Conceptual and practical approaches to aid the conservation of cetaceans

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Abstract

Cetaceans are a diverse group of marine mammals with a global distribution and at risk of a of number natural and anthropogenic threats. There are gaps in our knowledge for some species (e.g., distribution, abundance), largely due to some species being more difficult to study because of their behaviour, rarity, or habitat remoteness. My thesis fills some of these conservation gaps. First, I present a review of known and emerging threats to species, highlighting the importance of distinguishing between threats acting at individual and population levels (Chapter two); threats acting at a population level are considered process thresholds, compromising the survival of a population or species. Secondly, I explore an example of a well-known anthropogenic threat, shipping, via the novel application of a terrestrial road ecology framework in the marine environment (Chapter three). This provides new insights to develop mitigation measures to reduce impacts from increased global shipping. Thirdly, I investigate the role of citizen science as a complementary tool for whale conservation (Chapter four). I demonstrate the benefits of citizen science-based studies as a robust, cost-effective and citizen empowering approach to monitoring wildlife over long time periods. Finally, I explore the use of emerging technologies such as Unmanned Aerial Vehicles (UAVs, or drones) for marine megafauna conservation via the development of a purpose-built low-cost drone for collecting whale lung microbiota (Chapters five and six). This approach can be used to provide a non-invasive, remote assessment of individual whale health and supporting data for long-term monitoring of population health.

Statement of Originality

I, Vanessa Pirotta, certify that this thesis entitled "Conservation of cetaceans" is an original piece of work and has not been submitted in whole or in part for a higher degree at any institution other than Macquarie University. This work was undertaken in the Marine Predator Research Group in the Department of Biological Sciences at Macquarie University under the supervision of Professor Robert Harcourt (Macquarie University), Dr. Ian Jonsen (Macquarie University) and Dr. Alana Grech (James Cook University). Funding was provided by an Australian Postgraduate Research Award.

This thesis was prepared and written by me. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself. All assistance in the preparation of this thesis has been acknowledged and all references and sources of information used in this thesis are listed within. Animal ethics approval was granted for the work presented in this thesis by the Macquarie University Animal Ethics Committee, under animal research authorities 2016/010-2 and 2016/010-4.

Vanessa Pirotta

July 2018

Statement of Authorship

Chapter Two: 'When threats become process thresholds'

This chapter identifies conservation priorities for cetaceans and provides a decision tree and framework for directing conservation action. Conception of the paper was by myself, Professor Rob Harcourt, Dr. Alana Grech and Dr. Ian Jonsen. I prepared the manuscript. Dr. Alana Grech, Dr. Ian Jonsen and Professor Robert Harcourt contributed critically to the final version of the manuscript and are listed as co-authors on the paper.

Chapter Three: 'Marine roads: Consequences of global shipping traffic for marine giants'.

This chapter presents a terrestrial road ecology framework to understand and mitigate the impacts of shipping activity on marine giants (the great whales, basking and whale sharks). Conception of this paper occurred at the Genes to Geosciences Enrichment Program at Macquarie University in 2015 with myself, Professor William Laurance and Dr. Ian Jonsen. I prepared the manuscript which has been accepted for publication in the journal *Frontiers in Ecology and the Environment* in June 2018. Significant contribution was provided by Professor Rob Harcourt, Dr. Alana Grech, Dr. Ian Jonsen and Professor William Laurance who are listed as co-authors on the paper.

Chapter four: 'A citizen science approach to long-term monitoring of humpback whales (*Megaptera novaeangliae*) off Sydney, Australia'.

This chapter explores the role of citizen science to support conservation research and demonstrate how it can be used to estimate whale population growth. Conception of the paper was by myself, Mr. Wayne Reynolds, Mr. Geoffrey Ross, Professor Rob Harcourt, Dr. Ian Jonsen, Dr. Alana Grech and Dr. David Slip. Data was collected by Mr. Wayne Reynolds and contributed to by myself, Mr. Geoffery Ross, Dr. David Slip and Professor Robert Harcourt. Statistical guidance was provided from Dr. Ian Jonsen. Data analysis was performed by myself and Dr. Ian Jonsen. I prepared the manuscript with significant contribution from Dr. Alana Grech, Professor Rob Harcourt, Dr. Ian Jonsen, Mr. Wayne Reynolds, Mr. Geoffery Ross and Dr. David Slip who are listed as co-authors on the paper.

Chapter five: 'An Economical Custom-Built Drone for Assessing Whale Health'.

This chapter describes a custom-built aerial drone for collecting whale lung microbiota (blow) as a tool for remote assessment of whale health. Paper conception was by myself, Mr. Alastair Smith, Professor Rob Harcourt, Dr. Martin Ostrowski, Dr. Ian Jonsen and Dr. Alana Grech. Together, myself and Mr. Alastair Smith designed, developed and constructed the waterproof drone with remotely operated flip lid petri dish. Experimental design was myself, Mr. Alastair Smith, Professor Rob Harcourt and Dr. Ian Jonsen. Field work and data (sample) collection offshore Sydney conducted by myself, Mr. Alastair Smith and Professor Rob Harcourt. I conducted laboratory techniques to process the samples with the assistance of Mr. Dylan Russell and Dr. Martin Ostrowski. Analysis and interpretation of data was conducted by Dr. Martin Ostrowski, myself, Professor Rob Harcourt, Dr. Ian Jonsen, Dr. Alana Grech and Mr. Dylan Russell. I prepared the manuscript with contribution from Dr. Martin Ostrowski, Professor Rob Harcourt, Dr. Ian Jonsen, Dr. Alana Grech and Mr. Alastair Smith.

Chapter six: 'Virological Sampling of Inaccessible Wildlife with Drones'.

This chapter characterised the virome of whale blow samples collected using an aerial drone. Paper conception was by Dr. Jemma Geoghegan, myself, Mr. Alastair Smith, Professor Rob Harcourt and Professor Edward Holmes. Field work and sample collection offshore Sydney conducted by myself, Mr. Alastair Smith and Dr. Jemma Geoghegan. Sample validation by Dr. Jemma Geoghegan, Dr. Jan Buchmann, Dr. John-Sebastian Eden, Ms. Erin Harvey and Professor Edward Holmes. Formal analysis by Dr. Jemma Geoghegan, Ms. Erin Harvey, Dr. John-Sebastian Eden and Dr. Jan Buchmann. Writing and original draft preparation by Dr. Jemma Geoghegan, myself, Professor Rob Harcourt and Professor Edward Holmes. Writing-review by Dr. Jemma Geoghegan, myself, Ms. Erin Harvey, Dr. Martin Ostrowski, Professor Rob Harcourt and Professor Edward Holmes.

Dr. Jemma Geoghegan, myself and Ms. Erin Harvey all contributed equally to the final publication.

Presentations

- **Pirotta, Vanessa.** Flying Science and whale snot off Sydney, Australia. Sydney Institute of Marine Sciences public lecture. 5 July 2018.
- **Pirotta, Vanessa.** Thar she blows: An economical custom-built drone for assessing whale health. Australian Marine Sciences Association 2018 Conference, Adelaide, South Australia. Monday 3 July 2018.
- **Pirotta, Vanessa.** Using drones to collect whale snot. International Famelab final and semifinal, Cheltenham Science Festival, United Kingdom, 8 June 2018.
- **Pirotta, Vanessa.** Using drones to collect whale snot. FameLab National final. Octagon theatre, University of Western Australia, Perth, Australia. 10 May 2018.
- **Pirotta, Vanessa.** Using drones to collect whale snot. FameLab NSW semi-final. Powerhouse Museum. 11 April 2018.
- **Pirotta, Vanessa.** *Thar she blows*, using drones for whale conservation. 3 Minute Thesis Competition. 7 September 2017.
- **Pirotta, Vanessa.** Whale Snot, Microbes and Ecosystem Health. Joint Academic Microbiology Seminar, Sydney, Australia. 24 April 2018.
- **Pirotta, Vanessa.** Whale hello Sydney: Whale observations and research, The Calyx, Royal Botanic Gardens, Sydney, Australia. November, 2017.
- **Pirotta, Vanessa.** Singapore Eco Film Festival, invited speaker. Arts Science Museum, Singapore. 31 August- 3 September 2017.
- **Pirotta, Vanessa.** Whales, Antarctica and Research. CSIRO onboard the *RV Investigator* voyage to Antarctica. January 2017.
- **Pirotta, Vanessa.** *Thar she blows*, using drones for whale conservation. 3 Minute Thesis Competition. Department of Biological Sciences. 28 August 2017.
- **Pirotta, Vanessa.** Use of unmanned aerial vehicles as tools for assessing whale health. Higher degree research conference, Department of Biological Sciences, Macquarie University. 2017.
- **Pirotta, Vanessa.** Highways of the sea: consequences of global shipping traffic for marine megafauna**.** Department of Biological Sciences Higher Degree Research annual conference, Macquarie University. 2016.
- **Pirotta, Vanessa.** Conservation for cetaceans in Australian waters**,** 2015. Department of Biological Sciences Higher Degree Research annual conference, Macquarie University.

Scientific posters

- **Pirotta, Vanessa**; Smith, Alastair, Ostrowski, Martin, Russell, Dylan, Jonsen, Ian; Grech, Alana and Harcourt, Robert. Whale snot, microbes and ecosystem health. Presented at the 7th Annual Joint Academic Microbiology Seminar conference, March 2018, Sydney, Australia.
- **Pirotta, Vanessa**; Jonsen, Ian; Grech, Alana and Harcourt, Robert. When is a threat a threatening process? Presented at 22nd biannual Society of Marine Mammalogy conference, October 2017, Halifax, Nova Scotia, Canada.

Awards

- Winner of the NSW Office of Environment & Heritage and the Ecological Society of Australia award for outstanding outreach for 2018. $AUD400
- **Winner** of the Sea World Research and Rescue Foundation best student oral in the area of Science and Conservation of Marine Vertebrates. Australian Marine Sciences Association 2018 Conference, 5 July 2018, Adelaide, South Australia. $AUD600.
- FameLab **International Runner Up**, 8 June 2018, Cheltenham Science Festival, United Kingdom.
- FameLab Australia **National Winner**, 10 May 2018, University of Western Australia, Perth, Australia. $AUD1000
- FameLab NSW Semi-Final, **Audience choice**, 11 April 2018, Sydney Power House Museum, Sydney, Australia.

I am passionate about science communication and the FameLab competition was one of my greatest achievements during my Ph.D. I was extremely proud to have won the Australian national competition (left picture) and to have represented Australia internationally in the United Kingdom (right picture, myself with winner Siti Khayriyyah Binti Mohd Hanafiah from Malaysia and second runner up Veli Vural Uslu from Germany).

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Awards continued:

- **Winner of best poster** at the 7th Annual Joint Academic Microbiology Seminar conference, March 2018, Sydney, Australia. $AUD500
- Student travel grant to the 22nd biannual Society of Marine Mammalogy conference, October 2017, Halifax, Nova Scotia, Canada. $USD1000
- **Winner** of the Faculty of Science and Engineering Three Minute Thesis Competition Final, September 2017, Sydney, Australia. $AUD700
- **Best presentation** of modelling/theoretical based research 2016, Department of Biological Sciences Higher Degree Research annual conference, Macquarie University. $AUD100

Additional publications (completed whilst doing Ph.D.)

- Tulloch, V., **Pirotta, V.**, Jonsen, I., Grech, A., and Harcourt, R. 2017. National Assessment of Cetacean Entanglements in Fishery Gear in Australia: Final Report. Macquarie University, Sydney, Australia.

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My Ph.D. has been a wonderful and positive journey. Never have I had a time in my life where I have been able explore my passion and develop my career professionally along the way. My enthusiasm for cetaceans and conservation has driven me throughout. During this journey, I have been supported by many wonderful people who I would like to thank.

Firstly, I would like to thank the people who have been there since the start, my supervisors, the dynamic trio; Rob Harcourt, Ian Jonsen and Alana Grech. Rob, it may have taken six months worth of phone calls but you delivered on your promise to offer me a research assistance position back in 2012. You have provided me with so many wonderful opportunities and I am so thankful to have taken them all on. I am so happy to now have the opportunity to officially thank you for it. THANK YOU! I have never looked back since commencing this wonderful journey and I intend to keep this alive. I thank you for your optimism, support, and encouragement and for always being that cheer squad throughout this journey. I am so thankful I was finally able to see killer whales with you in Canada and I will always remember that as a wonderful celebration of our journey together. I hope to continue our friendship throughout the next stage of my career.

