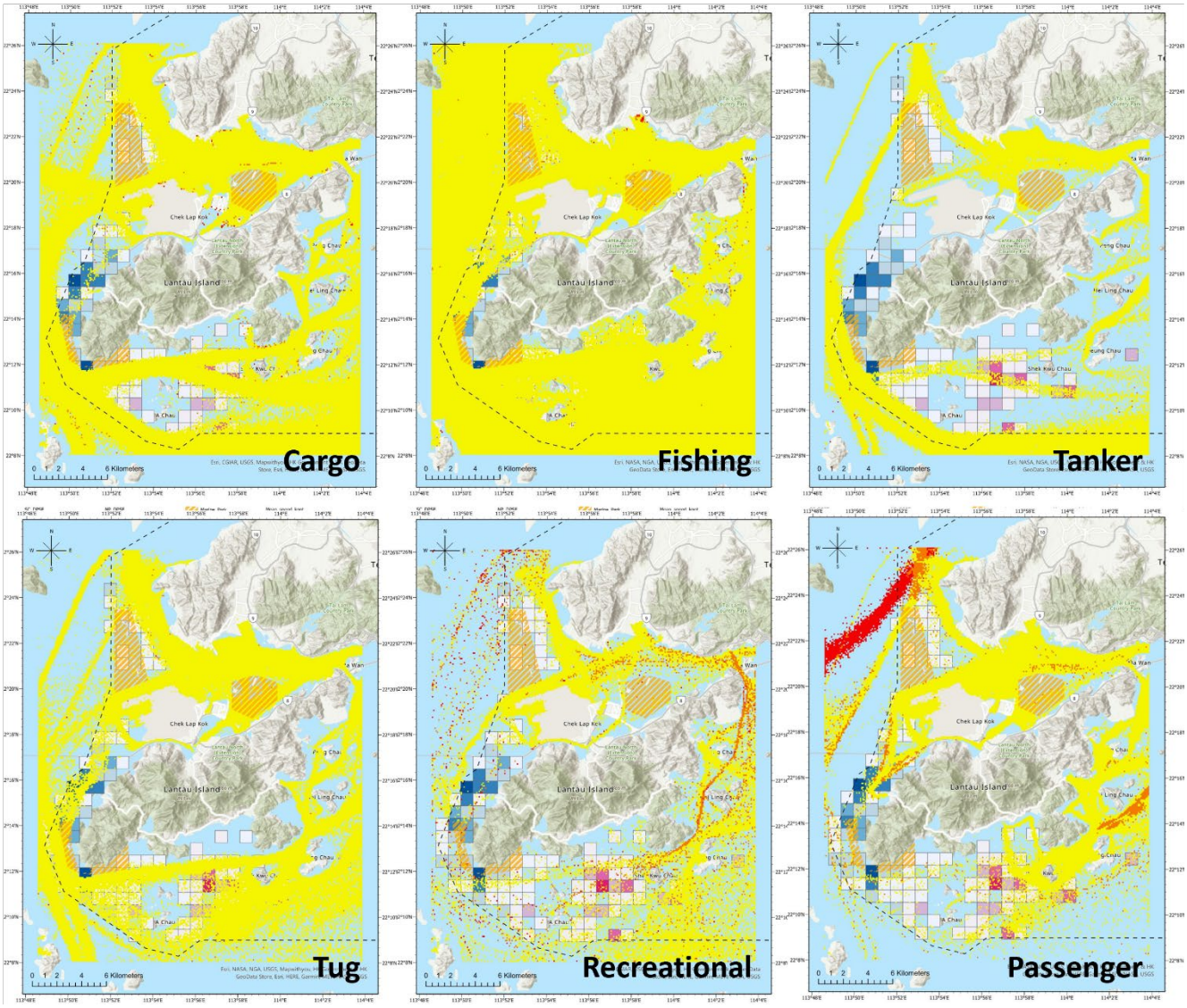


Fig. 7. Maps of Lantau waters showing cetacean-vessel encounter 'hot spots' overlaid with marine vessel transit density in 2021 as categorized by vessel type. Vessel speed is shown in yellow (1–7 knots), orange (7–14 knots), or red (>14 knots, the safety threshold).

With the standardisation of cetacean-vessel encounter risk analysis, prospective data from 2022 onward, are anticipated to be acquired for further investigation. Theodolite and Passive Acoustic Monitoring data will also be included.

E. 2022 International Cetacean Symposium

Since 2019, with the support from MEEF, the project team had organized annual symposia on cetaceans, namely "International Cetacean Symposium". In 2022, the project team had organized an academic symposium on 17 June with the theme of "Resilience", which was a follow up to its predecessor "Interaction" in 2021. A total of 13 scientific sessions presented by 14 international cetacean experts were held in online mode via Zoom (Fig. 8). Invited speakers shared their experiences and insights with the community on a range of topics regarding human impacts and diseases on cetaceans and their behavioural responses. In total the symposium attracted over 230 participants worldwide.

Fig. 8. Group photo of the invited speakers for the 2022 International Cetacean Symposium on 17 June 2022.

F. Stakeholder forum

The outbreak of the Covid-19 has caused major disruptions to the global maritime industry, which cross-boundary ferry vessel had been hafted for 2.5 years. This provided a unique opportunity for the project team to study the dynamic of vessel traffic pre- and during Covid-19 era. For an integrated, scientific-based investigation on marine vessel-Hong Kong cetacean interaction, it is crucial to bring together as many of the stakeholders that influence and effect the habitat and cetaceans as possible. As a wrap-up of the 3-year project, a stakeholder forum was organized on 21 June 2022, which gathered 38 local stakeholders, including researchers (academics from local universities, representatives from cetacean research institute, environmental consultancy and Ocean Park Hong Kong), policy groups (lawyer, representatives from AFCD and WWF Hong Kong), economics and stakeholders (representatives from the Hong Kong International Airport, ferry, dolphin watching and ecotourism companies) from different organizations (Fig. 9). After the presentations by the project PI, AFCD and WWF Hong Kong on the current status of local cetaceans and marine traffic, different parties engaged in sharing and discussion on the obstacles and strategic solutions to mitigate cetacean-vessel interactions (Fig. 10). Potential collaborations among stakeholders from different sectors were proposed, and a mitigation plan summarizing the discussion is prepared to be published in the form of journal manuscript in late 2023.

Fig. 9. Group photo of the stakeholder forum held in the Concord Room of Renaissance Harbour View Hotel Hong Kong on 21 June 2022.

Fig. 10. Discussion on the obstacles and strategic solutions to mitigate cetacean-vessel interactions among different parties during the stakeholder forum on 21 June 2022.

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;	√	100
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;	√	100
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	√	100
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;	√	100
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.	√	100

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				
2022				Brian C.W. Kot*, Heysen H.N. Ho, Edgar K.C. Leung, Tabris Y.T. Chung, Henry C.L. Tsui	Characterisation of *Crassicauda fuelleborni* nematode infection in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) using postmortem computed tomography. International Journal for Parasitology: Parasites and Wildlife 2022:18:68-75.	Yes	Yes (Appendix 2)
2022				Brian C.W. Kot*, Heysen H.N. Ho, Paolo R. Martelli*, Sarah M. Churgin, Nimal Fernando, Foo Khong Lee, Henry C.L. Tsui, Tabris Y.T. Chung	An Indo-Pacific humpback dolphin (*Sousa chinensis*) severely injured by vessel collision: live rescue at sea, clinical care, and postmortem examination using a virtopsy-integrated approach. BMC Veterinary Research 2022:18:417.	Yes	Yes (Appendix 3)
		√		Brian C.W. Kot*, Jessie W.Y. Yeong, Aurora S.Y. Kwan, Gabrielle Y.H. Ho, Heysen H.N. Ho, Henry C.L. Tsui, Tabris Y.T. Chung	Illustrated cross-sectional computed tomography anatomy of the abdomen in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*). Frontiers in Marine Sciences	Yes	Yes (Appendix 4)
			√	Brian C.W. Kot*, Tabris Y.T. Chung, Henry C.L. Tsui, Heysen H.N. Ho	Measurement of lumbar vertebral bone mineral density in Indo-Pacific finless porpoises using quantitative computed tomography. Animals	No	Yes
			√	Brian C.W. Kot*, Tabris Y.T. Chung, Henry C.L. Tsui, Heysen H.N. Ho	Characterisation and prevalence of lungworm infection in stranded Indo-Pacific finless porpoises using virtopsy-led postmortem investigation. Animals	No	Yes
			√	Brian C.W. Kot*, Tabris Y.T. Chung, Henry C.L. Tsui, Heysen H.N. Ho	Three-dimensional forensic scene investigation of stranded cetaceans. Animals	No	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)*
Jan / 2022 / Hong Kong	Contribution of virtopsy to aquatic animal conservation: small steps and giant leaps	2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems	Yes (Appendix 5)	Yes
Jan / 2022 / Hong Kong	Helminth in the peribullar sinus of Indo-Pacific finless porpoise: morphological and molecular characterization	2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems	Yes (Appendix 6)	Yes
Jan / 2022 / Hong Kong	Pattern of flipper bone ossification for cetacean bone age estimation: a virtopsy approach	2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems	Yes (Appendix 7)	Yes
May / 2022/ Virtual	Bycaught or not: Postmortem computed tomography as a tool to assess peracute underwater entrapment in stranded cetaceans	2022 International Association for Aquatic Animal Medicine Virtual Conference	Yes (Appendix 8)	Yes
Jun / 2022 / Hong Kong	Three-dimensional forensic scene investigation of marine vessel interaction in Indo-Pacific humpbacke dolphins and Indo-Pacific finless porpoises in the Hong Kong waters	2022 International Cetacean Symposium	Yes (Appendix 9)	Yes
Jun / 2022 / Hong Kong	Cetacean-Vessel Interaction 'Hot Spots' in Hong Kong Waters: A Preliminary Study	2022 International Cetacean Symposium	Yes (Appendix 10)	Yes
Jun / 2022 / Hong Kong	Application of three-dimensional surface scanning in cetacean stranding investigation: a step-by-step guide for surface documentation	2022 International Cetacean Symposium	Yes (Appendix 11)	Yes
Aug / 2022 / USA	Spatiotemporal pattern of vessel-cetacean collision risk in Hong Kong waters before and during the COVID-19 pandemic	24th Biennial Conference on the Biology of Marine Mammals	Yes (Appendix 12)	Yes
Aug / 2022 / USA	Morphological revision and molecular characterization of helminths in the respiratory tract of the Indo-Pacific finless porpoises in Hong Kong waters	24th Biennial Conference on the Biology of Marine Mammals	Yes (Appendix 13)	Yes
Aug / 2022 / USA	Measurement of lumbar vertebral bone mineral density in Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) using quantitative computed tomography	24th Biennial Conference on the Biology of Marine Mammals	Yes (Appendix 14)	Yes
Aug / 2022 / USA	Computed tomography anatomy of the abdomen of the Indo-Pacific finless porpoises (*Neophocaena phocaenoides*)	24th Biennial Conference on the Biology of Marine Mammals	Yes (Appendix 15)	Yes

