#### Marine Ecology Enhancement Fund (MEEF) Declaration

To: The Secretariat of the MEEF

Reference No.: MEEF2017014

Virtopsy for characterization and documentation of injury and death caused by human interaction, in stranded Indo-Pacific **Project Title:** humpbacked dolphins (*Sousa chinensis*) in the Hong Kong waters

Name of Project Leader: Brian Chin Wing Kot

I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorisation has been obtained in respect of information owned by third parties.

Signature:

Project Leader, Brian Chin Wing Kot

Date: 0.12.2018

For Use by the Secretariat: <u>MEEF2017014/L01</u> (Reference Number)

#### MARINE ECOLOGY ENHANCEMENT FUND (MEEF)

#### **Completion Report for Year 2017-2018**

Report for the period ending 30 June 2018

#### Part A: Executive Summary

#### 1. Executive Summary

Throughout 12 months, from 1 July 2017 to 30 June 2018, project entitled "Virtopsy for characterization and documentation of injury and death caused by human interaction, in stranded Indo-Pacific humpbacked dolphins (*Sousa chinensis*) in the Hong Kong waters", has progressed satisfactorily and its objectives have been 40-60% accomplished. All supporting staffs (1 part-time RAsso, 2 RAs and 2 part-time student RAs) were recruited to assist the workflow of this project. The realization of the 4 project objectives are illustrated as below:

- a) To characterize virtopsy based markers that aid in the identification and prognosis of injury and death caused by human interaction (HI) in stranded Chinese White dolphins (CWDs).
- b) To discern antemortem injury from postmortem injury and to infer such injury characteristics from HIs using virtopsy and conventional necropsy findings in stranded CWDs.

As of 30 June 2018, 36 out of 40 Hong Kong (HK) stranded cetaceans (90%) were performed virtopsy, including 6 CWDs, 24 Indo-Pacific finless porpoises, 1 Indo-Pacific bottlenose dolphin, 1 common bottlenose dolphin, 1 short-beaked common dolphin, 1 pygmy sperm whale and 1 undetermined cetacean species. In addition, 2 individual fetuses found inside the body of stranded cetacean carcasses (1 CWD and 1 Indo-Pacific finless porpoises), were also performed virtopsy. All the virtopsy findings were verified by subsequent necropsy, with supplemented information given for the analysis of HI related injury and death of stranded cetaceans. The remaining 4 stranded cetaceans (10%) were recognised to be non-transportable and inappropriate for virtopsy by cetacean stranding response team of the Ocean Park Conservation Foundation Hong Kong (OPCFHK). Onsite necropsy and sample collection were directly performed to these cetacean carcasses by the team of OPCFHK.

In these 36 cases, findings of 19 stranded cetaceans (3 CWDs, 14 Indo-Pacific finless porpoises, 1 Indo-Pacific bottlenose dolphin and 1 pygmy sperm whale) were sufficient to assign the cause of death with confirmed, probable or suspect category associated by HIs (53%), which included evidence of fishery (e.g. fishing gear entanglement/digestion), and vessel interactions (e.g. sharp chopped wound). The rest 17 stranded cetaceans (4 CWDs, 10 Indo-Pacific finless porpoises, 1 common bottlenose dolphin, 1 short-beaked common dolphin and 1 undetermined species) were likely associated with different causes of death, e.g. respiratory diseases, drowning and natural death. Most virtospy findings were shared bilingually on the stranded cetacean blog, allowing general public and international experts to obtain first-hand information and share their opinions on local stranding events.

c) To document and describe the types of HIs taking place using 3-D surface documentation techniques, virtopsy and conventional necropsy findings, leading to injury and death in stranded CWDs.

In order to undergo corrective virtopsy procedures and interpretation of postmortem radiological images, all supporting staff underwent training by the P.I. in the first month of employment for PMCT/PMMRI imaging techniques and 3-D volume reconstruction and rendering processes, as well as familiarisation of

highly specific cetacean anatomy and pathology. Apart from the PMCT/PMMRI imaging techniques, 2 commonly employed 3-D surface documentation techniques i.e. 3-D photogrammetry and 3-D surface scanning, were explored for the documentation of the external conditions, particularly on types of HIs taking place in carcass. Trials regarding the applications of 3-D photogrammetry and 3-D surface scanning were performed on dried skeletal specimen and live dolphins in fall 2017 while the procurement of hardware and software for 3-D surface documentation were completed in early December 2017, and subsequent trainings on 3-D surface scanner operation and post-processing technique were performed in December 2017 and January 2018. To date, 7 members in our laboratory were trained and they were all competent in performing 3-D surface documentation on stranded carcass.

Trials of 3-D surface scanning of HK stranded cetacean carcass was conducted in spring 2018. Preliminary results of 3-D models demonstrated an excellent color-texture information, allowing further data postprocessing and measurement in a true-to-scale 3-D model. A standardised protocol on 3-D surface scanning of cetacean carcass was established and its content was subsequently presented by P.I. and his team as a conference proceedings entitled "Three-dimensional surface scanning in postmortem investigation of stranded cetaceans: A step-by-step guide for carcass surface documentation", in an international conference in May 2018.

Various subprojects with the use of 3-D surface scanning on HK stranded cetacean carcass and the inflicting tools were branched from the core project for final year undergraduate students in Tung Wah College (Forensic science and Basic Medical Sciences major, 2014 cohort) to experience how practical inter-disciplinary research project works with effort contributed from respective expertise. Instead of being merely a school assignment, this provided them an opportunity to demonstrate their work at the international aquatic animal medicine workshop and conference platform and a pathway to enter global research arena.

d) To compile postmortem findings collected by original notes, 3-D surface documentation techniques, virtopsy and conventional necropsy, and transcribe into virtopsy and postmortem multimedia platform for first time all-in-one real-databased models of the stranded CWDs for geometric comparison of patterned injuries with the presumed types of HI.

At the inception stage of database development, various procurement and technical procedures had been undergone in order to establish a web-based server connected with server-based image viewing system for the establishment of a virtopsy and postmortem multimedia platform. The purchase of hardware (i.e. server) and software (i.e. TeraRecon's Aquarius package installed in the server) were completed in end of October 2017. Data compilation of 118 virtopsy cases (from March 2014 to 30 June 2018, included 31 CWDs, 76 Indo-Pacific finless porpoises and 11 other cetacean species) and respective links between the web server and DICOM viewer were completed. Inclusion of the corresponding stranding information and other multimedia of retrospective cases are in progress.