Ian, thank you for your continued support, guidance, and grounded advice. I remember back to the marine mammal conference in San Francisco 2015 where the majority of talks made reference to Jonsen et al. I was and still am so proud to have you as my supervisor! I have thoroughly enjoyed working with you. I have always loved your contribution to science and how you help us understand animal movements in the ocean. This is fascinating and obviously beneficial to how we protect our marine wildlife. Thank you. And finally, Alana, thank you. I am so proud to have been supervised by such a dynamic, vivacious, influential, positive, strong woman. You are a true role model for me as a female finishing her Ph.D. Thank you for always having a can-do approach to everything and for always be supportive and encouraging throughout my Ph.D. journey. I am also incredibly proud of the work you do for protecting our marine environment and to have had you as a supervisor. Thank you!

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To my very beautiful sister Tania. Thank you. You are so special to me and I am forever grateful of your support. Thank you for always being there to direct me and advise me on everything. You have always been there for me. Thank you also to Richie, little George, and Louis for their loving support.

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Finally, to my late mother Rosa. Thank you for providing me with the most wonderful opportunities in life. Your strength, energy, positivity, dedication, commitment and love truly made me into who I am today. Your sudden passing gave me a realisation to make the most of everything and has given me the determination to achieve every goal. My Ph.D. is a testament to this. I wish you could have seen my journey. Out of everyone I know you would have been the proudest, especially when I had the opportunity to represent Australia in the FameLab international finals and when interviewed on TV. This is exactly the kind of journey we would

joke about. Unfortunately, I will never be able to share this with you but you will forever be in my memory and I have always thought of you during my most memorable achievements throughout my Ph.D. Thank you.

<div align="center">Dedication</div>

This thesis is dedicated to my late mother Rosa and to a man who has done so much for the conservation of wildlife and is also a great science communicator, Sir David Attenborough. I wrote to David at the start of my Ph.D. and he kindly wrote back…..

<div align="center">8.2.15</div>

Dear Vanessa Pirotta,

Thank you for your letter. I am glad to know that you think well of my programmes. It was very kind of of you to have written to tell me so.

I wish you every success in achieving your bucket list

Yours sincerely

David Attenborough

from David Attenborough

<div align="center">I will cherish this forever.</div>

"When everything seems to be going against you, remember that the airplane takes off against the wind, not with it."

— Henry Ford

"Do the best you can until you know better. Then when you know better, do better."

— Maya Angelou

Chapter One

1.1 General Introduction

The conservation of wildlife

Wildlife conservation requires learning about individuals, populations, and species in order to conserve them. Conservation is defined as the preservation, protection, or restoration of wildlife (Oxford Dictionary 2018); the subgenre of conservation biology is concerned with the biology of species, communities, and ecosystems impacted either directly or indirectly by natural or anthropogenic factors (Soulé 1985). A goal of conservation biology is to provide tools and principles for preserving biological diversity (Soulé 1985). Conservation biology is also known as a crisis discipline, often requiring conservation action before important information is known or when there is considerable uncertainty (Soulé 1985, Bottrill et al. 2008).

Knowing when and where to direct conservation resources is important to ensure conservation actions are prioritised and resources are not misdirected. A well-known international model for assessing wildlife conservation priorities is set by the *International Union for Conservation of Nature* (IUCN) (IUCN Red List 2018). The IUCN Red List of Threatened Species has an established set of criteria that can be used to systematically assess species' extinction risk. This can be used to identify the conservation status of wildlife internationally, which can then be used to help direct conservation resources at national, state, and local levels where species reside.

Where conservation falls short

Despite international attempts to identify wildlife most in need, conservation priorities and resources are sometimes misidentified and misdirected. This may be a result of challenges in identifying threats to populations and difficulties in acquiring population information for wildlife e.g., data deficient species or species which detract from real conservation issues (i.e., "welfare cases"; Bradshaw and Bateson 2000). As an example of the latter, resources may be directed towards particular cases which humans find distressing due to animal welfare concerns under the excuse of conservation needs (McMahon et al. 2012). As a result, conservation efforts may be misdirected to species with a lower extinction risk, rendering more threatened species vulnerable to extinction. In contrast, for cases where conservation needs have been correctly identified, efforts may fall short due to logistical challenges, lack of funding/resources or conservation efforts may be required over prolonged periods of time.

Conservation in the terrestrial and marine environment

Wildlife differ physiologically, behaviourally, inhabit different environments, have different community structures and population dynamics, and varied lifecycles (or life stages) (Deem et al. 2001, Festa-Bianchet 2003). As a result, no single blanket approach exists for wildlife conservation. At the highest level, wildlife is protected under international and national legislation e.g., The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the United States Endangered Species Act, and the Australian *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) (Nurse 2015). Legislation is often informed by scientific research used to better understand wildlife and their conservation needs. Information gained from scientific research methods may also be used to inform the location of protected areas and conservation management (McGowan et al. 2017). For example, understanding conservation needs for terrestrial wildlife may include methods such as visual observations, camera traps (He et al. 2016), capturing individuals (Royle et al. 2017), aerial surveys (Gonzalez et al. 2016), and molecular techniques e.g., DNA from animal hair, faeces, urine, feathers and environmental DNA (Waits and Paetkau 2005, Bohmann et al. 2014). Similarly, conservation efforts of marine wildlife may use a number of techniques implemented in the terrestrial environment specifically adapted to monitoring wildlife in the ocean e.g., acoustic monitoring (Sousa-Lima et al. 2013, Costello et al. 2017), satellite tracking (Block et al. 2011), light-level geolocation (Hill and Braun 2001), and acoustic telemetry (Hussey et al. 2015). In addition, habitat modelling may be used to inform threat assessments and protected area designations. For example, cetacean sighting data, including location and time of year can be used within statistical modelling to help inform habitat use and identify areas in need of protection (Redfern et al. 2017, Storrie et al. 2018). Habitat models have also proven successful in predicting cetacean distributions in data-poor environments, where ecosystem-specific models have been used to predict important blue whale habitat, which can be used for future research and monitoring efforts (Redfern et al. 2017).

Conservation of cetaceans

Cetaceans (whales, dolphins, and porpoises) are a diverse group of marine mammals with a global distribution. Biologically, cetaceans are divided into two groups; Odontocetes or toothed whales e.g., sperm whales, beaked whales, dolphins, and porpoises, and Mysticetes or baleen whales (toothless whales). Species are found throughout polar regions i.e., the Arctic and Antarctic and temperate and tropical waters. Some species persist locally, with a relatively

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small distribution e.g., many coastal dolphin populations, while others move across large geographical ranges each year during their annual migration e.g., baleen whales. Such widespread distribution and species diversity presents conservation challenges for cetaceans. There is a lot of information about some species and very little about others leading to gaps in knowledge that limits our ability to focus conservation efforts. Many species are logistically challenging to study due to their behaviour, remoteness or rarity and as a result, many have not yet been studied and/or remain data deficient (Allen and Singh 2016). This makes it difficult to understand the impact of threats for some species and implement conservation action.

International protection of cetaceans

The global range of the cetacean clade means species exposed to risk from a multitude of threats within the marine environment. These include both natural threats such as extreme weather events (e.g., cyclones and hurricanes), and threats from anthropogenic activities e.g., fishing, vessel disturbance, ship strike, and marine pollution (Fossi et al. 2018, Peel et al. 2018). The International Whaling Commission (IWC) has a global responsibility for the conservation and management of cetaceans (International whaling Commission 2018). The *International Convention for the Regulation of Whaling* (1946), also known as the Convention, includes a legally binding Schedule which set catch limits for commercial and aboriginal subsistence whaling (International whaling Commission 2018). The Convention is supported internationally by 87 Governments from countries around the world as signatories, including Australia (International whaling Commission 2018).

Australia's protection of cetaceans

Australia is host at least 45 species of cetaceans (10 large whales, 20 smaller whales, 14 dolphins, and one porpoise) (Australian Government 2018). Australia has comprehensive legislation intended to provide a high level of protection of cetaceans and other marine fauna under its jurisdiction, supported by efforts to increase understanding through research. Australia also plays a leading role in a number of international treaties and conventions including the IWC, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Convention on the Conservation of Migratory Species of Wild Animals (CMS), and the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Harcourt et al. 2014).

At the National level, all cetaceans are protected under the EPBC Act, which is Australia's primary environmental legislation (Harcourt et al. 2014). To protect cetaceans throughout Australian waters, the Australian Whale Sanctuary has been established which includes all Commonwealth waters from the three nautical mile state waters limit out to the boundary of the Exclusive Economic Zone i.e., out to 200 nautical miles and further in some places (Figure 1.1) (Australian Government 2018). Remote areas such as Antarctica are also included in the Australian Whale Sanctuary to account for migratory species, which are species known to pass within Australian waters during their annual migration. Species identified as migratory are those identified in the CITES (also known as the Bonn Convention, Appendices I and II) (Australian Government 2018). In addition, cetaceans are also protected in state and territory waters, within three nautical miles of the coastline (Australian Government 2018).
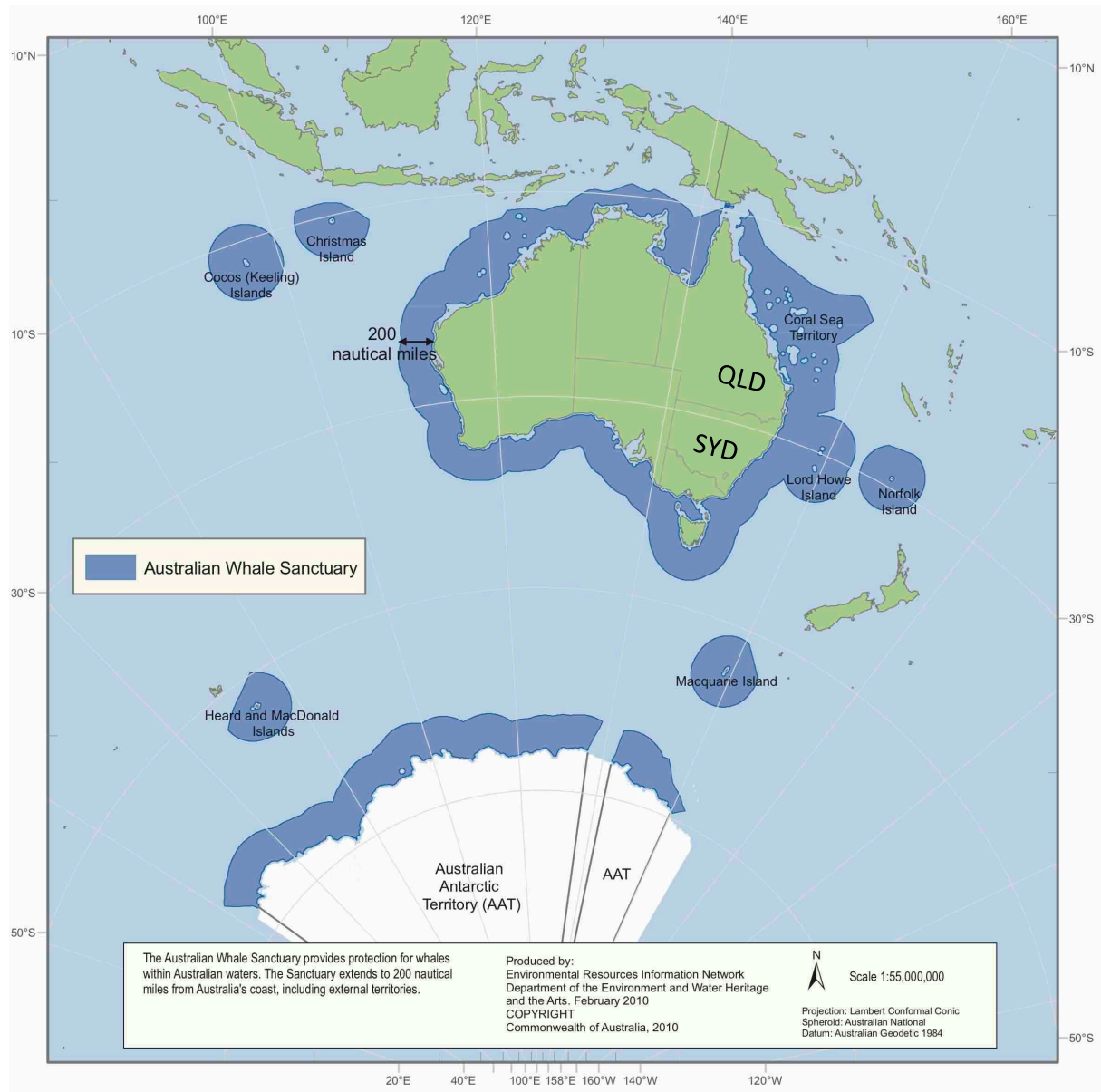
Figure 1.1: The Australian Whale Sanctuary. The extent of the Australian Whale Sanctuary (dark blue), surrounding Australia, areas of Antarctica and small surrounding islands. This is to ensure protection of migratory species. The states of Sydney (SYD) and Queensland (QLD) are marked. From Australian Government, 2018.