14. Other impact

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

N/A

Part E: Summary and Way Forward

Throughout 12 months, from 1 July 2021 to 30 June 2022, 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 ongoing progressively and its objectives have been 100% accomplished. Various research tasks (spatiotemporal analysis on vessel-cetacean interaction, virtopsy and postmortem investigation on stranded cetaceans, 3DSS on stranded cetaceans and marine vessels) related to the 5 objectives of the project were conducted within the 12-month timeframe, with deliverables reported in various means. Two manuscripts regarding the postmortem investigation of local cetaceans using virtopsy were published on peer-reviewed journals. Eleven conference proceedings were also published in 4 international conferences. Findings of the project had also been shared to the public via different media and outreach activities (7 workshops, 1 academic symposium and 1 stakeholder forum).

The first important step in cetacean-vessel interaction risk analysis is the identification of high risk areas, where high number of animals and high number of vessels converge. The spatiotemporal analysis of cetacean distribution and marine vessel traffic from 2019 to 2021 was completed. Cetacean sighting datasets from different sources were consolidated and normalized for the first time in the region, which were overlaid with marine vessel traffic datasets to reveal the interaction 'hot spots'. Vessel-related factors, including vessel types and speed, were also included in the analysis to provide a more realistic evaluation on the risk of interaction. With the standardisation of cetacean-vessel encounter risk analysis, prospective data from 2022 onward, are anticipated to be acquired for further investigation, in which theodolite and Passive Acoustic Monitoring data will also be included. In the future, animal-related factors like surfacing patterns, hearing capabilities and behavioural responses to vessels may be addressed via observational studies, which is lacking for the local resident cetaceans.

Forensic scene investigation on stranded cetaceans and marine vessels using virtopsy and 3DSS will be continued as the established postmortem investigation protocols from the captioned study has been successfully incorporated into the Hong Kong cetacean stranding investigation programme that co-run by AFCD, OPCFHK and our team (Aquatic Animal Virtopsy Lab) in CityU. Routine virtopsy and 3DSS procedures on stranded cetacean carcasses provide 3-D models of the injury victims of vessel-cetacean interaction, while the standardized protocol for 3DSS on marine vessels established in this project provide 3-D models of the suspected injury-inflicting instruments. Despite logistic difficulties and social distancing restrictions due to the COVID-19 pandemic, the project team will continue to acquire data from more vessel types for a more comprehensive investigation. The results will be combined for matching analysis to simulate vessel-cetacean interactions in a 3-D forensic manner, which will provide scientific evidence for better mitigation strategies to reducing injuries to cetaceans, vessel crews, and the vessels.

Overall, the project has taken the spatiotemporal distribution of vessel traffic and local resident cetaceans, the vessel types and speed that pose interaction risk, the interaction 'hot spots', the 3-D geometry of stranded cetacean carcasses and suspected injury-inflicting instruments, and the postmortem findings of stranded cetaceans from virtopsy and conventional necropsy altogether, to investigate the topic of vessel-cetacean interaction in Hong Kong waters. While the cetacean-vessel encounter risk analysis in this project was conducted in a focal "hot-spot" zone, i.e. Lantau waters, cost for acquiring the AIS data and time for data analyses were optimized. Such standardisation of methodology provides a definite round for the cetacean-vessel encounter risk analysis to conduct with the extension of area of concern to a wide scope, i.e. the entire Hong Kong waters, as well as the adjacent waters including the Guangdong–Hong Kong–Macao Greater Bay Area, to provide an overview of the threat – vessel interaction on the largest known population (> 2500 individuals) of Indo-Pacific humpback dolphins in the Pearl River Estuary.

Data from postmortem investigation on stranded cetaceans has been archived and evaluated on the web-based "Cetacean Postmortem Multimedia Analysis Platform" database. By identifying the 'hot spots' and vessel types of high interaction risk and simulating the interaction in a 3-D forensic manner, mitigation management plans including maritime routing, establishment of marine protected area and speed control zones have been discussed among stakeholders from different sectors, and will be suggested to governmental agencies for injury prevention and effective conservation of the vulnerable local resident cetaceans 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:-

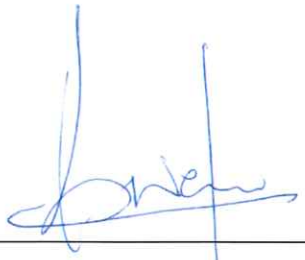
1. *do not deal with, and are not in any way associated with, any country or organisation or activity which is or may potentially be relevant to, or targeted by, sanctions administered by the United Nations Security Council, the European Union, Her Majesty's Treasury-United Kingdom, the United States 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.*

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."

Principal Investigator

Signature : 

Name of Project Leader : Brian Chin Wing Kot

Date : 4 July 2022

Official Chop : 

Appendix 1: List of project assets bought

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

Appendix 2. Peer-reviewed journal publication

International Journal for Parasitology: Parasites and Wildlife 18 (2022) 68–75

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Characterisation of *Crassicauda fuelleborni* nematode infection in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) using postmortem computed tomography

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ABSTRACT

Nematodes of the genus *Crassicauda* are parasites that infect various body tissues of cetaceans, including the mammary glands which can influence the reproductive output and hence threaten the survival of endangered cetacean populations. In this study, postmortem computed tomography (PMCT) was used to characterise lesions related to *Crassicauda fuelleborni* infections in stranded Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) from Hong Kong waters. Using PMCT and subsequently verified by conventional necropsy, *Crassicauda*-related lesions were found in 52% of finless porpoises examined (n = 13/25), including both males and females. These parasitic lesions were mostly located in the ventral abdominal muscles in both sexes and situated in proximity to the mammary glands in females. *C. fuelleborni* infections were also found in the male reproductive organs, which to our knowledge have not been reported in this cetacean species previously. PMCT characteristics of the lesions were also correlated with the gross appearance observed at necropsy and the chronicity of the parasitic infections. In conclusion, this study established the use of virtopsy, particularly PMCT, to characterise *C. fuelleborni* infections in stranded finless porpoises for the first time, which is non-invasive and can be used prior to conventional necropsy to aid disease diagnosis and targeted sampling. This technique can be extended to other species of cetaceans and parasites, as well as being used in the retrospective analysis of past PMCT scans to deepen our understanding of the prevalence, health impacts, and ecological implications of parasitic infection in cetaceans.

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 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 units in this study. Special thanks to Dr Akira Shiozaki and Dr Priscilla Leung for their assistance on the parasitological analysis in this study. This project is financially supported by the Marine Conservation Enhancement Fund (grant no.: MCEF20007), the CityU Strategic Research Grant (grant no.: CityU 11104721), and the Marine Ecology Enhancement Fund (grant number: MEEF2019010, MEEF2019010A, MEEF2019010B), 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; and views of HKLTL, CAPCO and HK Electric, and the Marine Conservation Enhancement Fund.

Appendix 3. Peer-reviewed journal publication

Kot et al. BMC Veterinary Research (2022) 18:417
https://doi.org/10.1186/s12917-022-03511-1

BMC Veterinary Research

CASE REPORT Open Access

An Indo-Pacific humpback dolphin (*Sousa chinensis*) severely injured by vessel collision: live rescue at sea, clinical care, and postmortem examination using a virtopsy-integrated approach

Brian Chin Wing Kot[1,2*†], Heysen Hei Nam Ho[1†], Paolo Martelli[3*], Sarah M. Churgin[3], Nimal Fernando[4], Foo Khong Lee[3], Henry Chun Lok Tsui[2] and Tabris Yik To Chung[2]

Abstract

Background: Vessel collision induces blunt and sharp force traumas to aquatic animals and is a leading anthropogenic impact affecting cetaceans worldwide. Vessel collision is an important threat affecting vulnerable coastal cetaceans such as the Indo-Pacific humpback dolphins (*Sousa chinensis*) which reside in coastal waters of Hong Kong amongst heavy marine traffic.

Case presentation: A severely injured subadult *S. chinensis* was sighted in the waters off southwestern Hong Kong with four gaping incision wounds on its dorsum. It was in poor body condition and seemed unable to use the fluke effectively. The deepest wound located at the caudal peduncle near the base of the fluke and exposed the underlying fractured caudal vertebrae. The dolphin was monitored in the field over three weeks and eventually captured for medical intervention as veterinary assessment indicated progressive and life-threatening deterioration. During rehabilitation, the dolphin demonstrated initial signs of improvement over the first 36 hours as supported by diagnostic tests but then deteriorated rapidly. It was humanely euthanised after three days of rehabilitation. Postmortem investigation was carried out using virtopsy (postmortem computed tomography and magnetic resonance imaging) and conventional necropsy, with special attention to the traumatic musculoskeletal injuries caused by vessel collision and also revealed acute gastrointestinal compromise and respiratory disease that further hampered the rehabilitation.

Conclusion: In cetaceans, the prognosis for recovery from injuries caused by vessel collision depends on the extent, location, and gravity of the injuries (i.e., superficial, deep, penetrating, blunt vs. sharp, fresh vs. septic), as well as

†Brian Chin Wing Kot and Heysen Hei Nam Ho contributed equally to this work.