Three peer-review articles and 1 conference proceedings were published regarding a review of the currently available stranding and mortality database and virtopsy findings in this present project. A number of local and international print media have voiced rising concern on unusual surge of stranding numbers in the first 2 quarters of 2018, with supplemented virtopsy findings reported from P.I. and his team on cetacean stranding blog.

A significant increase in workload of the project team were recognised due to an unexpected surge of cetacean stranding in HK waters since January 2018. To date, as of 30 June 2018, 29 stranded cetaceans (4 CWDs, 22 Indo-Pacific finless porpoises, 1 common bottlenose dolphin, 1 short-beaked common dolphin and 1 undetermined cetacean species) were recovered in Hong Kong waters, which was recognizably higher than the same periods of previous years' records, with 21 carcasses found in average (In 2014: 27 cases; 2015: 26 cases; 2016: 16 cases; 2017: 14 cases). A rising concern has been voiced since 15 out of 29 stranded cetaceans (52%) were likely suspected to be associated with human interaction related injury and death. Although the project was under-resourced due to the unexpected surge of cetacean stranding since 2018, the project team had paid immediate efforts to convey conservative

messages via outreach activities, such as conducting interviews with different print media, holding public seminar, talks and workshops, which jointly organised with the Hong Kong Science Museum and universities, to elevate the public awareness on cetacean stranding response program, immediate threats the local cetaceans facing, injury and death of local cetaceans caused by human interaction, and the greater scope: marine conservation and interest in science and technology.

#### Part B: The Project and Investigator(s)

#### 2. Project Title

Virtopsy for characterization and documentation of injury and death caused by human interaction, in stranded Indo-Pacific humpbacked dolphins (*Sousa chinensis*) in the Hong Kong waters

利用影像解剖識別及紀錄在香港水域擱淺的中華白海豚因人類活動所造成的傷害和死亡的研究

#### 3. Project Period

From 1 July 2017 to 30 June 2018 (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

Evaluating marine mammals for signs of human interaction requires consistent, objective, thorough examinations by experts in various disciplines. This project is meant to apply virtopsy on characterization and documentation of human interaction related injuries and death in stranded Chinese white dolphin, and provide supplementary information to aid conventional necropsy, for determination of human activities contributed to the stranding event. World first all-in-one real-databased models of the stranded cetaceans for geometric comparison of patterned injuries with the presumed types of human interaction will also be established, thus providing a sound scientific basis for policy and management decisions in the Hong Kong waters.

一直以來,如欲評估及證實人類活動在海洋哺乳類動物身上所留下的痕跡,均需不同界別的專家進行一致及客觀的測試。本項目將利用影像解剖技術識別及紀錄擱淺的中華白海豚因人類活動而造成的損傷及死亡,並以這些額外的資訊為傳統解剖提供協助,從而判斷人類活動對擱淺所造成的影響。此外,本項目亦會建立全球首個一站式鯨豚類擱淺數據資料庫,有系統地比較 擱淺鯨豚身上的損傷及人類活動,為管理香港水域及制定相關政策提供科學根據和基礎。

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. Brian Chin Wing KOT, Assistant Professor	School of Medical and Health Sciences, Tung Wah College
Co-investigator	Dr. Paolo MARTELLI, Chief Veterinarian	Department of Zoological Operations and Education, Ocean Park Corporation
Co-investigator	Prof. Hock Gan HENG, Clinical Professor & Section Head, Diagnostic Imaging	Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, Indiana, USA
Co-investigator	Prof. Michael J. THALI, Director	Institute of Forensic Medicine, University of Zurich
Supporting bodies	Ms. Gendron SUZANNE Foundation Director	Ocean Park Conservation Foundation Hong Kong
Supporting bodies	Dr. Wai Chuen NG, Marine Conservation Officer	Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong SAR
Supporting bodies	Mr. Victor HUNG	TeraMed Ltd.
Supporting bodies	Dr. Richard BROWN, Associate Director (Production Animal Programmes)	College of Veterinary Medicine and Life Sciences, City University of Hong Kong

### 7. Timetable of the Project Progress according to the Proposed Work Schedule

Activities	Original Period	Progress
Recruitment of support staff	July 2017	Finished
Training of support staff	July – September 2017	Finished
Compilation of retrospective cases and prospective data collection	July 2017 – now	1 <sup>st</sup> phase completed
Stranded cetacean blog	October 2017 – now	1 <sup>st</sup> phase completed (commenced in Mid- August 2017)
3-D surface documentation workshop - HK SciFest 2018	22 April 2018	Finished
Press release/media tea reception	Early 2018	N/A
Public seminar - HK SciFest 2018	22 April 2018	Finished
Public talk - Biodiversity workshops	7 February, 24 March, 15 April, 6 June 2018	The 1 <sup>st</sup> – 4 <sup>th</sup> workshops (Finished)
Data consolidation and write up publications	April – July 2019	3 peer-review articles and 1 conference proceedings published
Handbook of virtopsy for stranded cetacean	June – July 2019	N/A

#### 8. Project Expenditure

Project expenditure details are not disclosed due to confidentiality reason.

#### Part C: Completion Report on Year 2017-2018 Project Progress

#### 9. Project Objectives

#### 9.1 Objectives as per Original Application

- a) To characterize virtopsy based markers that aid in the identification and prognosis of injury and death caused by human interaction (HI) in stranded Chinese White dolphins (CWDs).
- b) To discern antemortem injury from postmortem injury and to infer such injury characteristics from HIs using virtopsy and conventional necropsy findings in stranded CWDs.
- c) To document and describe the types of HIs taking place using photogrammetry, virtopsy and conventional necropsy findings, leading to injury and death in stranded CWDs.
- d) To compile postmortem findings collected by original notes, photogrammetry, virtopsy and conventional necropsy, and transcribe into virtopsy and postmortem multimedia platform for first time all-in-one real-databased models of the stranded CWDs for geometric comparison of patterned injuries with the presumed types of HI.

#### 9.2 Revised Objectives

- Minor amendment on specific terminology of 2 objectives was made to precisely reflect our techniques used for 3-D surface documentation of injury and death in stranded CWDs.
- c) To document and describe the types of HIs taking place using 3-D surface documentation techniques, virtopsy and conventional necropsy findings, leading to injury and death in stranded CWDs.
- d) To compile postmortem findings collected by original notes, 3-D surface documentation techniques, virtopsy and conventional necropsy, and transcribe into virtopsy and postmortem multimedia platform for first time all-in-one real-databased models of the stranded CWDs for geometric comparison of patterned injuries with the presumed types of HI.