Conservation gaps and monitoring as a tool for conservation

Protecting cetaceans in Australian waters from multiple anthropogenic threats and informing conservation actions is challenging (Harcourt et al. 2014). One way of understanding cetacean interactions with threats is monitoring. By definition, the purpose of monitoring is to set clear objectives and (1) to provide information on abundance of a species, at one or more locations at a number of points in time, (2) record responses to changes in the environment and (3) determine effectiveness of a form of management (Goldsmith 1991, Magurran et al. 2010).

Monitoring is usually undertaken to determine whether prevailing conditions (e.g. behavioural, physiological, ecological or environmental) match with previous standards or norms (Goldsmith 1991). It can also be used as a way of better understanding more about a species over time, which can be used to infer baseline information e.g. habitat use, abundance and seasonal distributions. In addition, monitoring may be implemented to assess policy or legislation effectiveness and to act as an early warning tool for changes in the population (Goldmsith 1991). However, understanding the impact of multiple threats for cetaceans is difficult to measure and predict. Monitoring cetaceans is challenging due to their distribution, behaviour, and for some species, remoteness or rarity (Grech 2009). New approaches to obtain information currently unavailable to better inform and improve the design of future management action is required (Grech 2009).

1.2 Thesis aims

The objectives of this thesis are to examine conservation threats to cetaceans, explore alternative methods for mitigation and develop, and refine new technologies for cetacean conservation. This thesis accomplishes this by focussing on four topics:

1. Identify conservation priorities for cetaceans and provide a decision tree and framework for directing conservation action (**Chapter two**).
2. Better understand and mitigate the impacts of shipping activity on the great whales, basking and whale sharks by presenting a road ecology framework to assess the ecological consequences of marine shipping corridors (**Chapter three**).
3. Explore the role of citizen science to support conservation research and demonstrate how it can be used to estimate whale population changes (**Chapter four**).
4. Develop new technologies, in this case drones, for remote assessments of whale health by collecting and describing their microbial communities and viruses (**Chapters five and six**).

1.3 Thesis structure

This thesis includes an introduction and three themed sections: (1) conservation principles, (2) conservation resources and (3) conservation technologies, comprised of four chapters. It concludes with a general discussion. Although each chapter has been written as a separate paper, all contribute to the overall theme of cetacean conservation.

Section one: Conservation principles

In **section one**, I explore themes of conservation principles, which form the basis of the thesis. This section includes chapters two and three.

In **chapter two**, I present a framework that can be used to inform the prioritisation of threats to cetaceans. This chapter sets the platform for the entire thesis. Here I clearly define the terms *threat* and process thresholds and provide examples where threats act at the individual and population level. These are terms commonly used in management frameworks for biodiversity both at an international level such as the IUCN and at a national level such as the Australian EPBC Act. However, there is occasional confusion over the term threatening process (process thresholds), which I define as an activity that has an impact at population level or above, rather than something causing harm to a few individuals of a population (a threat). Distinguishing the two is important when there are limited conservation resources, and this can help avoid misdirection of effort to individual welfare cases rather than a process in support of conservation goals. To help clarify this issue, I present a decision tree that can identify major threats to cetaceans and incorporates the use of a precautionary principle when there is major uncertainty to identify the threats with the greatest level of impact. Given the complexity and the interactions of multiple stressors on cetaceans, I also present a conceptual framework within which to consider the impact of cumulative threats.

In **chapter three**, I explore an example of one of these threats to cetaceans, the growth of shipping (vessel disturbance), which has been identified globally as a major threat to some cetaceans. Shipping was chosen as it is a good example to demonstrate the application of the clearly-defined terms identified in chapter one, as I present unequivocal evidence that shipping is acting as a threatening process for some populations but not for others. I apply ecological principles derived from the terrestrial road ecology literature to improve the way we mitigate shipping impacts on cetaceans. Using a terrestrial road ecology framework in the marine environment, mitigation principles are broader and are of a substantively different nature compared to when impacts are only assessed individually, based on what we understand from mechanical processes. I also demonstrate how road ecology can be used to help reduce the impact of future marine road expansion in a systematic fashion by incorporating knowledge of existing shipping impacts in the marine environment and their consequences for marine megafauna

Chapter three has been accepted for publication in the journal *Frontiers in Ecology and the Environment*:

Pirotta, V., Grech, A, Jonsen, ID, Laurance, William F. and Harcourt R (2018) Marine roads: Consequences of global shipping traffic for marine giants. Accepted. *Frontiers in Ecology and the Environment*.

Section two: Conservation resources

In chapters two and three I highlight the issue that even if cetacean populations are recovering, resources for assessing present and future impacts and monitoring recovery are usually limited. To help address this concern, I explore the role of citizen science in **chapter four** to be used as a way of leveraging information, collected with the efforts of the general public, about growing populations to support conservation management. By applying well-established ecological principles and by making data gathering processes simple and robust, we can employ citizen science to derive reliable data on population recovery and other aspects of cetacean monitoring. I provide a specific example for a cetacean population which is recovering post whaling in Australian waters, the east coast humpback whale. I analyse a 20-year dataset (1997-2017) of whale sightings off Cape Solander, Sydney, collected by citizen scientists during the Cape Solander Whale Migration Study. I explore what components of the process have allowed the dataset to be robust and compare the findings with systematic land-based surveys (focused on the same population) in Queensland (north of Sydney) (Figure 1.1). I demonstrate the utility of citizen science based studies like this, and show that they can provide a robust, cost-effective and citizen empowering approach to gathering simple measures for monitoring wildlife over the period necessary to detect change in a population.

Chapter four is currently in review for publication in the journal *Marine Mammal Science*:

Pirotta, V., Reynolds, W., Ross, G., Jonsen, I., Grech, A., Slip, David and Harcourt, R. (2018) A citizen science approach to long-term monitoring of humpback whales (*Megaptera novaeangliae*) off Sydney, Australia. *Under Review.*

Section three: Conservation technologies

Continuing the theme of limiting resources for assessing and monitoring, in **chapters five and six** I investigated the application of new and economical techniques for monitoring. I developed

bespoke technology that utilizes aerial unmanned vehicles (drones) to collect whale exhalation (blow) samples; these samples provided a direct means to assess the health of the recovering east coast humpback whale population. Despite the threat of ship strike and fishing gear entanglements of individuals, this population does not seem to be subject to any specific threatening processes and is a good model species for this research as there are large numbers of individuals which facilitates testing drone sampling on multiple animals. As part of this, I develop an experimental approach to assess whale health. I collaborated with a drone expert to develop waterproof, remotely-piloted aircraft/drones for sampling whale blows. This method is a much safer alternative for researchers and whales in comparison to methods for example that require a close vessel approach and a long pole with a collection device at the end. A unique feature of our drone is the flip-lid petri dish which opens and closes remotely to minimise sample contamination. This design is aimed at addressing several sampling challenges: accessibility; safety; cost, and critically, minimizing the collection of atmospheric and seawater microbiota and other potential sources of sample contamination.

In **chapter five**, I assessed microbiological communities from the blows of northward migrating humpback whales off Sydney, Australia. I used the drone's on-board camera to validate sample collection and attempted to identify individual whales based on their unique colours patterns and scars. To process the samples, I used a variety of laboratory techniques including DNA extraction methods and PCR to prepare the samples for next generation genetic sequencing. High throughput sequencing of bacterial ribosomal gene markers was used to identify respiratory tract microbiota. I used model-based comparisons with seawater and drone-captured air to demonstrate that the flip lid system on the drone minimised external sources of contamination and successfully captured material to identify whale blow-specific microbial taxa. From these findings, I describe whale-specific taxa and a baseline of respiratory tract microbiota profiles of east Australian humpback whales. This paper is published in the journal *Frontiers in Marine Science*:

Pirotta V., Smith A., Ostrowski M., Russell D., Jonsen I.D., Grech A. and Harcourt R. (2017) An Economical Custom-Built Drone for Assessing Whale Health. Front. Mar. Sci. 4:425. doi: 10.3389/fmars.2017.00425

Using the same collection method in the previous chapter, **chapter six** explores the virome of humpback whales. In a pilot study, I and my collaborators characterised the virome of 19

pooled samples of whale blow using a meta-transcriptome analysis. We attempted to identify novel viruses. To my knowledge, this is the first time a drone has been used to sample whale viruses. This paper has been published in the journal *Viruses*:

Geoghegan, J.L., **Pirotta, V**., Harvey, E., Smith, A., Buchmann, J.P., Ostrowski, M., Eden, J., Harcourt, R., Holmes, E.C. (2018). Virological Sampling of Inaccessible Wildlife with Drones. *Viruses,* 10, 300: 1-7, doi: 10.3390/v10060300

The methods developed and reported on in chapters five and six can be employed to monitor populations exposed to continuing anthropogenic stressors in different parts of the world e.g., the North Atlantic right whale and the southern right whale. Using drones has reduced the risk associated with collecting health information from whales (Apprill et al. 2017, Christiansen et al., 2016, Christie et al., 2016, Pirotta et al. 2017), and has wide application. For example, this technology would benefit existing whale research programmes such as Southern Ocean whale research.

The following provides a visual representation of my thesis structure:
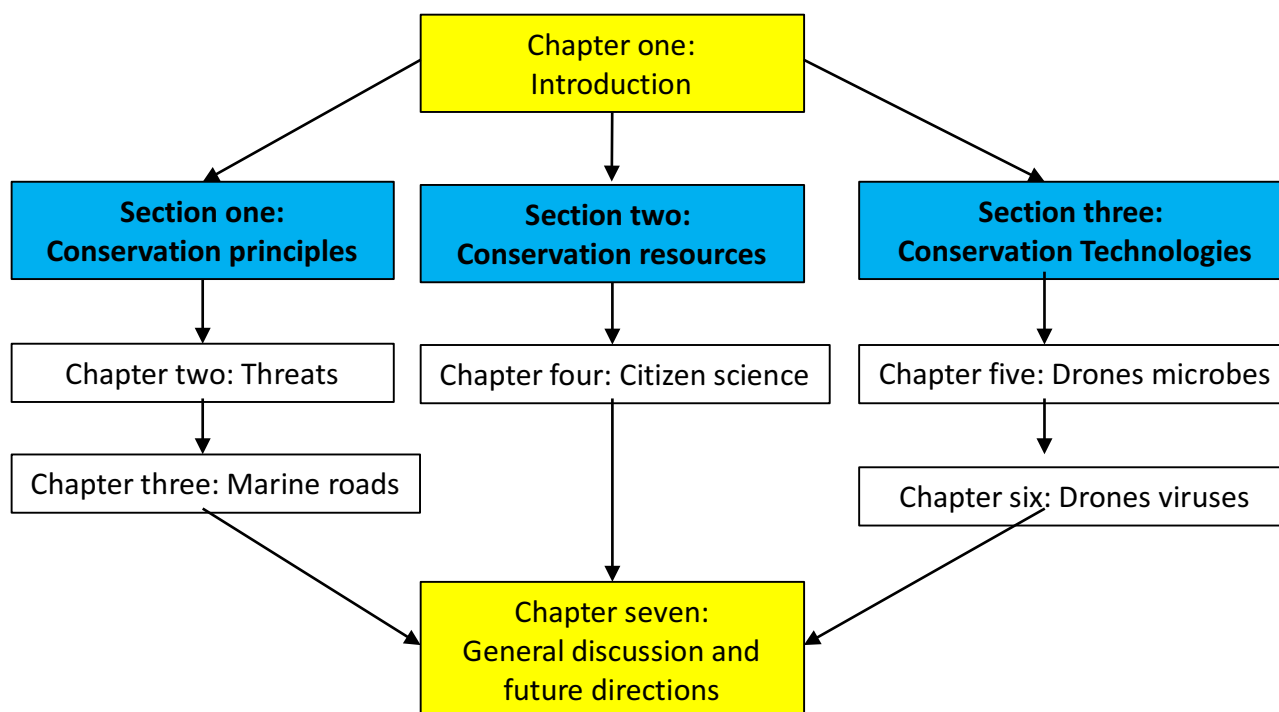
Figure 1.2: Summary figure of my thesis. This figure shows the overall structure of this thesis and the links between chapters.