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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 the Hong Kong cetacean stranding response programme. Sincere appreciation is also extended to the veterinarians, staff, and volunteers from the Ocean Park Conservation Foundation Hong Kong, the Marine Mammal Department of Ocean Park Hong Kong, as well as the Aquatic Animal Virtopsy Lab, City University of Hong Kong. Special gratitude is owed to technicians in the Hong Kong Veterinary Imaging Centre for operating the CT and MRI units in this study. Any opinions, findings, conclusions, or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee, and views of the Research Grants Council of the Hong Kong Special Administrative Region.

Funding

This project is financially supported by the Marine Ecology Enhancement Fund (grant numbers: MEEF2019010, MEEF2019010A, MEEF2019010B) of the Marine Ecology & Fisheries Enhancement Funds Trustee Limited, the Research Grants Council of the Hong Kong Special Administrative Region, China (grant number: CityU 11104720), and the CityU Strategic Research Grant (grant number: CityU 11102619). All of the fundings were awarded to BCWK (project leader and principal investigator of the funded projects).

Appendix 4. Peer-reviewed journal publication under review

Illustrated cross-sectional computed tomography anatomy of the abdomen in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*)

Brian Chin Wing Kot[1,2*†], Jessie Wei Yeng Yeong[3†], Aurora Shuk Yee Kwan[1†], Gabrielle Yick Hey Ho[3†], Heysen Hei Nam Ho[1], Henry Chun Lok Tsui[2], Tabris Yik To Chung[2]
[1]Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong
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†These authors have contributed equally to this work.

Abstract
This study describes the cross-sectional imaging anatomy of the abdomen of the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) using postmortem computed tomography (PMCT). PMCT scans of finless porpoises stranded in Hong Kong waters were reviewed, of which two freshly dead cases, one male and one female, were selected for illustration. In addition, a contrasted PMCT scan was performed on the female subject as a trial for a postmortem contrast study in cetaceans, also known as PMCT-angiography. A total of 18 axial PMCT images were acquired at selected vertebral levels in the abdomen and supplemented with a series of corresponding labeled anatomical diagrams. Using different image rendering techniques, most osseous and soft tissue structures in the finless porpoise abdomen were successfully depicted on PMCT and annotated, including the male and female reproductive organs in the caudal abdominal region, for the first time in cetaceans. The application of contrast medium in PMCT created artificial radiodensity differences which improved the ability to visualize and differentiate soft organs and vasculature. The merits and limitations of CT compared to other imaging modalities, as well as the future directions of PMCT in stranding investigation, were discussed. The findings from this study significantly enhance the applications of CT in cetaceans by assisting researchers and veterinarians in the interpretation of cetacean abdominal CT for morphological and pathological assessment during clinical or postmortem examination.

Fundings
This project is financially supported by the Marine Ecology Enhancement Fund (grant numbers: MEEF2019010, MEEF2019010A, MEEF2019010B), the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. All the funding was awarded to BCWK (project leader and principal investigator of the funded projects).

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 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 units in this study. Any opinions, findings, conclusions, or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee; and the views of the Marine Conservation Enhancement Fund.

Appendix 5. Abstract of conference proceedings

Oral presentation at the 2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems in January 2022

Contribution of virtopsy to aquatic animal conservation: small steps and giant leaps

Kot B.C.W., Tsui H.C.L., Chung T.Y.T., Cheng A.C.S., Ho G.Y.H., Ho H.H.N., Kwan A.S.Y., Leung E.K.C., Robles-Malagamba M.J., Wong H.C.H., Yeong J.W.Y.

Abstract

"Virtopsy", also known as virtual necropsy, is the frontier in human and veterinary postmortem investigation, which utilizes imaging technologies such as computed tomography, magnetic resonance imaging and 3-dimensional surface scanning to document and assess the carcasses prior to conventional necropsy. Since 2014, routine implementation of virtopsy was pioneered in Hong Kong, which effectively supplement conventional necropsy by precisely locating diverse pathological lesions for targeted sampling, as well as provided additional insights into biological health and profiles of aquatic animals. The radiological appearances of lesions, fractures and tissue destruction on PMCT were suggested to correlate with the progress, chronicity or severity of the pathologies and trauma. Such information could facilitate critical review on retrospective virtopsy data on stranded aquatic animals worldwide. The prevalence and level of infections of related diseases, as well as the serious injuries and deaths caused by anthropogenic impacts on the animals, could be determined consequently. Knowledge on biological health and profiles in aquatic animals will educate the society on the whole spectrum of threats the animals are facing, particularly as these threats are likely to interact and are cumulative. From an epidemiological perspective, evaluation of the pathologies and trauma is an important part of assessing the health of free-ranging aquatic animals, which provides important information to weigh the impact of various threats in order to evaluate the conservation status of aquatic animals. Virtopsy findings will enrich the aquatic animal postmortem multimedia analysis platform regarding impact of parasites on the natural mortality and population trends for research, education and conservation policy making.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Conservation Enhancement Fund (grant no.: MCEF20007), the CityU Strategic Research Grant (grant no.: CityU 11104721), the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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; and views of HKLTL, CAPCO and HK Electric, and the Marine Conservation Enhancement Fund.

Appendix 6. Abstract of conference proceedings

Oral presentation at the 2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems in January 2022

Helminth in the peribullar sinus of Indo-Pacific finless porpoise: morphological and molecular characterization

Edgar KC. Leung, Priscilla TY. Leung, Tabris YT. Chung, Gabrielle YH. Ho, Heysen HN. Ho, Aurora SY. Kwan, Maria Jose Robles Malagamba, Henry CL. Tsui, Jessie WY. Yeong, Brian CW. Kot

Abstract
The prevalence of cranial sinus helminths is high in Indo-Pacific finless porpoises *Neophocaena phocaenoides* stranded in Hong Kong waters, but information on the helminth species and their pathogenicity is scarce. In heavy infections, these helminths can cause cranial osseous lesions, which may lead to stranding of the cetaceans. Conventional morphological assessment of helminth is often hindered by the loss of the species-specific characters due to advanced decomposition. In this study, we present species identification of cranial sinuses helminths of 33 *Neophocaena phocaenoides* stranded in Hong Kong waters. Helminths were found in 19 porpoises (58%) and examined with morphological and molecular approaches. Three nematode species were morphologically identified from intact specimens: *Pharurus sunameri*, *Pharurus asiaeorientalis* and *Stenurus nanjingensis*. Nematode fragments that were unable to be identified morphologically were successfully identified with the molecular approach. Three major clusters were revealed, which were in accordance with morphological identification. Among the 19 infected porpoises, 3 cases (16%) showed infection of all 3 nematode species, 9 cases (47%) had co-infections of 2 species, while 7 cases (37%) were singly-infected. *COI* and *ITS-2* sequences of nematodes were also evaluated on their phylogenetic relationships within the genera *Pharurus* and *Stenurus*. This study recorded *S. nanjinensis* and *P. asiaeorientalis* infection for the time in Hong Kong waters, not only enriched the genetic data of endoparasites in cetaceans, but also facilitated species identification in mixed infections, especially in advanced decomposed carcasses.

Acknowledgement
The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Conservation Enhancement Fund (grant no.: MCEF20007), the CityU Strategic Research Grant (grant no.: CityU 11104721), the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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; and views of HKLTL, CAPCO and HK Electric, and the Marine Conservation Enhancement Fund.

Appendix 7. Abstract of conference proceedings

Oral presentation at the 2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems in January 2022

Pattern of flipper bone ossification for cetacean bone age estimation: a virtopsy approach

Aurora SY. Kwan, Tabris YT. Chung, Ashton PH. Ling, Henry CL. Tsui, Brian CW. Kot

Abstract
Age estimation of cetaceans is an important element for the interpretation of their biological data, pathological findings, and population dynamics. Conventional method using growth layer groups in teeth is however invasive and labour intensive. With the increasing popularity and availability of diagnostic imaging modalities in zoological and cetacean stranding response settings, the pattern of age-dependent skeletal ossification can be effectively assessed with computed tomography during routine clinical or postmortem investigation. In this study, the stages of epiphyseal ossification at the humerus, radius and ulnas on flippers of 60 Info-Pacific finless porpoises stranded in Hong Kong waters between 2014 and 2020 were assessed, described and scored to correlate with body length of the animals, which is another validated age-dependent morphometrics. Sexual dimorphism, with males presenting higher ossification scores than females, was observed in adolescent animals. This novel radiological assessment is objective, non-invasive and fast, which can be applied on both live and deceased cetaceans. With additional knowledge of their developmental biology, the health and pathological conditions of free-ranging cetaceans can be better understood.

Acknowledgement
The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 8. Abstract of conference proceedings

Oral presentation at the 2022 International Association for Aquatic Animal Medicine Virtual Conference in May 2022

Bycaught or not: Postmortem computed tomography as a tool to assess peracute underwater entrapment in stranded cetaceans

Heysen HN. Ho, Maria Jose Robles-Malagamba, Henry CL. Tsui, Tabris YT. Chung, Brian CW. Kot

Abstract

Fishery bycatch is one of the leading anthropogenic causes of mortality affecting cetaceans around the world. Proper documentation of bycatch incidents is essential, yet often challenging due to the difficulties in differentiating bycatch-related mortality during the postmortem investigation of stranded cetaceans. Peracute underwater entrapment (PUE) refers to the acute and agonal process during which bycaught cetaceans become entangled with fishing gear and eventually die of forced submersion and drowning.[1] To date, the only widely accepted and reliable pathological indication of PUE is the presence of fresh ligature marks or injuries indicating acute entanglement.[1-5] However, this is not always present in bycaught cetaceans, while other pathological features such as pulmonary changes are often non-specific and only provide circumstantial evidence for the diagnosis of bycatch-related mortality in cetaceans.[1-5]

In humans, postmortem computed tomography (PMCT) has been used to study the pathological features of drowning victims.[6] As part of the virtopsy technique, Kot and his team have pioneered the use of PMCT in the postmortem assessment of stranded cetaceans.[7] Since 2017, PMCT has routinely been used to investigate natural and anthropogenic causes of death in stranded cetaceans from Hong Kong and adjacent waters, and have preliminarily been demonstrated to be useful in assessing fishery-related mortality.[8] To advance the application of PMCT, this study aimed to characterise and diagnose PUE or bycatch-related features in stranded cetaceans using PMCT. Similar studies have been conducted with conventional necropsy,[1-5] but to the best of our knowledge have not been attempted with virtopsy.