#### **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

A part-time research associate (RAsso) and 2 full-time research assistants (RAs) and were recruited in July and August 2017 respectively for a period of about 11 months. The part-time RAsso took up the role as a project manager to assist P.I. to oversee overall management and administrative work of the project, with service, research and public education components. One RA took up the role as a project assistant to assist P.I. to oversee project logistics arrangement, liaison and coordination among other team members, departments and collaborators, data collection and management (i.e. and data gathering and archiving) and publication preparation. Another RA took up the role as an in-house programmer to tailor-develop a cetacean virtopsy and postmortem multimedia platform (i.e. Virtopsy database connected with TeraRecon's Aquarius solution) and oversaw its administration and maintenance in the project. Two part-time student RAs were employed to assist the P.I. and his team on project operation, such as stranding response participation, on-site virtopsy examinations at the imaging center, and stranded cetaceans blog establishment and update.

#### **B.** Training of the supporting staff

All supporting staff (1 part-time RAsso, 2 RAs and 2 part-time student RAs) underwent training by the P.I. in the first month of employment for PMCT/PMMRI imaging techniques and 3-D volume reconstruction and rendering processes, as well as familiarisation of highly specific cetacean anatomy and pathology. They were also advised the project should be executed according to timelines, with proper documentation maintained throughout the project. P.I has also arranged mentorship to junior members of the team, and motivated and supervised project team members from time to time to enable them to carry out their assigned work effectively. Following the initial training period, the supporting staff understood the stranding procedures, assisted all postmortem imaging procedures, stranded cetacean carcass logistics and liaison, and managed data gathering and archiving.

#### C. Establishment of a stranded cetacean blog and media coverage

Diverse social media accounts regarding this project were developed, including Facebook, Instagram Facebook ("Cetacean and YouTube channel. Both page Virtopsv Lab". https://www.facebook.com/cetavirtolab/: Instagram ("dolphin cvlab". Fig. 1) and https://www.instagram.com/dolphin\_cvlab/) were officially launched in middle of August 2017, with prior notification to the secretariat of the MEEF management committee and Agriculture, Fisheries and Conservation Department (AFCD), the government of the HK Special Administrative Region (HKSAR). These social media accounts acted as a world-first stranded cetacean social platform 1) to document stranding incidents (highlight HI related injury and death) with virtopsy-driven stranding response effort in HK waters; 2) to allow clinicians, scientists, researchers, governmental officials and general public to archive first-hand information and share their views on HK local cetacean stranding cases; 3) to promote regional seminar and workshop to facilitate knowledge exchange on stranded cetacean postmortem investigation with HI related injury and death; and 4) to deepen engagement with social networks around marine ecology, conservation and related research interests. Up to 30 June 2018, the Facebook page has reached over 32,000 users with 313 followers and 50 posts were published.



Fig. 1. Stranded cetacean blog, Facebook page of Cetacean Virtopsy Lab, providing diverse information regarding virtopsy of stranding cetacean in Hong Kong waters.

The renowned Sea Mammal Research Unit, University of St. Andrews conducted an interview with P.I. and his team on 13 September 2017 in preparing a video presentation for the 22<sup>nd</sup> Biennial Society for Marine Mammalogy Conference on the Biology of Marine Mammals. A video talk entitled, "A journey from analogue to digital: The intertwining paths of scientific investigation and media reporting across a twenty years study of Hong Kong's iconic dolphin", regarding virtopsy and its significant contribution made to understand stranded cetaceans was presented in the captioned conference, which 2017 held 22-27 October in Nova Scotia, Canada was on (https://www.youtube.com/watch?v=bBSrAt55B3k).

Apart from the promotion of cetacean stranding blog, Apple Daily (one of the highest shares paper in the Hong Kong newspaper market), published a news article on 3 February 2018, entitled "上月4宗

江豚擱淺 解剖見漁鈎魚絲食道亂纏", with in-depth interviews to P.I. and his team regarding the virtopsy and postmortem investigation on local stranded cetaceans. The news article also highlighted the incidence of injury and death caused by HI, including fishery and vessels interactions, in local stranded cetaceans from the middle of 2017 to January 2018. Common pathologies observed were also noted including parasitic infection (Cestoda: Tetraphyllidea) at mammary glands and pneumonia (<u>https://hk.news.appledaily.com/local/realtime/article/20180203/57786364</u>, <u>https://hk.news.appledaily.com/local/realtime/article/20180203/57786465</u>).</u>

Various local and international print media, including Apple Daily (蘋果日報), Ming Pao (明報), Oriental Daily (東方日報), Sing Tao Daily (星島日報), Headline Daily (頭條日報), Hong Kong Economic Journal (信報), The Standard (英文虎報), and Coconuts Hong Kong, have voiced rising concern on unusual surge of stranding numbers in the first 2 quarters of 2018, with supplemented virtopsy findings reported from P.I. and his team on cetacean stranding blog. All the related articles were listed as below:

Apple Daily (蘋果日報) 2018/02/03 "上月4宗江豚擱淺 解剖見漁鈎魚絲食道亂纏" https://hk.news.appledaily.com/local/realtime/article/20180203/57786364

2018/02/03"【話你知】江豚屬易危物種估計港最多僅152條" https://hk.news.appledaily.com/local/realtime/article/20180203/57786465

2018/02/04 "因填海失棲息地 4江豚疑遭誤捕 纏網吞魚鈎亡" https://hk.news.appledaily.com/local/daily/article/20180204/20294894

2018/02/21 "今年11條鯨豚擱淺 小江豚魚網纏身幸獲好心人解救" https://hk.news.appledaily.com/local/realtime/article/20180221/57856921

Oriental Daily (東方日報)

2018/02/21 "生態災難 新春假5海豚擱淺 兩月累計逾10條" http://hk.on.cc/hk/bkn/cnt/news/20180221/bkn-20180221104732950-0221\_00822\_001.html

2018/02/22 "3日內5鯨豚擱淺影像解剖揭疑遭船撞" http://orientaldaily.on.cc/cnt/news/20180222/00176 045.html

Sing Tao Daily (星島日報) 2018/02/21 "狗年伊始3日內5海豚擱淺" http://std.stheadline.com/instant/articles/detail/645012

Headline Daily (頭條日報) 2018/02/22 "三日五鯨豚擱淺疑遭螺旋槳擊殺" <u>http://hd.stheadline.com/news/daily/hk/647766/</u> (Acknowledgement of MEEF's support was listed in this news article)

Coconuts Hong Kong 2018/02/21 "Researchers lament 'forgotten' porpoises as five dead cetaceans found over 3 days" https://coconuts.co/hongkong/news/five-cetaceans-founds-over-3-days-up-from-previous-year/