Section one:

Conservation principles
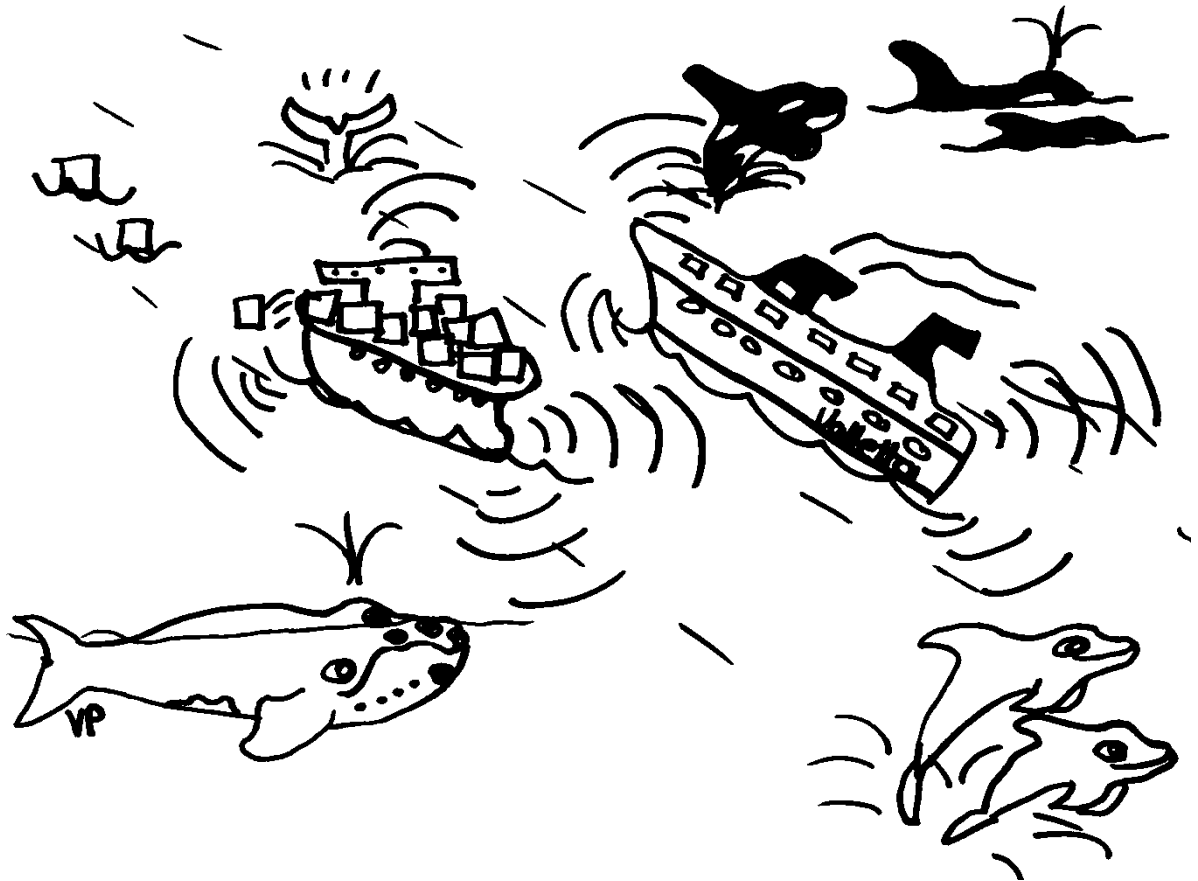
Chapter Two

2.1 When threats become process thresholds

This chapter has yet to be submitted for publication:

Pirotta, V., Grech, A, Jonsen, ID, and Harcourt R (2018) When threats become process thresholds.

Abstract

Wildlife throughout the world are exposed to multiple anthropogenic threats [1]during their lifetime. Threats are processes that harm or kill individuals (individual level impact) and/or threaten the existence of an entire population (population level impact). In order to prioritize conservation needs, it is important to correctly identify threats acting at an individual level or population level (when the majority of the population is impacted) because threats at a population level may result in loss of populations or extinction of the species. Cetaceans (whales, dolphins and porpoises) are a diverse group of marine mammals that may require different conservation actions for each species. There is a disproportionate amount of biological information about some species of cetaceans relative to others (e.g., data deficient species), complicating conservation decision making. Here we provide a concise review of threats to cetaceans based on their proximate drivers (what causes the threat) and pressures (the impact) of known threats. The aim of this paper is to act as an improvement for listing threatened species and preparing recovery plans. To do this, we present a decision tree to attribute the appropriate (based on degree of impact) level of effect and hence unambiguously define how a process acting at the population level is threatening. Given the complexity and the interactions of multiple pressures on cetaceans, we also provide a conceptual framework for considering cumulative impact from multiple threats. Use of the decision tree and framework could be beneficial to conservationists and policy/decision makers when prioritizing conservation resources for cetaceans in data poor environments as it can be used to identify knowledge gaps and conservation needs.

2.2 Introduction

Wildlife conservation aims to protect populations, species or communities of wild animals to ensure their long-term survival. The primary goal of conservation is to reduce the interaction

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[1] Also known as a stressor (Halpern et al. 2008).

between wildlife and their threats throughout their lifetime (Baillie et al., 2004). A threat is defined as an action likely to cause damage or danger, such as physical injury or mortality (Oxford Dictionary 2018). Threats to wildlife may be a result of natural processes (e.g., a predator or lack of food) or induced by anthropogenic pressures (e.g., habitat fragmentation, pollution, trophy hunting). Threats are either well-described or emerging, and for the latter, the impact on wildlife are not yet understood. For example, human poaching is a recognised threat to African rhino populations (white *Ceratotherium simum* and black *Diceros bicornis*) where individuals are killed for their horns and other products (Hübschle, 2017). By contrast, tuberculosis in African rhino populations is an example of an emerging threat as the extent of its impact is not yet known (Miller et al., 2017). When defining threats to wildlife, it is critical to identify the level at which the threat is acting because a threat may impact a few individuals (individual level) or an entire population (population level), and the consequences are different for species persistence. Threats acting at the population level may threaten the survival of populations and even threaten the existence of an entire species (Mace and Lande, 1991; Burgman et al., 2007).

Getting it right for conservation: distinguishing a threat from a process threshold
Distinguishing threats from a process threshold enhances the likelihood of taking appropriate conservation actions and species prioritization, with the aim of preventing population losses or extinction (Brooks et al., 2006). For example, any threat that negatively affects the critically endangered Javan rhino (*Rhinoceros sondaicus*) or the world's rarest mammal, the vaquita porpoise (*Phocoena sinus*) is a process threshold due to the small population size and restricted distribution of both species (Jaramillo-Legorreta et al., 2017; Setiawan et al., 2017). By contrast, threats to individuals in much larger populations e.g., entanglement in fishing gear of Australian humpback whales *Megaptera novaeangliae*, are no longer considered a process threshold because their population has largely recovered (Bejder et al., 2015).

Correctly identifying threats and process thresholds is integral to ensuring conservation resources are managed appropriately at both a species and community level. To support this, there are existing processes for determining species at risk of threats e.g. the threatened species list criteria listed under the Australian Federal Guidelines for assessing the conservation status of native species under the EPBC Act. Despite this, resources may be directed towards particular cases which humans find distressing on the basis of animal welfare concerns, yet under the guise of conservation needs (McMahon et al., 2012). For example, the release of

Keiko the killer whale was forced due to public concern with health implications of a single individual. Yet the release was argued for frequently under the banner of killer whale conservation (Kellow, 2007). Arguably freeing a single individual at a cost of millions of dollars is less important in comparison with the conservation needs of entire killer whale populations, e.g., the endangered southern resident killer whales (Ford et al., 2018), and may be problematic if limited resources are redirected. In order to identify a process threshold, detailed knowledge about a species is required, particularly information about their biology, behaviour, population abundance, distribution, and identification of known/emerging threats and their impacts (Fryxell et al., 2014; Allen and Singh, 2016). As a result, conservation actions are often directed to species that are data rich and do not account for, or overlook, species with knowledge gaps (data deficient) (Morais et al., 2013).

Gaps in knowledge: The conservation of cetaceans

Cetaceans (whales, dolphins and porpoises) are a diverse group of marine mammals, some of which are distributed globally. Their mobility means cetaceans are at risk of multiple threats within the marine environment. These include natural threats such as severe weather events (e.g., cyclones and hurricanes) and anthropogenic threats such as vessel disturbance, bycatch, and marine pollution. However, the level at which cetaceans are at risk from threats varies with their ecology and in particular their distribution. For example, some species are locally resident with a relatively small distribution e.g., coastal dolphin populations such as the resident bottlenose dolphin population in Port Stephens, Australia, which are vulnerable to vessel interactions throughout their home range (Steckenreuter et al. 2012). While others migrate across large geographical ranges each year during their annual migration e.g., baleen whales, where threats may vary with location and intensity. In addition, species with a large geographic range might be just as susceptible to impacts of a threat if that threat affects a critical aspect of their life history, such as displacement from key feeding or breeding grounds, which might only make up a small portion of their total range. For example, North Atlantic right whales are susceptible to ship strike throughout most of their range however, during times of breeding, this risk is seasonally heightened due to shipping traffic being within close proximity of breeding grounds (Silber et al. 2012, van der Hoop et al. 2015). We have knowledge gaps for some species, which limits our ability to inform conservation actions. In addition, many species are logistically challenging to study due to their behaviour, remoteness or rarity and as a result, many are data deficient (Allen and Singh, 2016). This makes it difficult to understand the impact of threats for some species and adequately inform activities to benefit their conservation.

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Understanding and prioritizing the impact of threats to cetaceans

The goal of this paper is assist existing processes for assessing threatened species by providing a framework to inform the prioritisation of threats to cetaceans by clearly defining the difference between a (1) threat, an adverse process operating at the individual level, and (2) process threshold, an adverse process operating at the population level. Distinguishing between a threat and a process threshold will provide improved confidence for conservation management when limited conservation resources are allocated to populations or species. A better understanding of populations or species identified at the process threshold level will enable conservation management to direct resources towards protecting those most in need. This may include allocating funds into to understanding why populations or species are at risk such as funding for monitoring e.g. abundance estimates/surveys. In contrast, this framework can be used as an early warning tool by conservation management by encouraging early assessment of threats to populations or species before they transition into population level consequences.

We provide a clear definition of threats and process threshold (Table 2.1) by presenting examples where threats are acting at the individual or population level. We reclassify threats to cetaceans based on their proximate drivers (what causes the threat) and pressures (the impact) (Table 2.1). We also highlight threats where the level of impact is not well understood i.e., threats known to cause harm to individuals but the level at which the threat is acting is difficult to infer. To help identify the major threats to cetaceans, we present a decision tree to attribute the appropriate level of effect and hence unambiguously define what is and what is not a process threshold. We use this decision tree, while incorporating the precautionary principle when there is major uncertainty, to identify the threats with the greatest level of impact. Given the complexity and the interactions of multiple stressors, we finish by providing a conceptual framework within which to consider the impact of cumulative threats, when multiple threats are impact individuals but not at a level to cross the threshold into population level impacts.

Term	Definition	Reference
Threat	An action likely to cause damage or danger.	(Oxford Dictionary 2018)
Process threshold (also known as a threatening processes)	Threat or threats acting at the population level. Also known as a process that may detrimentally affect the survival, abundance, distribution, or potential for evolutionary development of a native species or ecological community.	(Burgman et al., 2007)
Proximate driver	Immediate/cause of a particular phenomenon to happen or develop.	(Oxford Dictionary 2018)
Stressor	An activity (e.g., dredging, netting) that causes a direct, physical or biological impact/effect on cetaceans.	(Maxwell et al. 2013)
Cumulative impact	Cumulative change that results from the synergistic interactions of multiple past, current and future activities and stressor.	(Spaling and Smit, 1993)
Risk	A function of the consequence of a threat and the likelihood that a threat event occurs.	(Oxford Dictionary 2018)

Table 2.1: Definitions of terms associated with environmental impact. The above are common terms discussed within conservation literature.

Threat (likely to cause damage or danger)	Proximate driver The source/what causes it (Immediate/likely cause of a particular phenomenon to happen or develop)	Stressor (the impact, an effect on cetaceans) (Maxwell et al. 2013)
Vessel disturbance	Marine industries e.g., tourism, shipping, fisheries, underwater construction, oil and gas exploration. Recreational vessel use.	Ship strike, acoustic pollution (noise) leading to physiological and behavioural effects (Rolland et al. 12 and 17), chemical pollution e.g., oil spills. Degradation of environment via the transmission of invasive by the release of ballast water.
Fisheries interaction	Fisheries. Placement of gear in marine environment.	Entanglement in fishing gear. Chronic injury from gear encounters.
Marine pollution	Land based activities, shipping, abandoned fishing gear, introduction of micro and macro plastics, underwater construction, oil and gas exploration, tourism.	Introduction of contaminants to the marine environment (runoff), ingestion, oil spills, entanglements
Climate change	Burning of fossil fuels for domestic and industrial purposes e.g., vehicle emissions. Natural based sources e.g., climatic events, cyclones, flooding.	Ocean warming and acidification altering ocean chemistry. Loss of prey species. Epizootic events e.g., disease outbreaks.
Habitat modification	Industry activities e.g., underwater construction, fisheries, shipping, coastal development, climate change.	Alteration and degradation of marine habitat. Changes in soundscapes. Reduced of prey availability. Removal of habitat important for critical behaviour - breeding or foraging areas.