We retrospectively analysed the PMCT scans of 54 known-bycaught narrow-ridged finless porpoises (*Neophocaena asiaeorientalis*), with reference to a list of PUE-related features that were established based on previous necropsy-based studies.[1-5] The PMCT findings were correlated and verified with the necropsy reports of these finless porpoises. Using relevant case studies and the data originated from these bycaught finless porpoises, we demonstrated that PMCT is effective in the visualisation and documentation of PUE-related features, especially for skeletal fractures and respiratory abnormalities such as froth or fluid deposition in the airways and pulmonary changes, as previously suggested.[9] Therefore, PMCT is a powerful tool that can be used prior to and in conjunction with conventional necropsy during the postmortem investigation of stranded cetaceans, particularly to improve the ability to diagnose PUE and bycatch-related mortality. Further directions of this study include establishing a set of diagnostic criteria[1] that integrate findings from both virtopsy and necropsy to facilitate better assessment of bycatch-related mortality in stranded cetaceans in Hong Kong and worldwide.

Acknowledgement

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 9. Abstract of conference proceedings

Oral presentation at the 2022 International Cetacean Symposium in June 2022

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

Brian Chin Wing Kot, Aris Cheng Cheuk Sing, Tabris Yik To Chung, Henry Chun Lok Tsui

Abstract

With a rapidly expanding economy and population, Hong Kong has been experiencing an inevitable conflict between development and the environment. The constant growing need for space in Hong Kong 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 resident cetacean species, namely the Indo-Pacific humpback dolphins (*Sousa chinensis*) and Indo-Pacific finless porpoises (*Neophocaena phocaenoides*), in Hong Kong waters, both with low abundance estimates and high incidences of stranding. Previous reports indicated vessel encounter as a significant cause of mortalities, with stranded carcasses presenting various traumatic injuries and strike deaths. Blunt and sharp force trauma were indicative of vessel interactions in the busy port with heavy marine traffic. Risk assessment and mitigation on marine vessel interaction for cetaceans residing in Hong Kong waters are crucial to implement effective management plan for injury prevention for vulnerable local resident cetaceans.

The aim of this project is to estimate the risk of cetacean interaction with vessels in Hong Kong waters by analysing information of the resident cetacean distribution, population density and habitat use, spatiotemporal patterns of vessel traffic in their core habitats, and direct evidence of vessel interaction in stranded cetaceans. Cetacean distribution, population density and habitat use data from surveys were collected from vessels, helicopters, theodolite tracking and passive acoustic devices. Retrospective and prospective sighting data had hinted the change in population trend of the cetaceans. Spatiotemporal distribution of marine traffic obtained from automatic ship tracking system were analyzed and overlaid with the cetacean data to obtain cetacean-vessel encounter probability, for insights on the potential impact of anthropogenic disturbances. Specific vessel types and speed that pose collision risk with cetaceans had been revealed, with interaction 'hot spot' for Indo-Pacific humpback dolphins and Indo-Pacific finless porpoises noted outside the Southwest Lantau Marine Park waters and in the Tai A Chau waters respectively.

Direct evidence of vessel interaction was analysed postmortem using virtopsy and conventional necropsy, with wounds of stranded cetaceans and the potential injury-inflicting parts of marine vessels documented by three-dimensional surface scanning for the first time. From 1 January 2017 to 15 June 2022, virtopsy was conducted in 192 out of 225 (85%) cetaceans stranded in Hong Kong waters, including 140 *Neophocaena phocaenoides*, 29 *Sousa chinensis*, and 23 of other species. In these 192 cases, the virtopsy findings of 60 stranded cetaceans (31%) were sufficient to assign the causes of death to be human-related, namely fishery interaction (e.g., evidence of fishing gear entanglement or underwater entrapment) and vessel interaction (e.g., sharp or blunt force trauma evidential of vessel collision), to varying degrees of confidence (confirmed, probable, or suspected). The remaining 132 stranded cetaceans (69%) were associated with various non-human related causes of death such as respiratory diseases, microbial or parasitic infections, and undetermined natural deaths. Such stranding investigation data before and during the COVID-19 pandemic were further compared to illustrate the potential influence of global marine mobility on cetacean mortality, in which virtopsy findings of 25 out of 105 stranded cetaceans (24%) and 4 out of 87 stranded cetaceans (5%) were associated with vessel interaction in 2017-2019 and 2020-2022 respectively. Resulting three-dimensional models will be used for geometric comparison and accident scene reconstructions of the traumatic collision.

This project has achieved useful outcomes to facilitate related government officials from Agriculture, Fisheries and Conservation Department and Hong Kong Marine Department to formulate conservation plans, marine vessel route diversion and speed control, and fellow stakeholders including relevant ferry service, shipping

companies, academics, green groups and cetacean experts to pose precious conservation measures and promote injury prevention for vulnerable local resident cetaceans in Hong Kong waters.

Acknowledgements
The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 10. Abstract of conference proceedings

Oral presentation at the 2022 International Cetacean Symposium in June 2022

Cetacean-Vessel Interaction 'Hot Spots' in Hong Kong Waters: A Preliminary Study

Aris Cheng Cheuk Sing, Brian Chin Wing Kot, Tabris Yik To Chung, Henry Chun Lok Tsui

Abstract

Indo-Pacific humpback dolphins (*Sousa chinensis*) and Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) are the endangered resident cetacean species in Hong Kong waters, both with low abundance estimates and high incidences of stranding. Previous reports indicated vessel encounter as a significant cause of mortalities, with stranded carcasses presenting various traumatic injuries and strike deaths. Blunt and sharp force trauma were indicative of vessel interactions in the busy port with heavy marine traffic.

Marine traffic data from Automatic Identification System (AIS) and cetacean distribution data from line-transect surveys were used to calculate the vessel-cetacean encounter rate from 2019 to 2021 to examine the spatiotemporal patterns of vessel-cetacean collision risk in Hong Kong waters. The vessel density of each classified vessel type with their speeds over ground around the Lantau Island waters were also estimated. From the AIS data, vessel types that the cetaceans were most likely to encounter with were revealed. The preliminary data across years were compared to illustrate the potential influence of global marine mobility on cetacean occurrence. With the consolidation of different data, the encounter rates between vessels and the resident cetacean species, and 'hot spots' were found.

Our results allow strategic planning of future infrastructural developments and marine routing via additional mitigation. Vessel traffic was noted within the existing Brothers Marine Park and the Sha Chau & Lung Kwu Chau Marine Park, which conceivably accounted for the diminished occurrence of humpback dolphins in these protected areas. High encounter risk areas for humpback dolphins and finless porpoises were noted outside the Southwest Lantau Marine Park waters and in the Tai A Chau waters respectively. Expansion of buffer zones and effective speed management are recommended to find a middle ground between urban development and protection of endangered cetaceans at risk. Further studies will be done on refining the collision risk model by including various factors, such as vessel types, speeds and structures, as well as cetacean behaviors, which can complement information provided by postmortem investigations and eye-witness reports. This is part of a multidisciplinary project, focusing on three-dimensional forensic scene investigation of cetacean-vessel interaction, by conducting matching analysis on injury on all cetaceans stranded in Hong Kong waters, which expected useful outcomes would facilitate related government departments and stakeholders to formulate and implement effective management plan for injury prevention for vulnerable local resident cetaceans in Hong Kong waters.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 11. Abstract of conference proceedings

Oral presentation at the 2022 International Cetacean Symposium in June 2022

Application of three-dimensional surface scanning in cetacean stranding investigation: a step-by-step guide for surface documentation

Tabris Yik To Chung, Brian Chin Wing Kot, Henry Chun Lok Tsui, Aurora Shuk Yee Kwan

Abstract

In human and veterinary medicines, radiological modalities like computed tomography (CT) and magnetic resonance imaging (MRI) have been used to document internal features of corpses or carcasses to indicate various pathological and traumatic conditions for postmortem (PM) investigation. Since 2014, "virtopsy" (virtual necropsy), involving PMCT and PMMRI, has been incorporated into the cetacean stranding investigation in Hong Kong. The non-invasive techniques create volumetric image datasets which can be rendered and reconstructed for postmortem diagnosis. Internal trauma, lesions and gas formation can be readily visualized by PMCT while delicate soft tissue anatomy particularly in the nerves system can be precisely depicted by PMMRI. External features, including features for species identification, haemorrhage and chop wounds, were conventionally documented by photography and manual measurements before necropsy. Photographs are limited in 2D nature and may not cover every part of the target, while measurements of morphological features must be performed before the carcass is irreversibly dissected.

In recent years, three-dimensional surface scanning (3DSS) has been applied in both human, veterinary and comparative forensic medicines. The technique produces a digital dataset of the surface conditions of the target, including shape, size and texture, which can be combined with PMCT or PMMRI datasets to visualize the target both internally and externally. The product is 3D in nature, allowing better illustration of forensic features like depth of wounds. Lighting is optimized during the real-time data acquisition in 3DSS, eliminating issues like shady or blurry images in conventional photography. Measurements can also be conducted on calibrated true-to-scale 3D models even after the carcass is dissected, facilitating morphometric studies on retrospective cases, including on features that were not of interest during carcass handling. The 3D models not only provide a more authentic presentation of the carcasses, but also enable matching analysis with injury-inflicting tools like propellers in cases of vessel-associated mortalities.

Since 2018, our team has conducted 3DSS on stranded cetaceans in Hong Kong waters to document their surface conditions to supplement virtopsy and necropsy findings. The additional scanning protocol was incorporated into the stranding response workflow before necropsy. On top of standard data acquisition and post-processing, there are precautionary measures and technical considerations to be taken while working on stranded cetacean carcasses of different status. By June 2022, 69 cetacean carcasses (52 Indo-Pacific finless porpoises, 12 Indo-Pacific humpback dolphins, 5 of other species) have been documented by 3DSS.