The Standard (英文虎報) 2018/02/22 "Boats suspected in five dolphin deaths" <u>http://www.thestandard.com.hk/section-news.php?id=193059&sid=4</u>

Hong Kong Economic Journal (信報)

2018/02/22 "云爾錄:三日內5豚擱淺 團體關注" http://www1.hkej.com/dailynews/views/article/1773310

Ming Pao (明報) 2018/04/01 "綠色生活:影像解剖還原海豚死亡真相" https://news.mingpao.com/pns/dailynews/web\_tc/article/20180401/s00005/1522519379797

Apple Daily (蘋果日報) 2018/06/23 "今年23宗擱淺創同期新高 懷孕江豚被船撞死「分屍」一屍兩命" https://hk.news.appledaily.com/local/realtime/article/20180623/58351989

#### D. Public seminar/talk/workshop

Four-day workshops jointly organised with the Hong Kong Science Museum, entitled, "Bloodless Necropsy - Local Cetaceans: Anatomy and Threats" was held on 7 February, 24 March, 15 April and June 2018 the Nature of the ΗK Science Museum 6 at Lab (http://hk.science.museum/ms/bdg/eworkshop.html). Each workshop day consisted of 2 biodiversity workshops, which aimed to provide an overview of cetacean stranding in HK waters and implementation of virtopsy to local stranding response program to the general public (Fig. 2). Throughout 8 workshops in 4 days, over 160 participants were recorded.



Fig. 2. Biodiversity workshop held at the Nature Lab of the Hong Kong Science Museum.

In addition, highlighted public seminar and 2 workshops entitled, "Bloodless Necropsy in Stranded Cetacean: Virtopsy Conservation" and (http://www.hk.science.museum/scifest2018/view\_detail.php?lang=en&act\_refno=128) and "Bloodless Necropsy Stranded Cetacean: 3-D Modelling in Techniques" (http://www.hk.science.museum/scifest2018/view\_detail.php?lang=en&act\_refno=129) respectively, were conducted on 22 April, Earth Day 2018, in the HK SciFest 2018 (Fig. 3; annual large-scale event to provide interesting scientific activities to elevate the public awareness and interest in science and technology, hosted by HK Science Museum http://hk.science.museum/scifest2018/). Over 180 participants attended the public seminar and 2 workshops. This participation was jointly organised among the HK Science Museum, Tung Wah College and the Hong Kong Polytechnic University, and was aimed to promote the modern non-invasive imaging techniques, i.e. virtopsy and 3-D surface scanning, in assisting the investigation of injury and death caused by HIs in stranded cetaceans. Acknowledgement of MEEF's support was included in the captioned event links. Full video of "Bloodless Necropsy in Stranded Cetacean: Virtopsy and Conservation" was also available: https://www.youtube.com/watch?v=aTM6MI2fZAA.



Fig. 3. Public seminar (Left) and workshops (Right) conducted in the HK SciFest 2018 on 22 April 2018.

## 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 (CPMAP)" has been developed since the project commencement. At the inception stage of database development, various procurement and technical procedures have been undergone in order to establish a web-based server connected with server-based image viewing system. The purchase of hardware (i.e. server) and software (i.e. TeraRecon's Aquarius package installed in the server) were completed in end of October 2017. Data compilation of 118 virtopsy cases (from March 2014 to 30 June 2018, included 31 CWDs, 76 Indo-Pacific finless porpoises and 11 other cetacean species) and respective links between the web server and DICOM viewer were completed. Inclusion of the corresponding stranding information and other multimedia of retrospective cases are in progress.

Prior to the official online launch of our web-based database, a demonstration of CPMAP prototype was conducted on 18 October 2017 to officials from AFCD, the government of HKSAR, which provided affirmative supports and constructive comments to the functions and applications of this database. Demonstration and training sessions to rest of the project team units was anticipated in spring 2018, for comments and testing.

#### **B. Surface documentation of carcass**

Apart from conducting virtopsy and necropsy as a routine on stranded cetaceans in the HK waters, 2 commonly employed 3-D surface documentation techniques, i.e. 3-D photogrammetry and 3-D surface scanning, were explored for the documentation of the external conditions, particularly on types of HIs taking place in carcass. Trials regarding the applications of 3-D photogrammetry and 3-D surface scanning were performed on dried skeletal specimen and live dolphins (Appendix 1) in fall 2017. The procurement of hardware and software for 3-D surface documentation were completed in early December 2017, and subsequent trainings on 3-D surface scanner operation and post-processing technique were performed in December 2017 and January 2018. To date, 7 members in our laboratory were trained and they were all competent in performing 3-D surface documentation on stranded carcass. Trials of 3-D surface scanning of HK stranded cetacean carcass was conducted in spring 2018 (Fig. 4). Preliminary results of 3-D models demonstrated an excellent color-texture information, allowing further data postprocessing and measurement in a true-to-scale 3-D model (Fig. 5).



Fig. 4. Trials of 3-D surface scanning on Hong Kong stranded cetacean carcass and its fetus.



Fig. 5. Results of 3-D surface scanning of carcass (Left) and its fetus (Right).

A standardised protocol on 3-D surface scanning of cetacean carcass was established and its content was subsequently presented by P.I. and his team as a conference proceedings entitled "Three-dimensional surface scanning in postmortem investigation of stranded cetaceans: A step-by-step guide for carcass surface documentation", in the 49<sup>th</sup> Annual International Association for Aquatic Animal Medicine (IAAAM) Meeting and Conference, held in Renaissance Long Beach, Long Beach, California, USA on May 2018 (Fig. 6 and Appendix 2).



Fig. 6. Oral presentation at the 49<sup>th</sup> Annual International Association for Aquatic Animal Medicine Meeting and Conference on the topic entitled, "Three-dimensional surface scanning in postmortem investigation of stranded cetaceans: A step-by-step guide for carcass surface documentation."

#### C. Virtopsy cases performed

From 1 July 2017 to 30 June 2018, 36 out of 40 HK stranded cetaceans (90%), included 6 CWDs, 25 Indo-Pacific finless porpoises, 1 Indo-Pacific bottlenose dolphin, 1 common bottlenose dolphin, 1 shortbeaked common dolphin, 1 pygmy sperm whale and 1 undetermined species, were performed virtopsy (Fig. 7). In addition to this, 2 individual fetuses were found inside the body of stranded cetacean carcasses (1 CWD and 1 Indo-Pacific finless porpoises), were also performed virtopsy. All the virtopsy findings were verified by subsequent necropsy, with supplemented information given for the analysis of HI related injury and death of stranded cetaceans. The remaining 4 stranded cetaceans (10%) were recognised to be non-transportable and inappropriate for virtopsy by OPCFHK. Onsite necropsy and sample collection were directly performed on these cetacean carcasses by the team of OPCFHK.