Table 2.2: Summary of known threats to cetaceans, their drivers (the source/what causes it), and stressors (the impact).

2.3 Review of threats to cetaceans

The following provides a concise summary of the known threats to cetaceans by identifying their proximate drivers and pressures and noting the circumstances under which each is considered a threatening process (Table 2.2). These examples were chosen as they represent well-known threats to cetaceans. A description of threats is useful for conservation managers when using the conservation decision tree (Figure 2.1) and cumulative assessment framework (Figure 2.3).

1.0 Vessel disturbance

Vessel disturbance is a well-documented threat to cetaceans, the most direct form of which is ship strike (Vanderlaan and Taggart, 2007; Peel et al., 2018; Pirotta et al., 2018). Ship strike can be fatal or result in serious trauma/injury (Laist et al., 2001; Van Waerebeek et al., 2007; IWC, 2015), and has mostly been documented for large whales, and less so for smaller cetaceans, (Doughty et al., 2016). Shipping has been identified as a process threshold for many species including the western grey (*Eschrichtius robustus*) (Bradford et al., 2009), Bryde's (*Balaenoptera edeni*) (Constantine et al., 2015), blue (*Balaenoptera musculus*) (Priyadarshana et al., 2015), and the north Atlantic right whale (*Eubalaena glacialis*). For the latter, ship strike is responsible for over half of all known mortalities in recent decades and is a major limiting factor to the species' survival (Meyer-Gutbrod and Greene, 2017). Ship strike has also been identified as a threat, but not a process threshold, for other species globally. This is because either a small number of animals are killed relative to the population size or the population remains data deficient and the extent of the threat has not been determined. Examples include the southern right (*Eubalaena australis*), sei (*Balaenoptera borealis*), minke (*Balaenoptera acutorostrata*), and sperm whale (*Physeter macrocephalus*) (Jensen et al., 2004; Peel et al., 2018). Vessel strike is less well documented for smaller and coastal cetacean species which more commonly interact with smaller vessels and appear less likely to interact adversely with larger vessels (Van Waerebeek et al., 2007). Vessel noise is also a form of vessel-related disturbance and is discussed in the *Acoustic pollution* section below.

2.0 Marine pollution

Pollution can be defined as any form of contamination in an ecosystem that can have negative impacts upon organisms (Clark et al., 1989). Marine pollution is a threat to cetaceans in many

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forms including marine debris (e.g., discarded fishing gear and plastics), environmental contaminants (e.g., vessel oils and oil spills, shipping emissions, land runoff), and noise (e.g., shipping, underwater construction, oil and gas exploration, seismic, sonar) (McKenna et al., 2012; Hassellöv et al., 2013; Fossi et al., 2018).

2.1 Marine debris

One of the most prevalent types of marine pollution is marine debris. This can impact whales via entanglement (bycatch) or ingestion, and has been documented in over 60% of all cetacean species (Fossi et al., 2018). Entanglement in fishing gear is one of the most widespread threats to marine mammals and can cause serious injury to cetaceans by physical harm (e.g., tissue damage, cuts and scarring), and energetic cost that may eventually lead to non-natural mortality (Read et al., 2006; Cassoff et al., 2011). Cetacean entanglements can involve active (fixed) or passive (ghost or discarded) fishing gear (Baulch and Perry, 2014), although identifying the source of entanglement is often impossible (Simmonds, 2012; Tulloch et al., 2018).

Ingestion of marine debris has the potential to obstruct the digestive tract, leading to reduced body condition, starvation, and likely death in cetaceans (Laist, 1987; Fossi et al., 2018). The ingestion of marine debris can also result in toxic contamination as chemicals such as UV stabilizers, flame retardants, heavy metals (e.g., lead), persistent organic pollutants (POPs) (e.g., dichlorodiphenyltrichloroethane (DDT), and polychlorinated biphenyls (PCBs)) adhere to plastics (Fossi et al., 2018). Additionally, consumption of contaminated prey items may lead to the bioaccumulation of toxic chemicals and plastics, which provide an indirect pathway via ingestion (Fossi et al., 2016). A review by Baulch and Perry (2014) found ingestion of marine debris had been documented in 56% of all cetacean species (48 species). This includes ingestion of fishing gear, plastic items (e.g., sheeting, bags, containers and other items), miscellaneous debris (e.g., fabric, rubber, paper, cellophane, polystyrene, and glass) and unidentified items (Baulch and Perry, 2014). Our understanding of the extent of marine debris ingestion is growing, with evidence to suggest ingestion of both macro and micro plastics are also potential problems (Fossi et al., 2014; Besseling et al., 2015; Fossi et al., 2016). Direct evidence of marine debris ingestion has come from cetacean necropsies, with plastic found in cetacean digestive systems (Unger et al., 2016). While these cases contribute to our knowledge of marine debris as a threat, our understanding of the impact of marine debris at the individual and population level for many cetacean species remains unclear (Gall and Thompson, 2015; Fossi et al., 2018).

2.2 Environmental contaminants

Environmental contaminants such as chemical pollution degrade the marine environment and are a known threat to cetaceans (Reijnders et al., 2009). Types of environmental contaminants include toxic chemicals such as Persistent Organic Pollutants (POPs) e.g., organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), oil and vessel discharge (oil spills, vessel emissions) and metals (Hassellöv et al., 2013; Bachman et al., 2014; Gajdosechova et al., 2016). Environmental containments such as POPs bio accumulate in cetacean blubber/tissue and are known to effect cetacean immunity and endocrine systems, rendering individuals with high contaminant levels vulnerable to infectious diseases or death (Jones and De Voogt, 1999; Aguilar et al., 2002; Gulland and Hall, 2007). Despite the banning of POPs in 2001, many of these toxic chemicals remain intact for years, widely distributed throughout the world via air, water, and soil (Stockholm Convention 2009). Sampling of species provides direct evidence for chemical presence in the marine environment. For example, killer whales (*Orcinus orca*) in the northeast Pacific Ocean are one of the world's most PCB-contaminated marine mammals, which presents a major concern for their health as PCBs do compromise reproductive cycles (Reijnders et al., 2009), and given the poor status of some populations e.g., the southern resident killer whales, has become a process threshold (Buckman et al., 2011).

In addition, oil spills are both a serious chronic and acute problem for cetaceans. Acute spills can have severe and prolonged affects. A large proportion of killer whales in the region of Alaska exposed to the 1989 'Exxon Valdes' oil spill died, presumably from inhalation of vapours/oils, oil contact with skin, and ingestion of contaminated prey. Subsequent population recovery has been poor, with this one event resulting in ongoing population-level impacts (process threshold) for two ecologically and genetically distinct killer whale populations (Matkin et al., 2008). The Deepwater Horizon spill in the Gulf of Mexico is another example of an acute event with ongoing impacts, which adversely affected 15 species of cetacean (Takeshita et al., 2017). In this case, pressures were documented at an individual level (e.g., inhalation, aspiration, ingestion and/or absorption of toxic oil) and statistical approaches were used to assess how injuries of individual animals may potentially have impacted the entire population (Takeshita et al., 2017). While it is common for the immediate pressures from oil spills to be documented (individual level consequences), a true understanding of long–term impacts and potentially population level impacts from oil contamination requires continued investigations and long-term monitoring, to improve our understanding of population level consequences

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from such events (Takeshita et al., 2017). Long-term monitoring is also required to understand the impacts of environmental contaminants over time making population-level impacts difficult to attribute (Takeshita et al., 2017).

2.3 Acoustic pollution

The introduction of non-natural noise to the ocean as a result of a variety of anthropogenic activities creates a noisier environment for cetaceans (Williams et al., 2015). Sources of anthropogenic acoustic pollution include underwater construction (oil drilling, pile driving, wind farms), sonar, seismic/airgun exploration for oil and gas, and vessel activity e.g., shipping and tourism (Hildebrand, 2009). Low frequency components of shipping noise are proportionally the largest contributor of anthropogenic noise in the ocean and can propagate kilometres from its source (Wilcock et al., 2014). These sounds range from 5 - 500 Hz, and as a broadband sources global shipping networks have added 12 dB to ocean ambient noise levels over the last few decades (Hildebrand, 2009); since a change of 6 dB is a doubling of sound energy, this represents a significant increase. Understanding the impacts of anthropogenic noise has been largely focused on individual level responses, with an acknowledgement of the potential for population level consequences (Fleishman et al., 2016). A major concern with increases in shipping noise is the potential to limit and/or interfere with whale vocal communication – known as masking (Cholewiak et al., 2018). For example, Byrde's whale communication space off New Zealand waters has been reduced by the sound form vessel passages by up to 87.4% (Putland et al., 2018). Some whales can respond to this noise exposure by changing their calling behaviour. During band-limited background noise, right whales (*Eubalaena spp*) shifted call frequencies, becoming louder in the presence of shipping (Parks et al., 2011), while male fin whales (*Balaenoptera physalus*) modified their song characteristics (Castellote et al., 2012). But such changes may bring a cost in terms of increased energy such as to call louder.

The identification of anthropogenic noise as an environmental stressor for cetaceans arose in the early 1970's due to evidence of disruption of baleen whale long range vocal communication (Payne and Webb, 1971). Anthropogenic pollution can impact cetaceans by reducing the available acoustic space for communicating over long distances and/or masking sounds, which may increase stress levels and lead to abandonment of important habitat (Weilgart, 2007). There is also evidence that exposure to anthropogenic noise may induce a number of behavioural modifications by cetaceans including avoidance, changes to foraging, altered movement patterns, increased stress levels, habituation, and disrupted communication

(Rolland et al., 2012; Burgess et al., 2016; Tennessen and Parks, 2016). In more extreme cases, strandings and death of some species e.g., beaked whales (family *Ziphiidae*) have been linked to naval sonar or seismic activity (Weilgart, 2007; D'Amico et al., 2009). While short term responses to anthropogenic sound may seem like a potential substitute for an understanding population-level impacts (Weilgart, 2007), such responses can be highly variable between contexts, species, different age classes, and behavioural states, (e.g., Gomez et al 2016) and may not be good predictors for long-term impacts (Rolland et al., 2012; Blair et al., 2016; Tennessen and Parks, 2016). In addition, understanding population level consequences arising from sound is challenging as it requires long-term observations of exposed cetaceans, conducted over large areas that encompasses the home ranges of migratory species (Weilgart, 2007).

3.0 Fisheries interactions

For some species of cetaceans entanglement in fishing gear is a leading cause of mortality, resulting in population decline i.e., a process threshold (Read, 2008; Moore, 2014). Interactions with fishing gear is a threat likely to increase over time due to human population growth and expansion of fisheries (Read et al., 2006). For example, the global bycatch of harbour porpoises is a well-known species impacted by fisheries interactions, resulting in legislative change and extensive research to mitigate interactions (Read et al. 2006). In addition, the North Atlantic right whales are also threatened at the population level by entanglement (Meyer-Gutbrod and Greene, 2017) in fixed fishing gear, including pots and gillnets which can cause drowning, severe tissue damage, infection and in some cases, mortality (Cassoff et al., 2011; Knowlton et al., 2012). A 30-year study on north Atlantic right whale entanglements found 83% of the population had experienced entanglement, many of which had been entangled multiple times (6 to 7 encounters), and across varying age classes (e.g., calves, juveniles and adults) (Knowlton et al., 2012). This suggests individuals previously entangled had not learnt from previous interactions with fishing gear (Knowlton et al., 2012).

Another a well-known species threatened at the population level by entanglement is the vaquita porpoise (Thomas et al., 2017). Entanglements in illegal gillnets targeting the totoaba fish (*Totoaba macdonaldi*) is the leading cause of injury and death through their limited range (Jaramillo-Legorreta et al., 2017). The small population has declined by 80% in four years and could face extinction if gillnetting continues (Jaramillo-Legorreta et al., 2017). By contrast, entanglements acting as a threat at an individual level, but not at a population level, have been

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documented for virtually all cetacean species, e.g., bottlenose dolphins (*Tursiops truncatus truncatus*), striped dolphins (*Stenella coeruleoalba*), and dusky dolphins (*Lagenorhynchus obscurus*) (Reeves et al., 2013; Adimey et al., 2014).