In 2020, our team further expand our 3DSS work to marine vessels in Hong Kong and their mechanical parts. By June 2022, underwater structures of over 20 vessels have been documented by 3DSS. The resulting models were used to reconstruction with cetacean carcasses with vessel-associated mortalities. The findings will provide a better understanding of how cetacean-vessel interactions occur, give insights on injury prevention for anthropogenic and natural causes, and facilitate effective management plans to safeguard the vulnerable wildlife populations.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 12. Abstract of conference proceedings

Oral presentation accepted at the 24th Biennial Conference on the Biology of Marine Mammals in August 2022

Spatiotemporal pattern of vessel-cetacean collision risk in Hong Kong waters before and during the COVID-19 pandemic

Brian CW. Kot, Aris CS. Cheng, Tabris Yik To Chung, Henry Chun Lok Tsui

Abstract

Indo-Pacific humpback dolphins (*Sousa chinensis*) and Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) are the endangered resident cetacean species in Hong Kong waters, both with low abundance estimates and high incidences of stranding. Previous reports indicated vessel encounter as a significant cause of mortalities, with stranded carcasses presenting various traumatic injuries and strike deaths. Blunt and sharp force trauma were indicative of vessel interactions in the busy port with heavy maritime traffic. Since the commencement of the COVID-19 pandemic, Governments all around the world have obliged to implement confinement and social distancing measures, which casted an impact on global maritime mobility. We used encounter rate model based on maritime traffic data from automatic identification system (AIS) and cetacean distribution data from line-transect surveys to examine the spatiotemporal patterns of vessel-cetacean collision risk in Hong Kong waters. From the AIS data, vessel types that the cetaceans were most likely to encounter with were revealed. The data before and during the COVID-19 pandemic were compared to illustrate the influence of global maritime mobility on cetacean occurrence. Our results allow for strategic planning of future infrastructural developments and maritime routing via additional mitigation. Vessel traffic was noted within the existing Brothers Marine Park and the Sha Chau & Lung Kwu Chau Marine Park, which conceivably accounted for the diminished occurrence of humpback dolphins in these protected areas. High encounter risk areas for humpback dolphins and finless porpoises were noted outside the Southwest Lantau Marine Park waters and in the Tai A Chau waters respectively. Expansion of buffer zones and effective speed management (e.g. establish and promote voluntary areas to be avoided, ATBAs, where speed reduction below 13 knots is encouraged) are recommended to find a middle ground between urban development and protection of the endangered cetaceans at risk.

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 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 units in this study. This project is financially supported by the CityU Strategic Research Grant (grant no.: CityU 11102619), the Research Grants Council of the Hong Kong Special Administrative Region, China (grant numbers: CityU 11104720), and the Marine Ecology Enhancement Fund (grant number: MEEF2019010, MEEF2019010A, MEEF2019010B), the Marine Ecology & Fisheries Enhancement Funds Trustee Limited. Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Research Grants Council of the Hong Kong Special Administrative Region; and the views of the Marine Ecology Enhancement Fund or the Trustee.

Appendix 13. Abstract of conference proceedings

Oral presentation accepted at the 24th Biennial Conference on the Biology of Marine Mammals in August 2022

Morphological revision and molecular characterization of helminths in the respiratory tract of the Indo-Pacific finless porpoises in Hong Kong waters.

Edgar KC. Leung, Brian CW. Kot, Tabris Yik To Chung, Priscilla TY. Leung

Abstract

Halocercus spp. were reported as parasites predilect in the cetaceans' respiratory tract, however, its severity, distribution and life cycle are scarcely known. Endohelminth were commonly found in the respiratory tract of Indo-Pacific finless porpoise stranded in Hong Kong waters, and were morphologically identified as *Halocercus pingi*. The identification was not completely certified, because the subtropical climate led the decomposition of specimens. In this study, we identified the *Halocercus* species recovered from the respiratory tracts of 29 stranded Indo-Pacific finless porpoise (unweand: n=5, weaned: n=24) in Hong Kong waters from 2020-2021. Helminths were found in six cases (21%) and comprehensively characterized by morphological and molecular methods. Two nematode species were morphologically identified from intact specimens: *Halocercus pingi* and *Halocercus sunameri*. Their morphological features and morphometrics for species specific identification were documented and illustrated. DNA Sequences of COI and ITS-2 genes of the recovered helminths were also achieved and analysed using phylogenetic relationships among the genus Halocercus. Two major clusters were resolved among the analysed COI and ITS-2 sequences, which is in accordance with morphological identification. Decomposed fragments which were unable to be identified morphologically, were successfully identified using molecular analysis. Among these six infected cases, two unweaned calves' tracheae (40%, 2/5) were infected by free *H. pingi*, four weaned animals' lungs (17%, 4/24) showed granulomatous *H. sunameri* infection. This study not only advances the morphology and genetic data on cetaceans' endohelminth, but also provides insights of the influence of host life stages, predilection sites and pathology. In addition, the first record of *H. sunameri* infection was reported in Indo-Pacific finless porpoise by this study.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Conservation Enhancement Fund (grant no.: MCEF20007), the CityU Strategic Research Grant (grant no.: CityU 11104721), the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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; and views of HKLTL, CAPCO and HK Electric, and the Marine Conservation Enhancement Fund.

Appendix 14. Abstract of conference proceedings

Oral presentation accepted at the 24th Biennial Conference on the Biology of Marine Mammals in August 2022

Measurement of lumbar vertebral bone mineral density in Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) using quantitative computed tomography

Heysen HN. Ho, Brian CW. Kot, Tabris Yik To Chung, Henry CL. Tsui

Abstract

Computed tomography (CT) can efficiently and non-invasively retrieve anatomical data, and has been increasing used in the postmortem assessment of marine mammals, especially cetaceans, to understand their biological and pathological profiles during stranding research. Bone mineral density (BMD) is an important parameter that reflect the health condition of an individual cetacean, as BMD plays an essential role in buoyancy and locomotion, as well as acts as indicators for nutritional status or diseases. Various vertebral osteopathies such as spondylitis and spondylosis have been documented in cetaceans. Abnormal BMD is associated with these diseases in humans and may act as a predictor for their occurrence in cetaceans. For wild cetaceans, abnormal BMD is also related to malnutrition and pollutant exposure and may reflect impacts from anthropogenic changes such as prey depletion and environmental pollution.

BMD is conventionally measured using dual-energy x-ray absorptiometry in clinical settings, which has been limitedly applied to cetaceans. Another method of measuring BMD is through the use of quantitative CT (qCT), which offers better characterisation of trabecular and cortical BMD without superimposition, and can be efficiently carried out alongside routine clinical or postmortem CT scans using appropriate set-up. However, reference methodology and value range have not been established in cetaceans. In this study, we developed a novel standardised method of using qCT to measure cortical BMD and trabecular BMD in the lumbar vertebrae of 22 Indo-Pacific finless porpoises (Neophocaena phocaenoides) stranded in Hong Kong waters. Our preliminary findings reported mean cortical BMD of 498.5±125.3 mg Ca-HA/mL and mean trabecular BMD of 284.4±74.3 mg Ca-HA/mL (n = 22). Our findings aid in the development of a standardised method for clinical or postmortem BMD evaluation using qCT, which improves the assessment of biological health and works toward establishing a reference range for the pathological evaluation of abnormal BMD in cetaceans.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 15. Abstract of conference proceedings

Oral presentation accepted at the 24th Biennial Conference on the Biology of Marine Mammals in August 2022

Computed tomography anatomy of the abdomen of the Indo-Pacific finless porpoises (*Neophocaena phocaenoides*)

Jessie WY. Yeong, Brian CW. Kot, Heysen HN. Ho, Gabrielle YH. Ho, Tabris Yik To Chung, Henry CL. Tsui

Abstract

Computed tomography (CT) is a non-invasive diagnostic imaging modality that has been increasingly applied in marine mammal medicine and research, as it provides high-quality and digitally storable data for assessing the anatomy, morphology, and pathology of marine mammals. Aside from clinical purposes, CT is applied in postmortem investigations as part of the virtopsy technique to enhance outcomes of conventional necropsy procedures.

To maximise the efficiency of CT, it is essential to establish proper imaging anatomy references for one to be able to distinguish pathology from normal morphology. In cetaceans, cross-sectional imaging anatomy studies with both CT and magnetic resonance imaging (MRI) have been conducted with the head. On the contrary, there are no published studies addressing the normal cross-sectional imaging anatomy of the cetacean abdomen, although it is an important area with multiple possibilities of pathology and injury. This study aimed to establish a reference for the normal CT cross-sectional anatomy of the cetacean abdomen. PMCT scans of two freshly dead (Code 2) Indo-Pacific finless porpoises, one male and one female, were performed in Hong Kong. Additional contrast study was performed with the female subject for comparisons with the non-contrasted PMCT scans. Using different image rendering techniques, most osseous and soft tissue structures were successfully depicted, including the male and female reproductive systems in the caudal abdominal region. Axial CT images were obtained at the medial plane of selected vertebral levels with optimised soft tissue windows (Subject 1: WW=560 & WL=200; Subject 2: WW=350 & WL=120). All selected transverse images were supplemented with corresponding hand-sketched labelled anatomical diagrams. This was the first study presenting a comprehensive documentation of cross-sectional PMCT imaging of the cetacean abdomen, which provided reference for CT clinical diagnosis and facilitated the integration of PMCT into postmortem investigation on diseases and anthropogenic impacts of these vulnerable species.

Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the continuous support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff, and volunteers from the Aquatic Animal Virtopsy Lab, Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong and CityU Veterinary Medical Centre. This project is financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A and MEEF2019010B), 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.

Appendix 16. Recruitment record

Recruitment record is not disclosed due to confidentiality reasons.

Appendix 17. Attendance record

Attendance record is not disclosed due to confidentiality reasons.