Fig. 7. Performing virtopsy (postmortem computed tomography) to HK stranded cetaceans at the Peace Avenue Veterinary Clinic, City University of Hong Kong.

In the aforesaid 36 cases, findings of 19 stranded cetaceans (3 CWDs, 14 Indo-Pacific finless porpoises, 1 Indo-Pacific bottlenose dolphin and 1 pygmy sperm whale) were sufficient to assign the cause of death with confirmed, probable or suspect category associated by HIs (53%), which included fishery and vessel interactions (Fig. 8). The rest 17 stranded cetaceans (4 CWDs, 10 Indo-Pacific finless porpoises, 1 common bottlenose dolphin, 1 short-beaked common dolphin and 1 undetermined species) were likely associated with different causes of death, e.g. respiratory diseases, drowning and natural death. Most virtospy findings were shared bilingually on our stranded cetacean blog (Facebook page: "Cetacean Virtopsy Lab", <u>https://www.facebook.com/cetavirtolab/</u>).



Fig. 8. Example on Virtopsy (postmortem computed tomography) findings in HK stranded cetaceans associated by HIs, included fishery (Left) and vessel interactions (Right).

#### **D. Publications**

First publication related to this current MEEF-funded project entitled, "Database documentation of marine mammal stranding and mortality: current status review and future prospects", was published in *Diseases of Aquatic Organisms* (DAO 2017 Nov 21; 126(3):247-256; abstract: <u>http://www.int-res.com/abstracts/dao/v126/n3/p247-256/;</u> Appendix 3) at the end of September 2017. The review article was to (1) describe and review the current status of marine mammal stranding and mortality databases worldwide, including the year established, types of database and their goals; and (2) summarise the geographic range included in the database, the number of cases recorded, accessibility, filter and display methods. This serves as a mean to critically review currently available database documentation of marine mammal stranding and mortality worldwide and thus allows us to establish a theoretical framework for our project. Acknowledgement of MEEF's support was listed in the accepted manuscript, and full text of the manuscript were shared with MEEF management committee on 22 November 2017.

Second publication related to this current MEEF-funded project entitled, "Morphological analysis of the foramen magnum in finless porpoise (genus *Neophocaena*) using postmortem computed tomography 3D volume rendered images" was submitted and accepted by *Marine Mammal Science* (MMS 2018 early view abstract: <u>https://onlinelibrary.wiley.com/doi/abs/10.1111/mms.12512</u>; Appendix 4) on 12 March 2018. This serves as an important baseline for the morphometric and typology of cranial characters in both finless porpoise species. These results may aid corrective diagnosis, classification, and treatment of diseases related to the foramen magnum in stranded or injured cetaceans in our project. Acknowledgement of MEEF's support was listed in the accepted manuscript, and full text of the manuscript were shared with MEEF management committee on 31 May 2018.

Third publication related to observation of atlanto-occipital dissociation in stranded cetaceans was published in *Scientific* Reports (SR 2018 May 31; 8:8474: full article: https://www.nature.com/articles/s41598-018-26866-8; Appendix 5) at the end of May 2018. The present study aimed to 1) describe a novel standardised method of determining the normal craniocervical relationships, and 2) define the 95% accuracy range of the normal craniocervical relationship in finless porpoises (genus Neophocaena), as obtained on reconstructed PMCT images for the diagnosis of AOD. This serves as a novel standardised method for determination of normal craniocervical relationships in finless porpoises was first described, which is used for atlanto-occipital dissociation diagnosis. Through identification of this observation, insight of injury and death caused by human interaction in stranded cetaceans can be obtained for our project. Acknowledgement of MEEF's support was listed in the accepted manuscript, and full text of the manuscript were shared with MEEF management committee on 31 May 2018.

11.	Evaluation	of the project	effectiveness	in achiev	ing the <b>j</b>	proposed	objectives	addressed t	to
dat	te								

<b>Objectives</b> (as per 9.1/9.2 above)	<b>Addressed</b> (please tick)	<b>Percentage</b> <b>achieved</b> (please estimate)
1. To characterize virtopsy based markers that aid in the identification and prognosis of injury and death caused by human interaction (HI) in stranded Chinese white dolphins (CWDs).	$\checkmark$	60
2. To discern antemortem injury from postmortem injury and to infer such injury characteristics from HIs using virtopsy and conventional necropsy findings in stranded CWDs.	$\checkmark$	60
3. To document and describe the types of HIs taking place using 3-D surface documentation techniques, virtopsy and conventional necropsy findings, leading to injury and death in stranded CWDs.	$\checkmark$	40
4. To compile postmortem findings collected by original notes, 3-D surface documentation techniques, virtopsy and conventional necropsy, and transcribe into virtopsy and postmortem multimedia platform for first time all-in-one real-databased models of the stranded CWDs for geometric comparison of patterned injuries with the presumed types of HI.	$\checkmark$	40

#### Part D: Research Output

12. Peer-reviewed journ	al publication(s)	arising directly	from this	research project
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The l	Latest Status	s of Publi	cations	Author(s)	Title and Journal/Book	Attached to	Acknowl
Year of	Year of	Under	Under	(denote the	(with the volume, pages and	this report	edged the
publicati	Acceptanc	Review	Preparati	corresponding	other necessary publishing	(Yes or No)	support
on	e (For		on	author with an	details specified)		of MEEF
	paper		(optional)	asterisk*)			(Yes or
	accepted						No)
	but not yet						,
	published)						
2017	Î Î			Derek K.P.	Database documentation of	Yes	Yes
				Chan, Henry	marine mammal stranding	(Appendix 3)	
				C.L. Tsui, Brian	and mortality: current status		
				C.W. Kot*	review and future prospects.		
					Diseases of Aquatic		
					Organisms. 126: 247-256		
					(2017).		
				Brian C.W.	Morphological analysis of	Yes	Yes
				Kot*, Derek	the foramen magnum in	(Appendix 4)	
				K.P. Chan,	finless porpoise (genus		
	2018			Adams H.L.	Neophocaena) using		
	2018			Yuen, Francis	postmortem computed		
				H.M. Wong,	tomography 3D volume		
				Henry C.L.	rendered images. Marine		
				Tsui	Mammal Science (2018).		
				Brian C.W.	Diagnosis of atlanto-	Yes	Yes
				Kot*, Derek	occipital dissociation:	(Appendix 5)	
				K.P. Chan,	Standardised measurements		
				Adams H.L.	of normal craniocervical		
2018				Yuen, Henry	relationship in finless		
2010				C.L. Tsui	porpoises (genus		
					Neophocaena) using		
					postmortem computed		
					tomography. Scientific		
					Reports. 8:8474 (2018).		