4.0 Climate change

Climate change, expressed as changes in sea temperatures (e.g., rising sea temperatures), reduction in sea ice, frequent extreme weather events (e.g., floods, cyclones), changes in ocean currents resulting in altered upwelling and productivity, rising sea levels, ocean acidification and the potential spread of marine diseases (Schumann et al., 2013; Burge et al., 2014; Simmonds, 2017), has a number of consequences for cetaceans (Simmonds, 2017). Impacts of climate change on cetaceans at individual and population levels vary. Climate change has implications for prey distribution causing some cetaceans to alter foraging locations and timing (Ramp et al., 2015). For example, minke whales (*Balaenoptera acutorostrata*) have altered where they feed to match changes in prey distribution as a result of increasing sea and bottom temperatures due to environmental changes off Iceland (Vikingsson et al., 2014). Fin (*Balaenoptera physalus*) and humpback whales (*Megaptera novaeangliae*) in the north Atlantic summer feeding ground off the Gulf of St. Lawrence, shifted their arrival earlier compared with previous years to coincide with earlier primary production as a result of earlier ice break-up and an increase in sea temperature (Ramp et al., 2015).

Climate change also has implications for ice-associated species that are part of the cetacean food chain. For example, reductions in sea ice habitat due to melting sea ice may drastically reduce available habitat for Antarctic krill (*Euphausia superba*), the main food source for many southern hemisphere baleen whales (e.g., humpback, Antarctic blue, and minke whales) (Nicol et al., 2008). In addition, reductions in krill abundance may also limit reproductive success and therefore recovery of southern right whales off Brazil (Seyboth et al., 2016). Climate change is also a particular concern for resident Arctic species such as the narwhal (*Monodon Monoceros*), beluga (*Delphinapterus leucas*) and bowhead whale (*Balaena mysticetus*) (Reeves et al., 2014). Reductions in sea ice may disrupt timing of prey abundance and range, e.g., phytoplankton production in areas, possibly affecting seasonal feeding opportunities in some areas for bowhead whales (Reeves et al., 2014). For example, changing sea ice patterns and prey dispersion has altered beluga whale migration and residency in Arctic areas, creating new threats by exposing them to killer whale predation, as killer whales can now enter these areas (O'Corry-Crowe et al., 2016). In a similar fashion, narwhals off Greenland have become more

vulnerable to hunting due to changed sea ice conditions providing earlier hunting access by small boats in the winter (Nielsen, 2009).

Reductions in sea ice also means other marine animals are able to move towards higher latitudes, potentially introducing novel pathogens and parasites into areas such as the Arctic, which could have long-term health implications for Arctic species (Reeves et al., 2014). This may have the potential for population level consequences (see *Viruses and Disease: Implications for* health) however, population level consequences are difficult to predict for cetaceans, as cetaceans are not easily monitored in comparison to other polar species e.g., polar bears (Laidre et al., 2008). Cetaceans are also exposed to pathogens as well as toxins (e.g., heavy metals, hydrocarbons, POPs, petroleum hydrocarbons, heavy metals and substances) due to the increased presence of anthropogenic activities in the Arctic (Burek et al., 2008). For example, the growth of vessel activity (e.g., shipping and tourism) increases the likelihood of oil spills, ship strike, acoustic pollution, atmospheric (e.g., greenhouse gas) and marine pollution, as well as bio invasions via the spread of invasive species released in ballast water (Seebens et al., 2013). In addition, greenhouse gas emissions generated from anthropogenic activities may contribute to changes in ocean chemistry resulting in ocean acidification (Hassellöv et al., 2013). Increases in absorbed atmospheric carbon dioxide cause the ocean to become more acidic, impacting cetacean prey dependent on calcifying species' ability to build calcium carbonate shells (e.g., molluscs, crustaceans, echinoderms, corals, large calcareous algae, foraminifera and some phytoplankton) (Raven et al., 2005). In addition, increased acidity in seawater propagates sound farther, with great amplitude, leading to noisier seas (Hester et al. 2008).

5.0 Habitat modification

Habitat modification in the marine environment is a result of a number of different factors (Table 2.1). For example, anthropogenic activities in the marine environment may modify the seascape for cetaceans e.g., vessels, fishing, acoustic pollution, physical changes to coastal habitats from coastal development and construction and reduced sea ice habitats (see vessel disturbance, marine pollution and climate change; Table 2.1). In addition, marine habitats may also become degraded as a result of anthropogenic activities e.g., marine pollution due to micro and macro plastics, introduction of toxic contaminants, greenhouse gas emissions, acoustic pollution, transfer of disease, increased ocean temperatures and acidity (see vessel disturbance, marine pollution and climate change). Together, habitat modification and

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degradation may also have implications for cetacean health, potentially leading to epizootic outbreaks (see cumulative impact example: viruses/disease below). A well-known cetacean example impacted by habitat modification are British Colombia killer whale populations, where a combination of anthropogenic activities such as increased vessel activity and environmental pollutants are degrading their habitat (Raverty et al., 2017, Reijnders et al., 2009). Changes in environmental conditions may also be contributing to prey availability of Chinook salmon, Southern Residents have already been documented foraging in different locations and shifting to different prey species (Shields et al. 2018). Habitat modifications in critical habitat may continue to contribute to disturb processes within important areas for these cetaceans (Shields et al. 2018).

6.0 Cumulative impacts

When multiple threats occur together and interact, either through the additive effect of individual pressures of the same driver or the interactive effect of multiple pressures or different drivers they are known as cumulative impacts (Table 2.1) (Spaling and Smit, 1993). A threat alone may not have population consequences for some species, but together or cumulatively with others, threats may have population level consequences.

Exposure to viruses and diseases is an emerging threat to cetaceans and can provide an example of a cumulative impact. Cetacean morbillivirus (CMV) is the most well-known virus to infect cetaceans, and is part of the genus *Morbillivirus* (family Paramyxoviridae) (Di Guardo et al., 2005). CMV includes three characterised strains first identified in porpoises (porpoise morbillivirus), dolphins (dolphin morbillivirus) and pilot whales (pilot whale morbillivirus) (Van Bressem et al., 2014). Morbilliviruses are highly contagious, inducing immunosuppression in their hosts and have been responsible for lethal disease outbreaks in cetacean populations globally (Van Bressem et al., 2014). Individuals infected with CMV may strand and display neurological or behavioural changes (Di Guardo et al., 2005; Stone et al., 2012). Morbillivirius may be spread via inhalation of expired blow droplets of adjacent individuals (horizontal transmission) and vertical transmission via mammary glands and possible transmission to foetuses and neonates (Van Bressem et al., 2014).

Vulnerability to viruses and diseases is worsened by environmental degradation due to exposure to anthropogenic activities e.g., chemical and biological contamination in the marine environment and interactions with fisheries and vessel activity, resulting in disturbance, stress,

traumatic injuries, or death (Van Waerebeek et al., 2007; Van Bressem et al., 2009b). Climate change is likely to facilitate the transmission of marine diseases, with abnormal climate events (e.g., extreme climate variability), possibly associated with morbillivirus, epizootics and mass mortalities (Van Bressem et al., 2014). However, predicting population level impacts is difficult due to changes in distribution of pathogens and patterns of diseases as a result of climate change (Burek et al., 2008). Interactions with anthropogenic activities have also disturbed the balance between populations and existing pathogens, resulting in lowered cetacean immune responses, increased stress (with perhaps concomitant immunosuppression; Lysiak et al. 2018, Rolland et al. 2012) and introduction and facilitation of new pathogens (Van Bressem et al., 2009a). Van Bressem et al. (2009) suggests inshore and estuarine species are at greater risk of contracting disease (e.g., compared with pelagic species of contracting morbillivirus epidemics, lobomycosis/LLD, toxoplasmosis, poxvirus-associated tattoo skin disease) compared with pelagic species due to habitats which incur higher frequencies of anthropogenic activities (Van Bressem et al., 2009a). The presence of viruses (e.g., morbilliviruses, papillomaviruses), bacteria (e.g., *Brucella* spp.) and parasites (e.g., *Toxoplasma gondii*) have the potential to impact at the population level if there is significant mortality, lowered reproductive success or by facilitating the transmission of other diseases (Van Bressem et al., 2009a).

The next section presents a decision tree for defining threats and process thresholds and a conceptual framework for considering cumulative threats.

Decision tree for defining threats and process thresholds
To inform threat prioritisation and to assist with existing processes for listing threatened species, we developed a decision tree to attribute an appropriate level of effect, by unambiguously defining a threat or a process threshold (Figure 2.1). The decision tree can be used while incorporating the precautionary principle when there is great uncertainty, to identify the threats with the greatest level of impact. It can also be used as a first point of establishing if species may be susceptible to only threats or process thresholds. The decision process begins by identifying the species of concern. This could be a population (e.g., small or large population of the same species) or an entire species, however, we suggest an assessment of only one population or species at a time to thoroughly assess the level at which threats are acting (we accommodate for cumulative impacts in the next section, cumulative impacts). It should also be noted that different populations and/or species may be vulnerable to the same threats, therefore multiple populations and/or species may be considered a conservation

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priority at the same time using this framework. Setting appropriate conservation priorities and deciphering where to allocate resources among populations or species which all meet the criteria of being vulnerable/threatened is a well-known challenge for conservation managers (Myers et al. 2000). Competition for limited conservation resources has resulted in many to ways to potentially deal with this issue such as creating 'biodiversity hotspots' or protected areas with high levels of endemic species to share limited conservation resources (Myers et al. 2000).