Handbook of 3D forensic scene investigation of marine vessel interaction in stranded cetaceans

Aquatic Animal Virtopsy Lab
City University of Hong Kong

Department of Chemistry
香港城市大學
City University of Hong Kong

Department of
Infectious Diseases and Public Health
香港城市大學
City University of Hong Kong

This project is financially supported by the **Marine Ecology Enhancement Fund**, the **Marine Ecology & Fisheries Enhancement Funds Trustee Limited** (grant numbers: MEEF2017014, MEEF2017014A, MEEF2019010, MEEF2019010A, MEEF2019010B). Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

Cetaceans in our waters

There are 2 **resident cetacean species** in Hong Kong waters

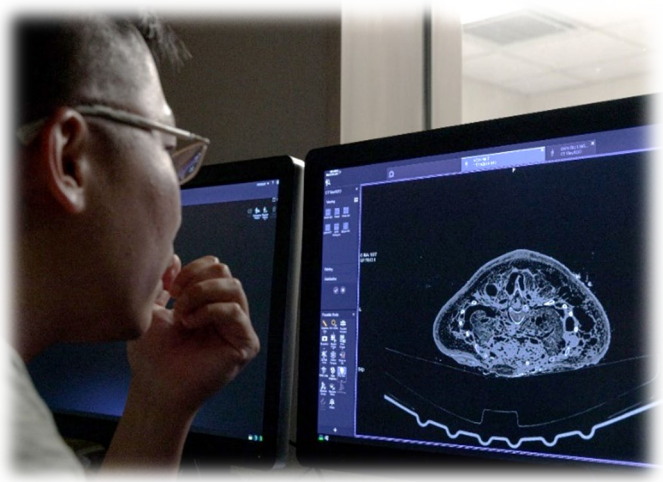
Indo-Pacific **humpback dolphins** in West/Southwest Lantau waters

Indo-Pacific **finless porpoises** in South Lantau / Eastern Hong Kong waters

Like other wildlife, cetaceans are facing various **natural** and **anthropogenic impacts**, including vessel interaction, fishery interaction, habitat degradation, prey depletion, infection and diseases, that result in **morbidity** and **mortality**

Aquatic Animal Virtopsy Lab

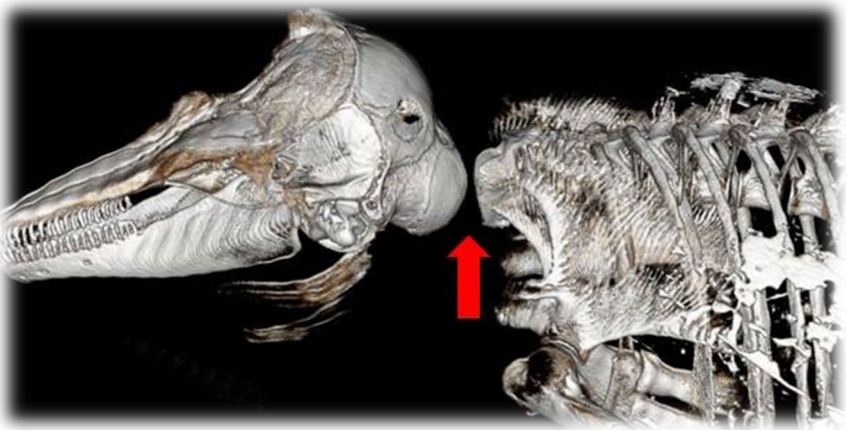
Since 2014, our team has implemented **virtopsy (postmortem CT/MRI)** into the cetacean stranding response program in Hong Kong to supplement conventional necropsy in their **postmortem investigation**



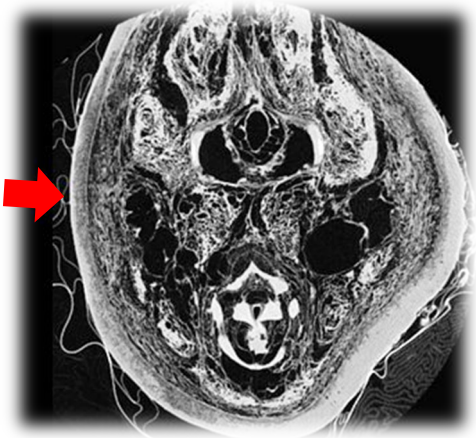
Internal documentation: Computed tomography (CT)

Postmortem CT is a **radiological technique** which can non-invasively visualize various internal conditions in stranded cetaceans, including **human-induced patterned injuries** (e.g. sharp- and blunt-force trauma), to supplement conventional necropsy in the evaluation of **biological health profile** and **cause of death** of these carcasses

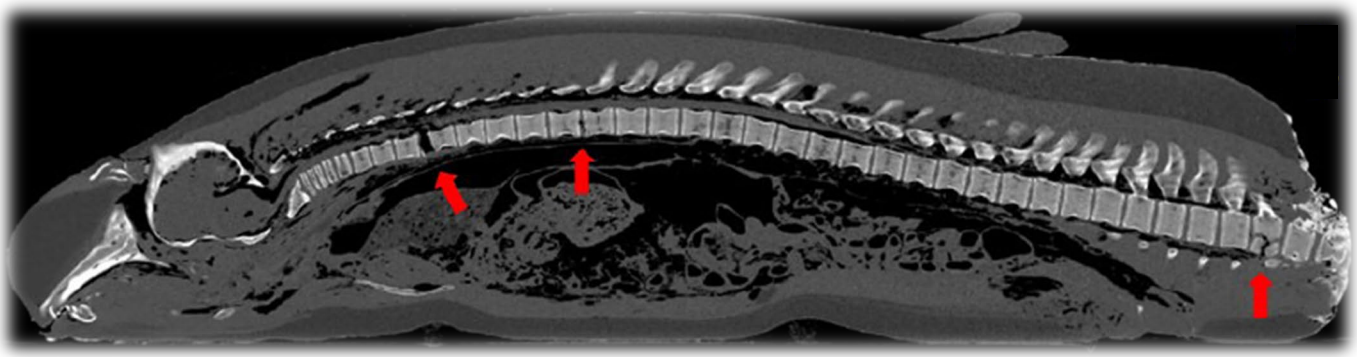
Examples of CT findings associated with blunt-force trauma:



Atlanto-occipital dissociation



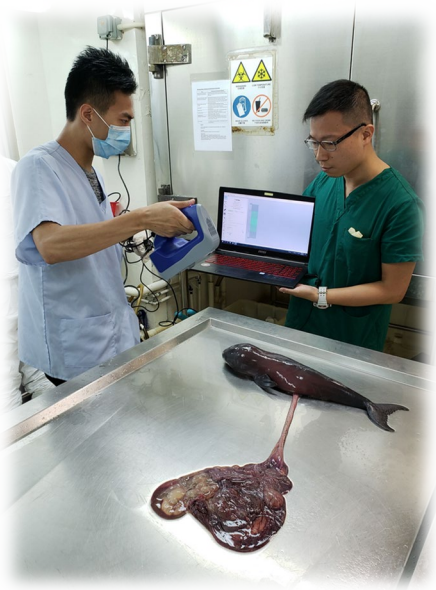
Hematoma

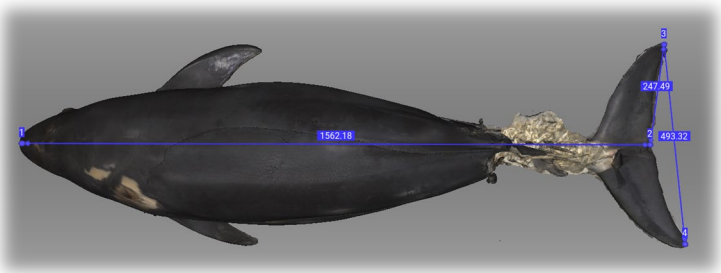


Multiple skeletal fractures

External documentation: 3D surface scanning (3DSS)

3D surface scanning is used in human, veterinarian, and forensic medicines to document **shape**, **size**, **texture** in 3D manner accurately

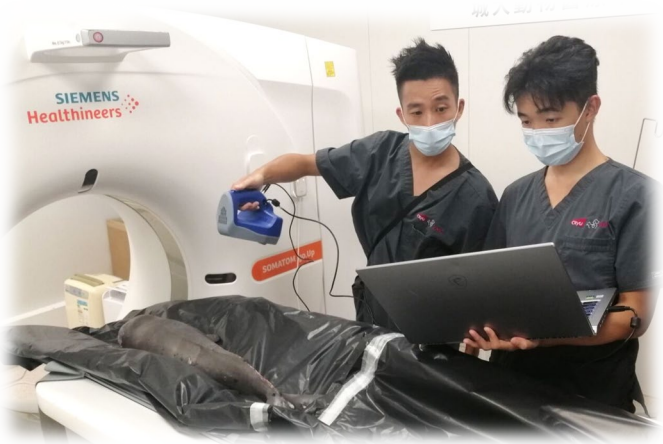




3DSS produces true-to-scale **3D models** for retrospective evaluation, morphometric measurements, and other purposes in virtual manner

3DSS of stranded cetaceans

Step 1 **Cetacean carcass** is positioned properly on CT table for scanning

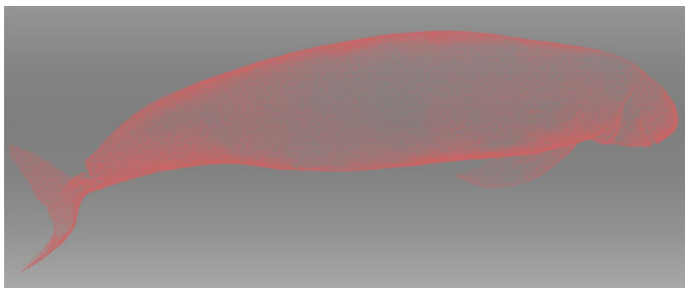


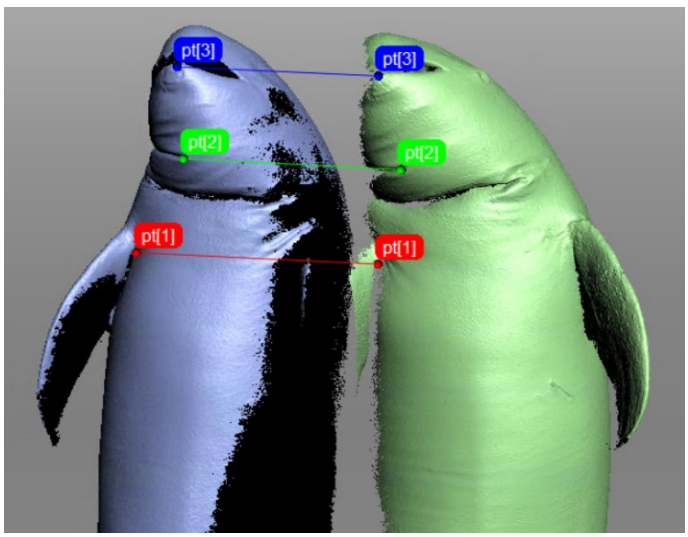
Step 2 **Handheld 3D scanner** is operated 360° around the carcass to capture the surface features