#### 13. Recognised 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	Title	Conference Name	Attached to this	Acknowledged
/			report (Yes or No)	the support of
Place			_	MEEF
				(Yes or No)
May 2018,	Three-dimensional Surface	International	Yes	Yes
Renaissance	Scanning in Postmortem	Association for	(Appendix 2)	
Long Beach	Investigation of Stranded	Aquatic Animal		
in Long	Cetaceans: A Step-by-step Guide	Medicine Meeting and		
Beach,	for Carcass Surface	Conference		
California,	Documentation			
USA				

#### 14. Other impact

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

From 30 October to 2 November 2017, P.I. were invited by the preparatory committee of the Chinese Association of Trainer and Veterinarian in Aquatic Animal Medicine as a keynote speaker to attend the "Second Term China Aquarium Development Forum", which was organized by the Ministry of Agriculture of the People's Republic of China and held in Polar Ocean World in Quan zhou, Shandong province, PRC. In this forum, P.I. and his team shared knowledge and experiences on technological advancement of marine mammal diagnostic imaging (Fig. 9), including how virtopsy (postmortem CT and MRI) helps characterising and documenting injury and death of cetaceans in HK and its adjacent waters.



Fig. 9. Second Term China Aquarium Development Forum, held in Polar Ocean World in Quan Zhou, Shandong province, PRC.

Various subprojects with the use of 3-D surface scanning on HK stranded cetacean carcass and the inflicting tools were branched from the core project for final year undergraduate students in TWC (Forensic science and Basic Medical Sciences major, 2014 cohort) to experience how practical inter-disciplinary research project works with effort contributed from respective expertise (Table listed below). Instead of being merely a school assignment, this provided them an opportunity to demonstrate their work at the international aquatic animal medicine workshop and conference platform and a pathway to enter global research arena. The TWC final year students and P.I. attended cetacean specimen documentation training workshop organized by the National Museum of Natural Science, Taichung, Taiwan (Fig. 10).

Student Name	Topic of Honours Year Project
Wilson Lai Leong	Standardized ideal protocol for 3-D surface scanning on cetacean skeletal
CHAU	structure
Lydia Ching Man LAM	Evaluation of three-dimensional surface scanning in the documentation of
	marine propellers used in Hong Kong waters
Yumi Tsz Ling WONG	Identification of cranial non-metric characters in indo-pacific finless porpoise
	(Neophocaena phocaeniodes): A comparison of dried skeleton, computed
	tomography, photogrammetry and surface scanning
Kelsy Fung Yee YEUNG	Application of three-dimensional surface acquisition on the study of vertebral
	abnormalities in stranded cetaceans around Taiwanese waters
Michael Cheuk Yin YU	Integration of three-dimensional surface scanning in postmortem investigation
	of stranded cetaceans: A step-by-step guide for carcass surface documentation



Fig. 10. Final year undergraduate students in Tung Wah College and P.I. participated a cetacean specimen documentation training workshop using 3-D surface scanning techniques organized by the National Museum of Natural Science, Taichung, Taiwan (Left), and trials of 3-D surface scanning on stranded cetacean carcass (Right) in February 2018.

#### Part E: Summary and Way Forward

#### 15. Summary and Way Forward

Throughout 12 months, from 1 July 2017 to 30 June 2018, project entitled "Virtopsy for characterization and documentation of injury and death caused by human interaction, in stranded Indo-Pacific humpbacked dolphins (*Sousa chinensis*) in the Hong Kong waters", has progressed satisfactorily and its objectives have been 40-60% accomplished. Various research tasks (e.g. virtopsy, 3-D surface documentation techniques) and outreach activities (e.g. 1 public seminar, 4 talks and 2 workshops) targeted to fulfill the captioned 4 objectives in this project were completed within the 12-month timeframe. Three peer-review articles and 1 conference proceedings were published regarding a review of the currently available stranding and mortality database and virtopsy findings in this present project. A number of local and international print media have voiced rising concern on unusual surge of stranding numbers in the first 2 quarters of 2018, with supplemented virtopsy findings reported from P.I. and his team on cetacean stranding blog.

For future prospect of the captioned project, there is an urging need to upgrade our established web-based database, CPMAP. At the inception stage of database development, the design of database (i.e. user interface) might not be user-friendly enough, and only provided some general and basic functions, such as import, export, searching. To make it more comprehensive and user-friendly, additional commercial web template would be expanded. Featured functions would be built, and focused on the development of display data tools, i.e. data filtering, statistical presentation and mapping. The statistical presentation would be included the tendency chart filtered by days, months and years, while the mapping would display the high-density stranding location. In addition to the user interface, new featured function, and additional license and image loading capacity could be upgraded in the AqNET client-viewer. A larger number of images that user allowed to attempt concurrently (up to 6,000 images) and more diverse 3-D reconstruction template setting and tool would be available in the upgraded version of CPMAP, which could facilitate a better presentation of injured and death findings.

With this, a consistent platform for collecting, collating, and visualizing cetacean biological health data would be provided, which could enhance visual identification of "hot spots" of cetacean injury and death, disease and health for better management decisions and evaluations through a dynamic and user-friendly tool. The updated CPMAP could also aid emergency management responses to natural and anthropogenic emergencies by assessing changes in cetacean health before, during, and following the event and enhance communication and collaboration amongst cetacean health scientists and veterinarians, resulting in better understanding of the relationship between health and environmental factors.

#### Part F: Complete statement of accounts

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

- 1. do not deal with, and are not in any way associated with, any country or organisation or activity which is or may potentially be relevant to, or targeted by, sanctions administered by the United Nations Security Council, the European Union, Her Majesty's Treasury-United Kingdom, the United States Department of the Treasury's Office of Foreign Assets Control, or the Hong Kong Monetary Authority, or any sanctions law applicable;
- 2. have not used any money obtained from the Marine Ecology Enhancement Fund or the related Top-up Fund (and any derived surplus), in any unlawful manner, whether involving bribery, money-laundering, terrorism or infringement of any international or local law; and
- 3. have used the funds received (and any derived surplus) solely for the studies or projects which further the MEEF Objectives and have not distributed any portion of such funds (including any derived surplus) to members of the recipient organisation or the public.