The first step (step 1) requires identification of known or emerging anthropogenic activities that the population or species are exposed to. The second step (step 2) identifies any stressors (impacts) arising from identified anthropogenic activities in the previous step. The final step (step 3) determines if the stressor/s are causing a change at the population level. If this is known, the pressure/s are identified as a process threshold. In contrast, if the pressure/s is not effecting the population but rather at an individual level, the population or species of concern is not threatened by this pressure/s at this time (non- process threshold). If there is any uncertainty (uncertain) of the level at which the pressure/s is acting, information regarding population or species size is required. This helps determine a basic understanding of the population or species being assessed. If the level at which the stressor/s is acting is known, the decision tree then requires information regarding the extent of the pressure/s e.g., does exposure to the stressor/s threaten the existence of the population or species? If so, then the threat/s are considered a process threshold. Alternatively, if there is no knowledge regarding the level at which the pressure/s impact on the population or species, then a precautionary principle must be applied. This is also the case if a population or species remains data deficient. Finally, this leads to the final action, which implements the Population Consequences of Disturbance (PCoD) model developed by New et al. (2015) to help identify potential population level impacts (Figure 2) (New et al., 2015).
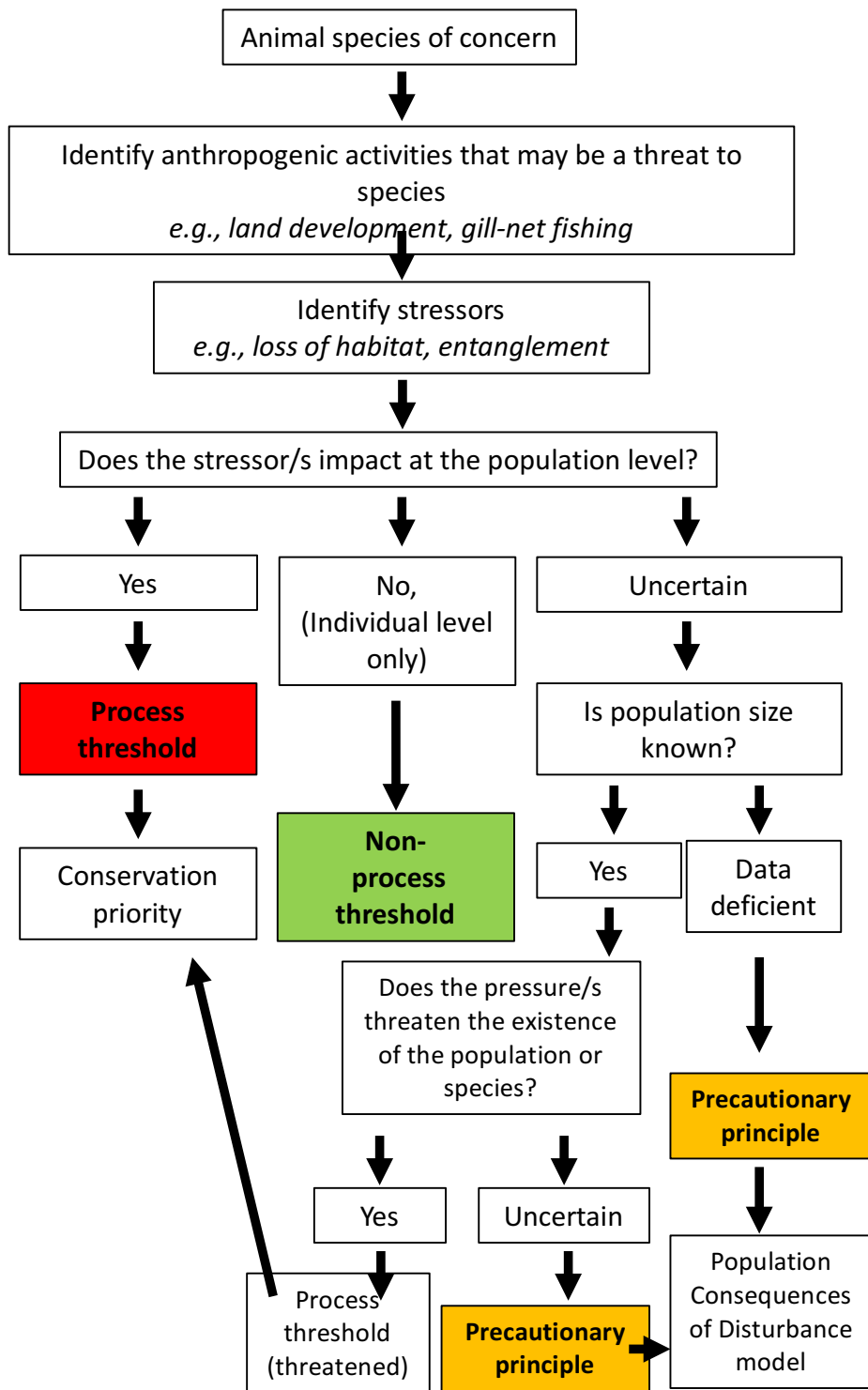
Figure 2.1: Decision tree for defining threats and process thresholds**.** The following decision tree has been developed to assist with the correct identification of when species are threatened at the population (process threshold) level and individual level (non- process threshold). Correct identification of these terms allows for targeted conservation actions for species in need. Uncertainty in the level at which threats are acting requires further action using the Population Consequences of Disturbance model developed by New et al. (2015) (Figure 2.2).
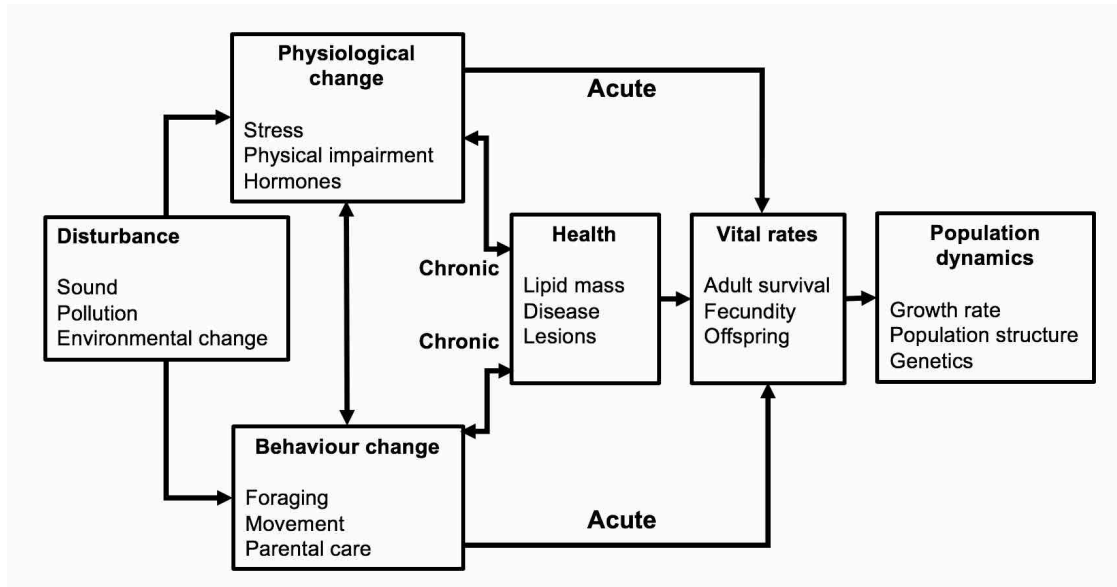
Figure 2.2: Population Consequences of Disturbance (PCoD) model developed by New et al. (2015). The PCoD is a tool to model demographic and population consequences of repeated disturbances. The model can be used in combination with the decision tree when there is uncertainty of the level at which the population or species is impacted i.e., unknown population size or if the pressure threatens the existence of an entire population or species is unknown. In both cases, a precautionary principle is advised and the PCoD model can be used to help identify areas where information is lacking and highlight potential long-term population level consequences arising from the disturbance. Reproduced with permission from Leslie New.

The PCoD model is a tool to identify and quantify possible impacts of anthropogenic activities on animals, providing an extension to the decision tree when there is uncertainty regarding the level at which pressures are acting (New et al., 2015). The model also takes into account when the threat is repeated at both the individual or population level (New et al., 2015). The conceptual model can be used to model the demographic and population consequences of repeated disturbance (e.g., vessel disturbance) from short term changes in individual animals (New et al., 2015). This helps identify areas where information is lacking and highlights potential long-term population level consequences arising from the disturbance. The model identifies potential sources of disturbance that can have an acute and immediate effect on vital rates (e.g., ship strike) and secondly, chronic effects (e.g., whale watching), which may affect vital rates through changes in individual health (New et al., 2015).

Specific examples using the decision tree

The following are two specific examples which demonstrate the use of the decision tree for defining threats and process thresholds (Fig. 2.1). This includes a local species example with a small home range and an example of a broader ranging species.

Species example with a small home range:

Bottlenose dolphins, Port Stephens, New South Wales, Australia (Steckenreuter et al. 2012).

Framework assessment

Identification of anthropogenic activities that may be a threat to species: industrial fishery, land development.

Identification of stressors: entanglement, increased interaction with vessels, risk of boat strike.

Does the stressor/s impact at the population level? Yes, small population living permanent within the area.

Outcome of the framework: Threshold process identified and therefore this population is considered a conservation priority.

Species example with a broad home range:

The Australian east coast humpback whale population (Group V).

Framework assessment

Identification of anthropogenic activities that may be a threat to species: industrial fisheries, coastal developments, shipping industry, underwater construction, oil and gas exploration (seismic).

Identification of stressors: entanglement, ship strike

Does the stressor/s impact at the population level? No, individual level only. This population is growing annually at 10.9% and numbers continue to grow each year.

Outcome of the framework: Non-threshold process.

A conceptual framework for considering cumulative threats

Given the complexity of threat exposure and the interactions of multiple stressors on species (fragile systems), we describe a conceptual framework within which to consider cumulative threats and their impact on cetaceans (figure 2.3). This framework can be used to assist with current processes already being used for listing threatened species and preparing recovery plans. The framework presents a cycle structure in three sections; (1) assessment, (2)

conservation action and (3) review (similar to the adaptive management framework of McCarthy and Possingham (2007)) to inform conservation priorities for species threatened by cumulative impacts.

Framework for assessing cumulative impacts
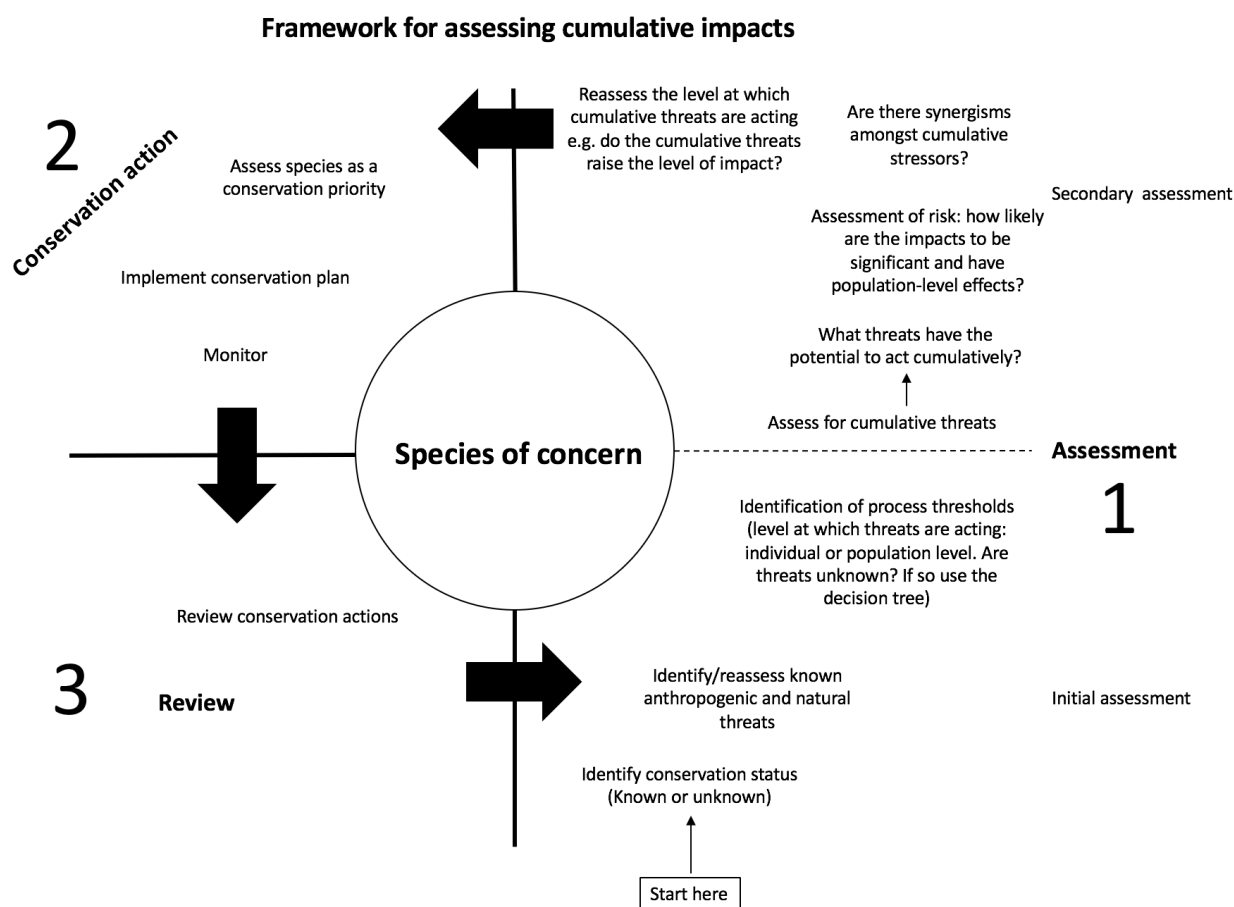
Figure 2.3: Framework for assessing cumulative impacts. This framework presents a cycle structure in three sections; (1) assessment, (2) conservation action, and (3) review to inform conservation priorities for species threatened by cumulative impacts.

1. Assessment

The first component of the cumulative impact framework is the assessment phase. This section provides an overall assessment of the species in question so that there is an understanding and prior knowledge of any pre-existing threats and identification of population level impacts (if applicable). The assessment phase is divided into two sections; initial and secondary assessment. In the initial assessment, the framework requires background information and identification of a species' status e.g., knowledge about the species' population size, biology,

ecology, abundance and distribution. Identification of all known anthropogenic and natural threats is also required to generate a list of existing threats and their proximate drivers and pressures. At this stage, the level at which threats are acting (e.g., individual or population level) should also be identified, providing a platform for assessing cumulative impacts in the next section (secondary assessment). If unknown, use of the decision tree previously described (figure 2.2) is recommended to assist with the identification of conservation priority.

The second (secondary assessment) component of the assessment phase is designed to collectively assess threats and identify those, which together (cumulatively) raise the level of impact for a species, increasing conservation priority. This section makes an assessment of risk and questions how likely are the impacts to be significant or have population-level impacts. It also tries to determine if there if there are any synergisms amongst cumulative stressors and highlights efforts to assess cumulative impacts which are likely to remain ongoing. This is important for conservation managers, who use a variety of approaches to better understand cumulative impacts, including cumulative impact assessments, which uses information generated from research and is communicated to decision-makers, resulting in rational decisions (Smit and Spaling 1995). In addition, there are different types of cumulative impacts, which may complicate our understanding of how cumulative impacts interact (Halpern et al. 2008). For example, within cumulative threats, there may be dominant (independent) threats which are elevated in conjunction with other threats (Halpern et al. 2008). These threats acting together may have a greater impact on individuals/populations compared when only acting alone (Halpern et al. 2008). For example, the threat of vessel disturbance (i.e., shipping) and marine debris (i.e., entanglement in fixed fishing gear) for North Atlantic right whales are independently process thresholds (Meyer-Gutbrod and Greene, 2017). Cumulatively, these threats act together to increase the level of severity experienced by individuals and therefore increase the conservation needs for the species. Once threats have been reassessed together for their *cumulative* potential and level of impact i.e., do threats now act a population level or not?, conservation planning is required.