Structured light emitted by the scanner is safe to operator

3DSS of stranded cetaceans

Step 3 After scanning, raw data is processed to generate **3D models**

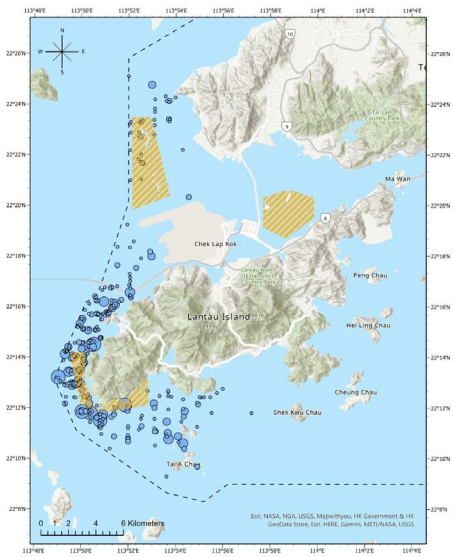




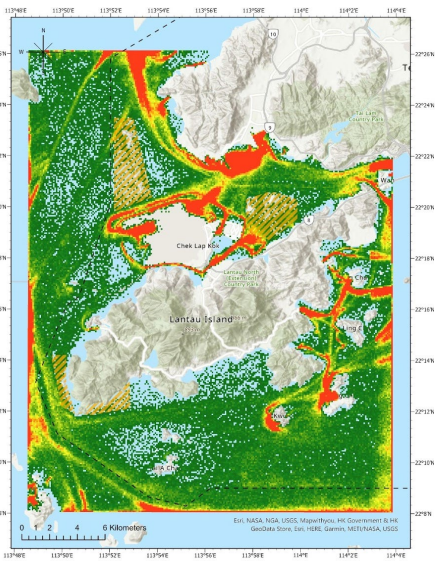
Examples of **cetacean carcasses** documented using 3DSS

Cetacean-vessel interaction

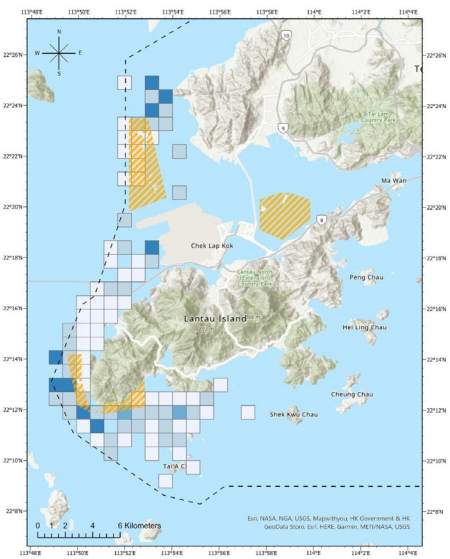
Spatiotemporal analysis using **cetacean distribution data** (from long-term monitoring programmes) and **marine traffic data** (from automatic identification system of vessels) can indicate high risk **interaction hotspots** in core cetacean habitats, **vessel types** and **speed** involved



Dolphin distribution data



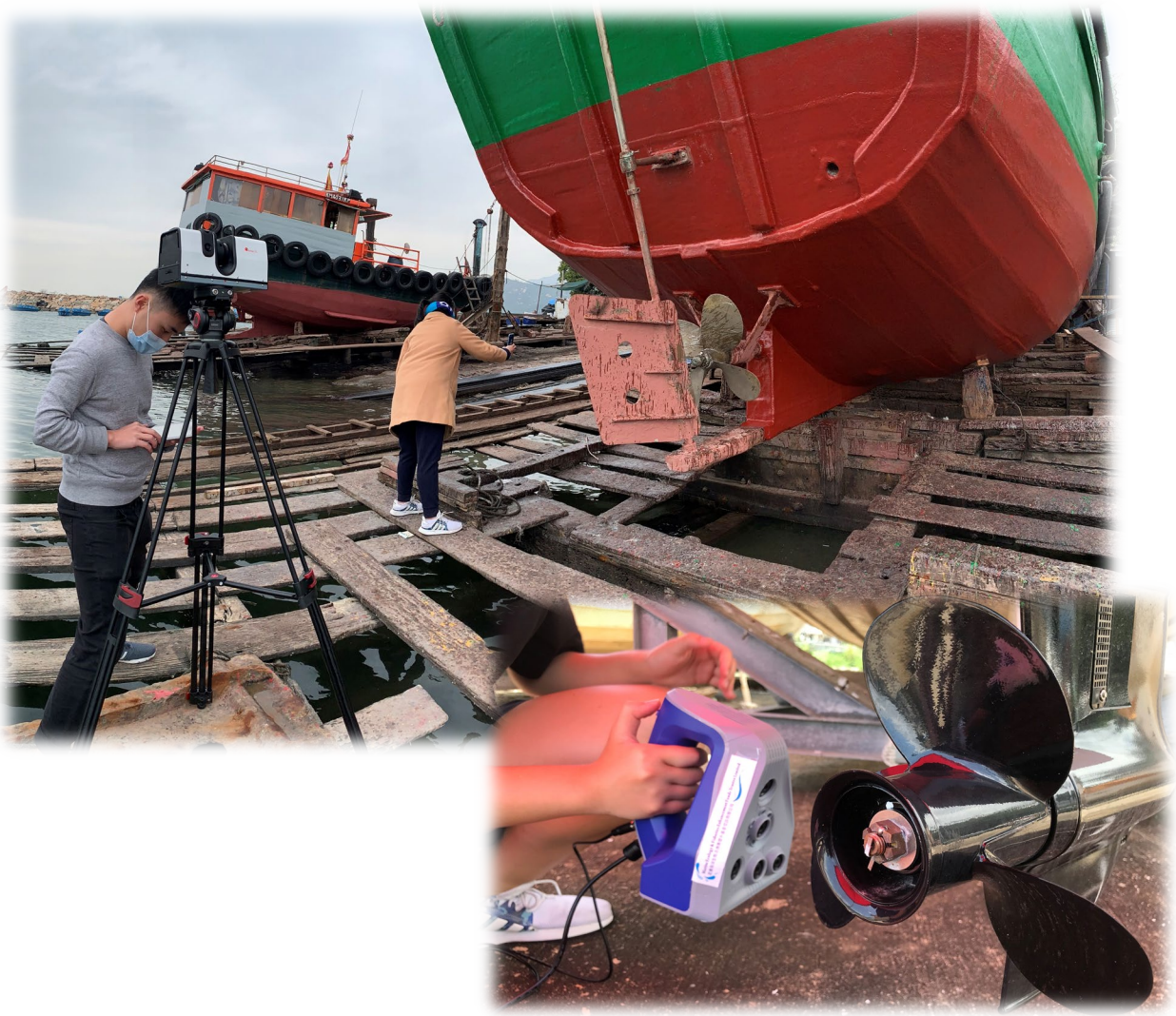
Marine traffic data



Relative dolphin-vessel encounter rate

3DSS of injury inflicting tools

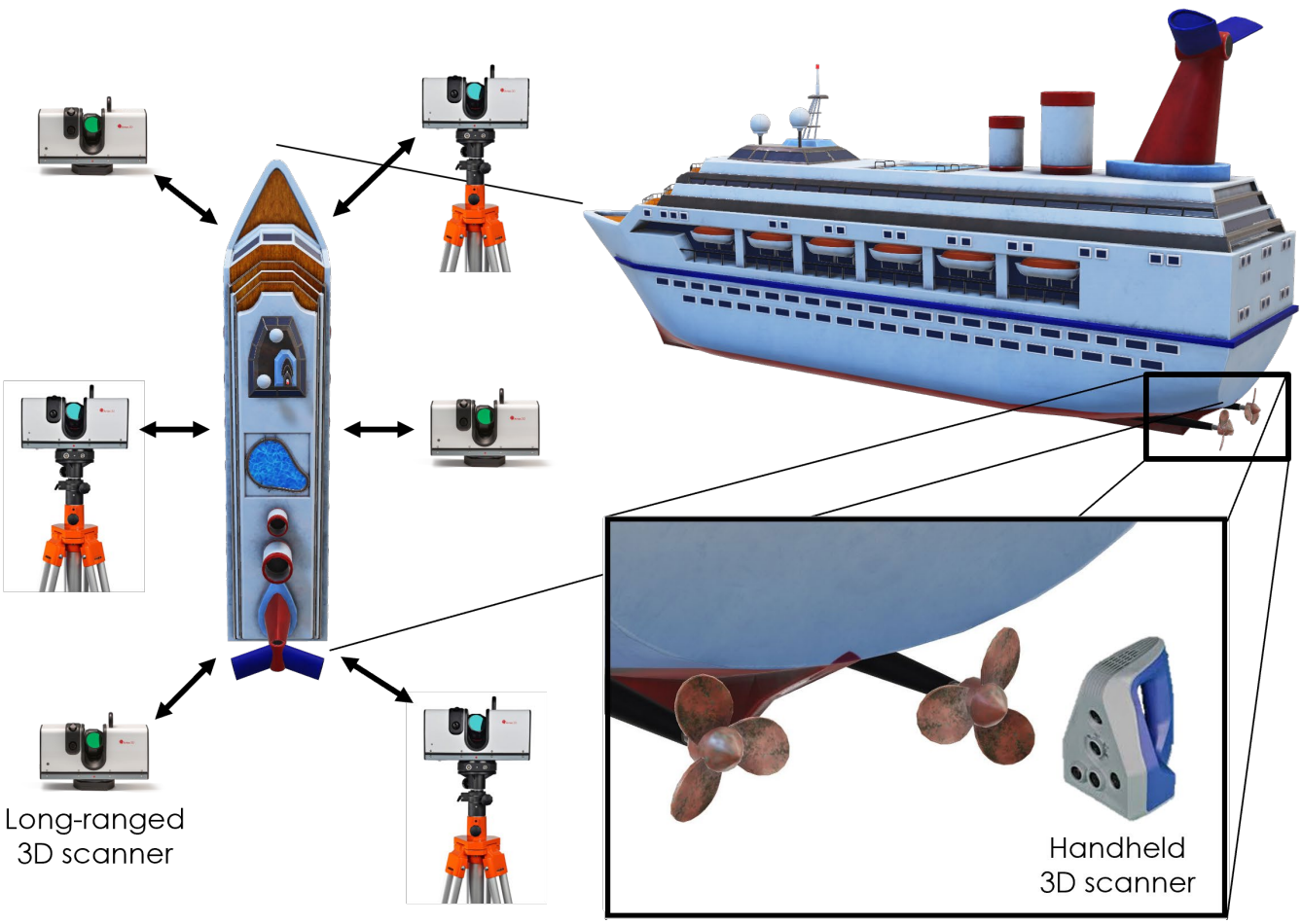
To understand and verify whether **pattern injuries** on cetacean carcasses are induced by vessel interaction, 3DSS is used to produce 3D models of **suspected injury-inflicting tools** (i.e. underwater parts and propellers) of marine vessels identified in the spatiotemporal analysis