**Principal Investigator** 

Signature :

Name of Project Leader :

Brian Chin Wing Kot

Date : 30 June 2018



Official Chop:

#### **Appendix 1: Trials of surface documentation of external body findings using various 3-D surface documentation techniques**

Exploration of different 3-D surface scanners prior to the item procurement



Application of 3-D photogrammetry on dried skull for 3-D model generation



Application of 3-D surface scanning technique on live dolphins in Ocean Park Hong Kong



#### **Appendix 2: Conference abstract for 2018** *International Association for Aquatic Animal Medicine* Meeting and Conference

#### IAAAM Proceedings 2018

http://www.iaaam.org/2018-iaaam-conference-proceedings/

Three-dimensional Surface Scanning in Postmortem Investigation of Stranded Cetaceans: A Step-by-step Guide for Carcass Surface Documentation

Brian C.W. Kot,  $^{1,2*}$  Derek K.P. Chan,  $^1$  Michael C.Y. Yu,  $^1$  Wilson K.L. Chau,  $^1$  Amy P.Y. Lau,  $^1$  and Henry C.L. Tsui,  $^3$ 

<sup>1</sup>School of Medical and Health Sciences, Tung Wah College, Homantin, Kowloon, Hong Kong SAR, China; <sup>2</sup>Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hunghom, Kowloon, Hong Kong SAR, China; <sup>3</sup>Office of the Vice-President (Development and External Relations), City University of Hong Kong, Kowloon, Hong Kong SAR, China

#### Abstract

Three-dimensional surface scanning (3DSS) documentation of external patterned injuries in human<sup>1</sup>, veterinary<sup>2,3</sup> and comparative forensic medicine<sup>4-6</sup> can help researchers to determine the shape and size of the injury, and enable the true-to-scale correlation of the wound and inflicting tool.<sup>7</sup> While routine cetacean virtopsy collected volumetric scans as a means of examining stranded carcass in a digital environment<sup>§</sup>, addition surface scans may reveal the external conditions including size, surface area, volume and possible texture. Like any measurement technique, 3DSS is subject to a number of limitations and methodological caveats, and thus should not be applied without any reference of standardization. The present study aimed to establish a new standardized 3DSS protocol for surface documentation of stranded cetaceans.

Stranded cetaceans in the Hong Kong waters were included in the present study. The operator scanned the carcass while it was first laid prone and then supine on a stainless steel necropsy table. The carcass was first scanned whole-body using the Artec Eva<sup>™</sup> (Artec Group, Inc., Luxembourg). Results from several scans were fused together with additional scans on head, flippers, dorsal fin/ridge and fluke using the Artec Space Spider<sup>™</sup> (Artec Group, Inc., Luxembourg). The 2 handheld scanners were used in conjunction with a high-performance laptop installed with a 3D modelling software, Artec Studio 12 (Artec Group, Inc., Luxembourg). Approximately 20 minutes was required to complete a full documentation of the carcass external conditions.

After acquisition of the 3DSS images, point clouds were generated to represent the scanned surface. Computation of point clouds into a polygon mesh structure of the 3D model required alignment, polygonization, mesh smoothing and thinning. Measuring deviations were first selectively eliminated (max error values >0.5 [Artec Space Spider<sup>TM</sup>] and >1.2 [Artec Eva<sup>TM</sup>]). Individual measurements were aligned based on point and surface. Measuring point clouds were then converted into a mesh of non-overlapping triangles, and were recalculated using the highest point resolution. Overlapping areas were deleted, and the resulting single measurements were merged into one polygon mesh. In smoothing and thinning processes, the polygon mesh data was shifted and reduced to minimize the measuring noise and outliners for important details preservation.

The 3DSS protocol developed has integrated into a virtopsy-driven stranding response workflow to provide surface documentation, which gives insights on potential cause of death, and pose precise conservation measure of local cetaceans with the external conditions caused by anthropogenic and natural injury.

#### Acknowledgements

The authors would like to thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for the support in the virtopsy project. Sincere appreciation is also extended to veterinarians, staff and volunteers from Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong for paying great effort on the stranding response and necropsy in this project. Special gratitude is owed to technicians from Peace Avenue Veterinary Clinic, City University of Hong Kong for operating the CT units to collect volumetric data in the present study. This project was financially supported by the Marine Ecology Enhancement Fund (Grant number: MEEF2017014/L01). Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee

## Appendix 3: Abstract of published review manuscript in *Diseases of Aquatic Organisms* and acknowledgements listed in the manuscript

*Diseases of Aquatic Organisms* 2017 Nov 21;126(3):247-256. http://www.int-res.com/abstracts/dao/v126/n3/p247-256/

Vol. 126: 247–256, 2017 DISEASES OF AQUATIC ORGANISMS Published November 2: https://doi.org/10.3354/dao03179 Dis Aqual Org Published November 2:
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#### REVIEW

#### Database documentation of marine mammal stranding and mortality: current status review and future prospects

Derek K. P. Chan<sup>1</sup>, Henry C. L. Tsui<sup>2</sup>, Brian C. W. Kot<sup>1,3,\*</sup>

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<sup>3</sup>Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hunghom, Kowloon, Hong Kong, SAR

ABSTRACT: Databases are systematic tools to archive and manage information related to marine mammal stranding and mortality events. Stranding response networks, governmental authorities and non-governmental organizations have established regional or national stranding networks and have developed unique standard stranding response and necropsy protocols to document and track stranded marine mammal demographics, signalment and health data. The objectives of this study were to (1) describe and review the current status of marine mammal stranding and mortality databases worldwide, including the year established, types of database and their goals; and (2) summarize the geographic range included in the database, the number of cases recorded, accessibility, filter and display methods. Peer-reviewed literature was searched, focussing on published databases of live and dead marine mammal strandings and mortality and information released from stranding response organizations (i.e. online updates, journal articles and annual stranding reports). Databases that were not published in the primary literature or recognized by government agencies were excluded. Based on these criteria, 10 marine mammal stranding and mortality databases were identified, and strandings and necropsy data found in these databases were evaluated. We discuss the results, limitations and future prospects of database development. Future prospects include the development and application of virtopsy, a new necropsy investigation tool. A centralized web-accessed database of all available postmortem multimedia from stranded marine mammals may eventually support marine conservation and policy decisions, which will allow the use of marine animals as sentinels of ecosystem health, working towards a 'One Ocean-One Health' ideal.

> Acknowledgements. Sincere appreciation is extended to veterinarians, staff and volunteers from Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong, and Tung Wah College for their great efforts with the stranding response programme in Hong Kong. We also thank the Agriculture, Fisheries and Conservation Department of the Hong Kong Special Administrative Region Government for continuous support in the pioneer cetacean virtopsy project. Special thanks to Dr. Michael Bradley for English editing of this manuscript. We thank the anonymous reviewers and responsible editor Dr. Stephen Raverty, who provided helpful comments on the refinement of this manuscript. This project was financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014/L01). Any opinions, findings, conclusions or recommendations expressed herein do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee. The pioneer stranded cetacean virtopsy project received financial support from the Hong Kong Research Grants Council (Grant number: UGC/FDS17/M07/14).

## Appendix 4: Letter of acceptance from *Marine Mammal Science* and acknowledgements listed in the manuscript

*Marine Mammal Science* 2018 Early view <u>https://onlinelibrary.wiley.com/doi/abs/10.1111/mms.12512</u>

<ul> <li>Marine Mammal Science - Editorial Decision for MMSCI-4537.R2</li> </ul>		Yahoo/Inbox 🖈
<ul> <li>Marine Mammal Science <onbehalfof@manuscriptcentral.com></onbehalfof@manuscriptcentral.com></li> <li>To: briankot@yahoo.co.uk, briankot@gmail.com, chankampingderek@gmail.com, yhladams@hotmail.com, cetaceanvirtopsylab@gmail.com and 1 more</li> <li>Cc: marinemammalscience@gmail.com</li> </ul>	0	12 Mar at 19:46 🔺

12-Mar-2018

Dear Dr. Kot:

I am very pleased to inform you that your revised Note entitled "Morphological analysis of the foramen magnum in finless porpoise (genus Neophocaena) using postmortem computed tomography 3D volume rendered images" has been accepted for publication in Marine Mammal Science. As noted in our email exchange, you will add the information about the availability of your data to the acknowledgement section of the final text file.

#### ACKNOWLEDGMENTS

185

All procedures in this study were reviewed and approved by the Agriculture, Fisheries and 186 Conservation Department (AFCD) of Hong Kong Special Administrative Region [AF GR 187 CON 09/68 PT.9]. AFCD has placed restrictions on the data that prevent it from being 188 shared publicly. Readers may contact Dr. Wai-chuen Ng (Marine Conservation Officer 189 [West]) from the AFCD to request the data. Interested readers can contact Dr. Wai-chuen 190 Ng at the following e-mail address: waichuen\_ng@afcd.gov.hk. This project was financially 191 supported by the Marine Ecology Enhancement Fund (Grant number: MEEF2017014/L01). 192 Any opinions, findings, conclusions, or recommendations expressed herein do not necessarily 193 reflect the views of the Marine Ecology Enhancement Fund or the Trustee. The pioneer 194 stranded cetacean virtopsy project received financial support from the Hong Kong Research 195 Grants Council (Grant number: UGC/FDS17/M07/14). The authors declare no competing 196 financial interests. The authors would like to thank the Agriculture, Fisheries and Conserva-197 tion Department of the Hong Kong Special Administrative Region Government for the 198 continuous support in this project. Sincere appreciation is also extended to veterinarians, 199 staff, and volunteers from Ocean Park Hong Kong, Ocean Park Conservation Foundation 200 Hong Kong, Tung Wah College and Cetacean Virtopsy Lab, for considerable effort on the 201 stranding response in this project. Special gratitude is owed to technicians from Peace Avenue 202 Veterinary Clinic, City University of Hong Kong, and Hong Kong Veterinary Imaging 203 Center for operating the CT units in the present study. Special thanks to Dr. Michael Bradley 204 for English editing of this manuscript. 205

Appendix 5: Abstract of published manuscript in *Scientific Reports* and acknowledgements listed in the manuscript

Scientific Reports 2018 May 31; 8:8474. https://www.nature.com/articles/s41598-018-26866-8

# SCIENTIFIC REPORTS

Received: 6 January 2018 Accepted: 21 May 2018 Published online: 31 May 2018

**OPEN** Diagnosis of atlanto-occipital dissociation: Standardised measurements of normal craniocervical relationship in finless porpoises (genus Neophocaena) using postmortem computed tomography

Brian C. W. Koto<sup>1,2</sup>, Derek K. P. Chan<sup>1</sup>, Adams H. L. Yuen<sup>1</sup> & Henry C. L. Tsui<sup>3</sup>

Due to the different craniocervical structures in humans and cetaceans, a standardised method assessing the normal craniocervical relationship in cetaceans is lacking, causing difficulties in defining the presence of atlanto-occipital dissociation (AOD) in cetaceans. The present study aimed to 1) describe a novel standardised method of determining the normal craniocervical relationships, and 2) define the 95% accuracy range of the normal craniocervical relationship in finless porpoises (genus Neophocaena), that allowed AOD diagnosis. Fifty-five out 83 stranded or by-caught finless porpoise carcasses were analyzed in term of their craniocervical relationship in dorsal-ventral and medial-lateral dimension, using postmortem computed tomography measurements. The normal craniocervical relationship in both dorsal-ventral (mean BD/OV: 0.87  $\pm$  0.24 [2 SD]) and medial-lateral dimension (mean VR/VL: 0.98 ± 0.17 [2 5D]) was first defined. The 95% accuracy ranges of the normal craniocervical relationship in dorsal-ventral (0.63-1.11) and medial-lateral dimension (0.82-1.15) were proposed. The baseline ranges could facilitate AOD assessment, and provide an objective means of record for AOD related injury and death of cetaceans caused by anthropogenic trauma. The technique developed may be applied to live cetaceans with abnormal craniocervical relationship to aid diagnosis and quide corrective therapy.

#### Acknowledgements

The authors would like to thank Agriculture, Fisheries and Conservation Department of the Hong Kong Special Administrative Region Government, and Institute of Hydrobiology, Chinese Academy of Sciences for the continuous support in this project. Sincere appreciation is also extended to veterinarians, staff and volunteers from Ocean Park Hong Kong, Ocean Park Conservation Foundation Hong Kong, Institute of Hydrobiology, Chinese Academy of Sciences, Tung Wah College and Cetacean Virtopsy Lab, for paying great effort on the stranding response, conventional necropsy and logistic arrangements in this project. Special gratitude is owed to technicians in Peace Avenue Veterinary Clinic, City University of Hong Kong, and Zhong Nan Hospital of Wuhan University for operating the CT units in the present study. Special thanks to Dr. Michael Bradley for English editing of this manuscript. This project was financially supported by the Marine Ecology Enhancement Fund (grant number: MEEF2017014/L01), Marine Ecology Enhancement Fund, 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. The pioneer stranded cetacean virtopsy project received financial support from the Hong Kong Research Grants Council (Grant number: UGC/FDS17/M07/14).