3DSS of injury inflicting tools

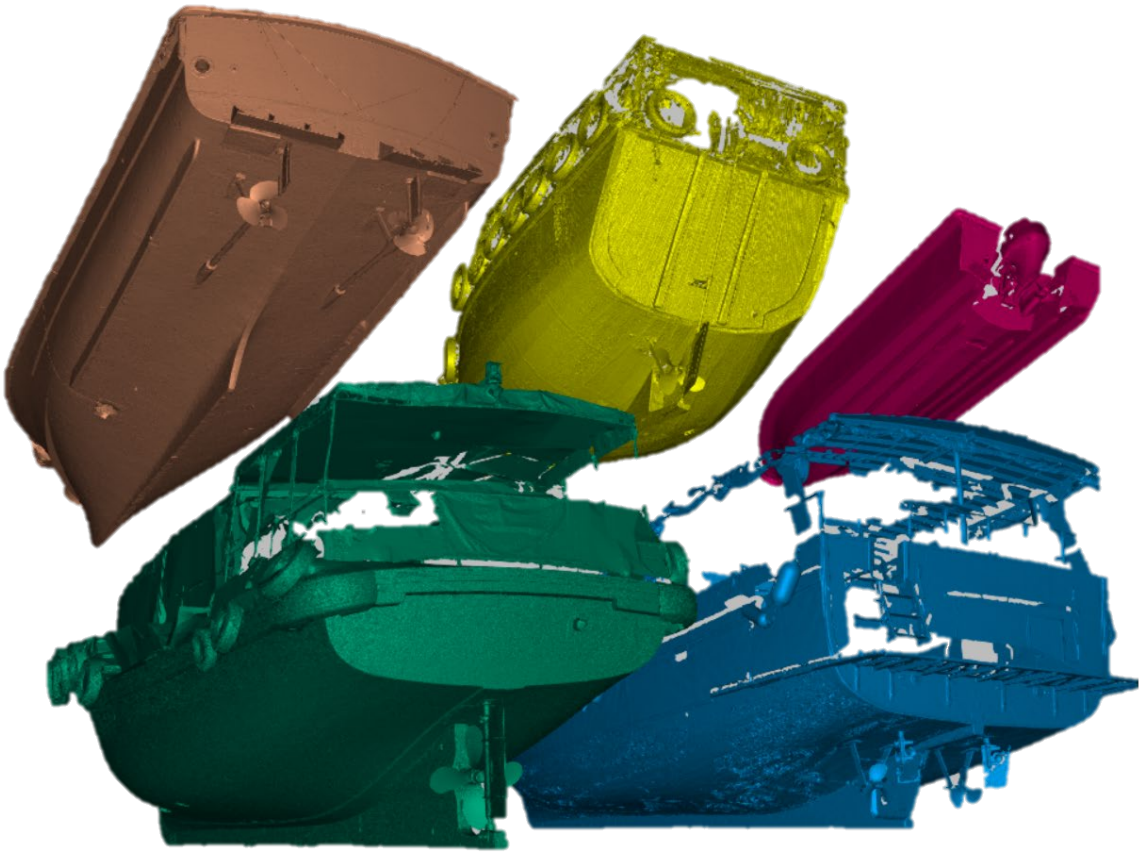
Long-ranged 3D scanner is used to document the entire marine vessel

Handheld 3D scanner is used to document small parts (e.g. propellers)



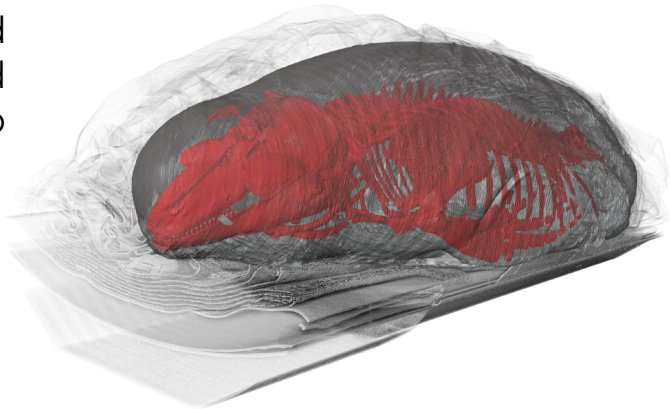
3DSS of injury inflicting tools

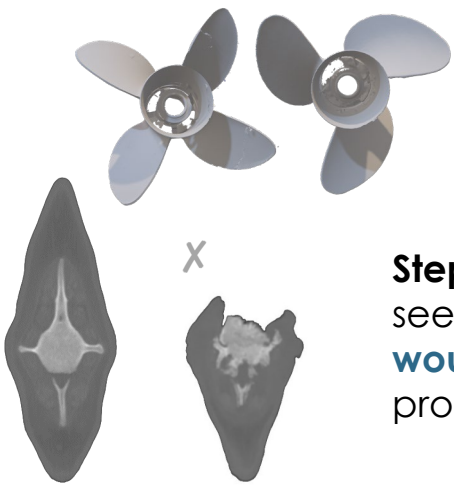
Examples of **marine vessels** and **propellers** documented using 3DSS



Forensic matching analysis

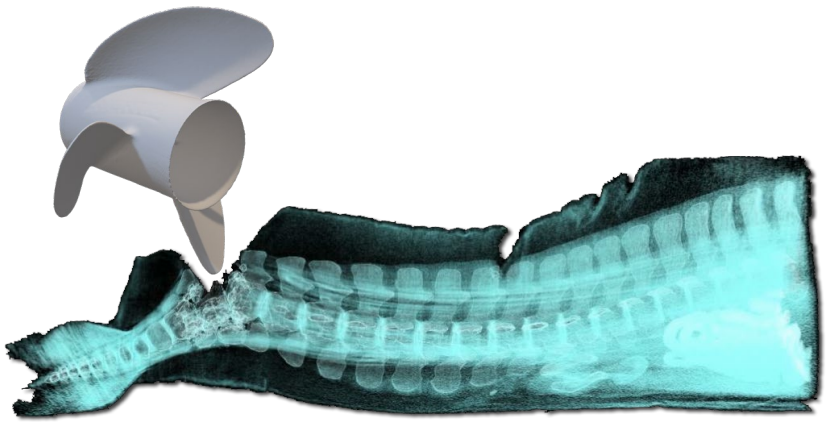
Step 1 Examination of external and internal conditions of stranded cetaceans using 3DSS and CT to search for **patterned injuries**





Step 2 Matching analysis using 3D models to see whether the size and shape of the **injury wound** and **suspected injury inflicting tools** (i.e. propellers) are complementary to each other

Step 3 Virtual **scene reconstruction** using the matching 3D models for better understanding of vessel interaction and **injury prevention**



Summary

Our team conducted **forensic scene investigation** of marine vessel interaction in stranded cetaceans using a multidisciplinary approach, in collaboration with various parties, including the **Agriculture, Fisheries and Conservation Department**, **Marine Department**, **Ocean Park Corporation** and **Ocean Park Conservation Foundation Hong Kong**

Postmortem CT is conducted to document the **internal condition** of stranded cetaceans, supplementing conventional necropsy in assessing the **biological health profile** and **cause of death**

3D surface scanning is conducted to document the **external condition** of stranded cetaceans and suspected injury-inflicting tools, allowing **matching analysis** and **virtual scene reconstruction**

Spatiotemporal analysis is conducted to identify cetacean-vessel **interaction hotspots**, **vessel types** and **speed** involved

Findings will serve as **scientific evidence** for government officials and related stakeholders to establish effective management strategies for **injury prevention** and **conservation** of vulnerable cetaceans in Hong Kong waters

Way forward

Stranded cetacean virtopsy is an interdisciplinary, professional work that comprises:

- **Radiology** (i.e. certificated radiographers)
- **Forensic medicine** (e.g. forensic pathologists, forensic scientists)
- **Veterinary science** (i.e. veterinarians)
- **Marine mammal medicine** (who experienced in cetacean anatomy and physiology)
- **Medical informatics** (i.e. information engineering that integrate and manage big data)
- **Stranding response personnel** (i.e. different researchers and volunteers)

To maintain a proper postmortem investigation and integrate virtopsy as a routine workflow, we need:

- A **standardized image acquisition**, i.e. the standardized positioning and scanning protocols
- A **standardized image post-processing, techniques and evaluation** (i.e. DICOM viewing system and image rendering techniques)
- A **centralized data storage and management**, i.e. big data depository

Let's join hands and endeavor to promote virtopsy-led cetacean postmortem investigation to improve diagnostic accuracy and enhance injury prevention!

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