2. Conservation action

This section incorporates a number of conservation actions to help alleviate impacts, protect population(s) and monitor the conservation needs i.e. offsetting, of species affected by cumulative impacts. This section can also be used for species not identified at risk of cumulative impacts, but rather as a precautionary principle approach. Species at risk of cumulative impacts

at the population level should be given conservation priority over species threatened at the individual level. This is also known as the adaptive management phase, where planning and decisions are made to implement conservation action to help mitigate immediate impacts e.g., fishery closures to reduce whale entanglement, movement of shipping lanes to avoid whale strike. After the implementation of conservation actions, monitoring of species is required to assess conservation actions.

3. Review

A review phase is the final step in the cycle and is required to evaluate the success of conservation actions that have been implemented. This includes an evaluation and report of what worked and what didn't in terms of mitigating cumulative impacts for species. The process then cycles back to the assessment section to review the level at which anthropogenic and natural threats are acting.

Summary

Setting conservation priorities for cetaceans is important to ensure conservation resources are appropriately directed to species most in need. This is done by prioritizing species where threats act at the population level as process thresholds and have the potential to limit population recovery or threaten extinction. Clarification of the conservation terminology contributes to the conservation literature by providing an approach to help avoid misdirecting effort to individual welfare cases rather than resourcing mitigation of a process with real conservation implications. However, identifying conservation needs for data deficient species remains challenging. Implementation of the decision tree and conservation framework may assist conservation managers to systematically assesses threats and consider cumulative impacts from multiple threats.

Chapter 3 (pp. 34-49) of this thesis have been removed as they contain published material under copyright. Removed contents published as:

Vanessa Pirotta, Alana Grech, Ian D. Jonsen, William F. Laurance, Robert G. Harcourt (2019) Consequences of global shipping traffic for marine giants, *Frontiers in Ecology and the Environment*, vol. 17, no. 1, pp. 39– 47. doi.org/10.1002/fee.1987

Section two:

Conservation resources

Chapter Four

A citizen science approach to long-term monitoring of humpback whales (Megaptera novaeangliae) off Sydney, Australia.

This chapter has been submitted:

Pirotta, Vanessa; Reynolds, Wayne; Ross, Geoffrey; Jonsen, Ian; Grech, Alana; Slip, David; Harcourt, Robert (2018) A citizen science approach to long-term monitoring of humpback whales (*Megaptera novaeangliae*) off Sydney, Australia. *Under Review.*

Abstract

The Cape Solander Whale Migration Study is a citizen science project that annually counts northward migrating humpback whales (*Megaptera novaeangliae*) off Cape Solander, Sydney, Australia. Dedicated observers have compiled a 20-year dataset (1997-2017) of shore-based observations from Cape Solander's high vantage point (31m). Using this long-term dataset collected by citizen scientists, we sought to estimate the humpback whale population trend as it continues to recover post-exploitation. We estimated an exponential growth rate of 0.099 (95% CI = 0.079-0.119) using a generalised linear model, based on observer effort (number of observation days) and number of whales observed, equating to 10% per annum growth rate since 1997. We found favourable weather conditions for spotting whales off Cape Solander consisted of winds <30km/hr, coming from a southerly through to a north westerly direction. Incidental observations of other cetacean species included the endangered blue whale and data deficient species such as killer whales and false killer whales. Citizen science based studies can provide a robust, cost-effective and citizen empowering approach to monitoring wildlife over the time necessary to detect change in a population. Information obtained from citizen science projects like this are critical to supplement State and Federal protection of cetaceans in Australian waters.

4.1 Introduction

Long-term field studies that monitor wildlife are essential to understanding trends in populations over time (Clutton-Brock and Sheldon 2010; Magurran et al. 2010). Long-term monitoring refers to any systematic field-based measurements of the same population or species collected over a substantial number of years (>10 years) (Clutton-Brock and Sheldon 2010; Hayes and Schradin 2017; Lindenmayer and Likens 2010). The purpose of long-term monitoring in biodiversity research is to provide information on abundance of a species at one or more locations, at a number of points in time (Magurran et al. 2010). With any long-term monitoring, it's important to have a specific research questions in mind to ensure long-term data series will capture relevant trends if they occur. There are multiple functions of long-term monitoring, including estimating reproductive success and survival, determining population trajectories, understanding complex social systems, and determining changes in habitat use and species distributions (Clutton-Brock and Sheldon 2010; Gagnon et al. 2011; Hayes and Schradin 2017; Lovett et al. 2007; Magurran et al. 2010; Smith et al. 2017). Long-term monitoring can also generate large biological datasets that can be used by scientists and policy/decision makers to develop informed conservation actions to help protect or recover populations (Magurran et al. 2010).

As whales are entirely aquatic, long-term monitoring can be logistically difficult and highly resource intensive. Some methods commonly used include aerial or vessel based surveys (Carroll et al. 2014; Gill et al. 2015; Robertson et al. 2016), long-term genetic monitoring (Carroll et al. 2015), shore-based observations (Durban et al. 2013; Ford et al. 2013; Miller et al. 2016; Noad et al. 2011b; Rugh et al. 2008; Sagnol et al. 2015), and counting whales from satellite imagery (Fretwell et al. 2014). For whales that follow a coastline for at least part of their migration, shore-based observations are a logistically feasible, non-invasive, and low-cost technique for long-term monitoring of population abundance. These observations provide a consistent means by which to count individuals from the same population over time, especially for those species following culturally-inherited migratory routes (Carroll et al. 2015; Pierszalowski et al. 2016).

An opportunity for shore-based whale counting
The east Australian humpback whale (*Megaptera novaeangliae*) population (Group V, Stock E1) migrate along the east coast of Australia each year from the cool feeding waters of Antarctica to the warm breeding grounds in northern Queensland (Chittleborough 1965). This population

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has been recovering post whaling (since Australia stopped commercial whaling in 1978) and is currently estimated to be approximately 30,000 individuals, with population growth rates recorded to be 11% per annum (95% CI 10.6%-11.3%) from intermittent but systematic monitoring undertaken in southern Queensland (Noad et al. 2016). During their northward migration off Sydney (May-August), humpback whales can be seen traveling close to shore, where they tend to be concentrated within a narrow migratory corridor (Pirotta et al. 2016). In comparison, movements during the southward migration (August-December) appear more varied, with some individuals travelling further offshore, most likely a result of the warm southward flow of the East Australian Current (Suthers et al. 2011). During their annual migration, individuals of this population encounter a variety of potential anthropogenic threats as they travel along the east Australian coastline. These include vessel interactions (e.g., shipping, recreational and tourism based activities), commercial and recreational fishing activities, and underwater construction (Gulesserian et al. 2011; Peel et al. 2018; Pirotta et al. 2016). The large number of anthropogenic threats faced by this population, which is currently listed as Vulnerable under the Australian Government's *Environment Protection and Biodiversity Conservation (EPBC) Act* 1999, and Australia's obligations under various international conventions, justifies careful monitoring of humpback whale recovery. However, resources for this monitoring are limited.

A citizen science approach to long-term monitoring

Citizen science encompasses the collection and analysis of data by the general public in collaboration with scientists (Dickinson et al. 2012; Follett and Strezov 2015; Silvertown 2009; Theobald et al. 2015). The number of research projects involving volunteers is growing rapidly in response to improved technologies, labour demands, and growing interest in science outreach (Silvertown 2009; Tulloch et al. 2013). The Cape Solander Whale Migration Study (CSWMS) is one of Australia's longest running citizen science based whale monitoring studies (Figure 4.1). The CSWMS consists of a number of volunteers (hereafter referred to as observers) who have recorded humpback whale numbers over the last 20 years from Cape Solander in the Kamay Botany Bay National Park, south of Sydney, Australia (Figure 4.2a, b and c).

Figure 4.1: Cape Solander Whale Migration observer watching whales from the Cape Solander whale platform Sydney, Australia. Whale numbers are recorded each day during the counting period from the 24th of May to the 31st of July each year.
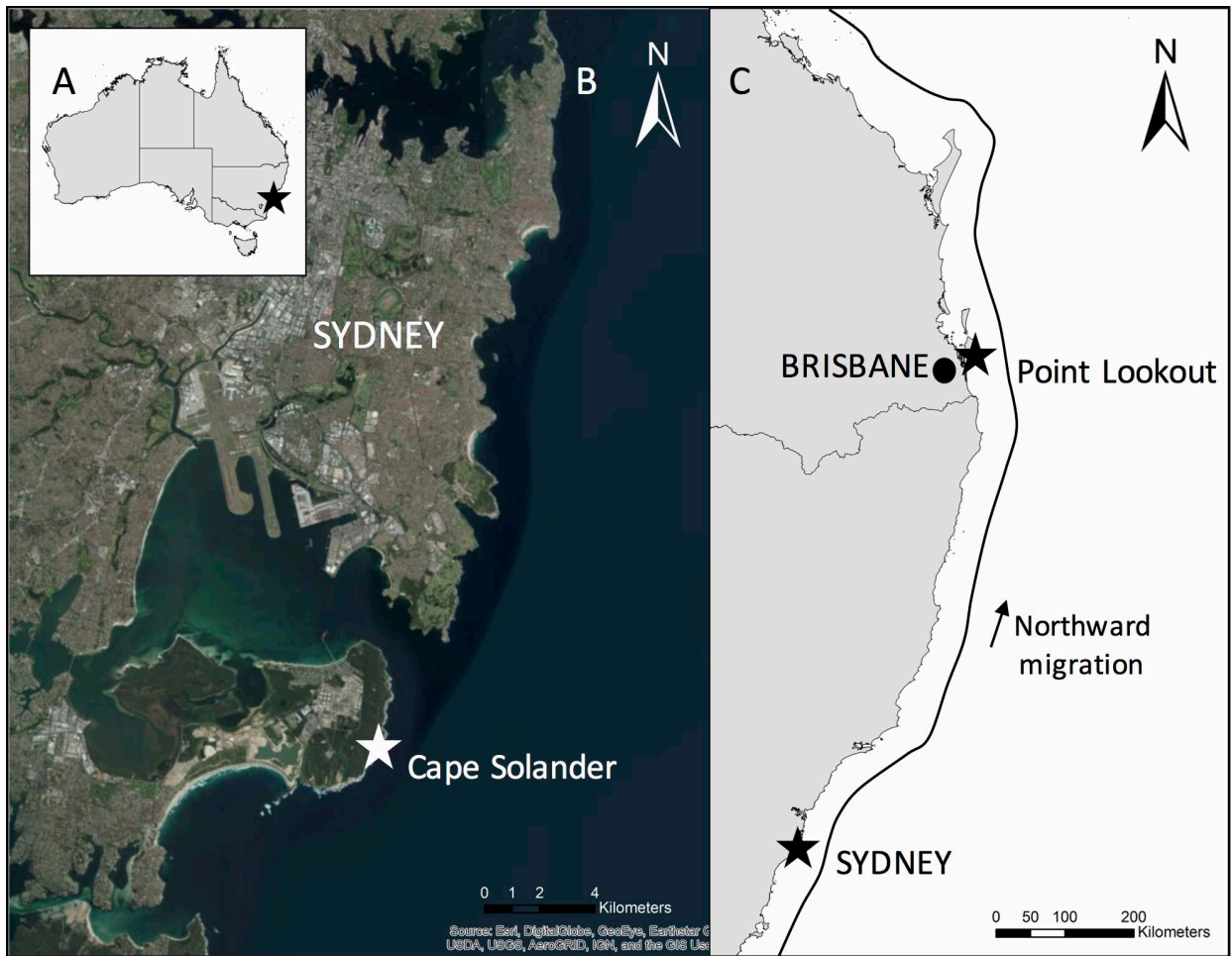
Figure 4.2: 4.2a) The Cape Solander Whale Migration Study area, Sydney, Australia. 4.2b) Location of the whale observation platform (indicated by the star) within the Kamay National Park, south of Sydney, Australia. 4.2c) Humpback whale northward migration route along the east coast of Australia. The bottom star indicates Cape Solander and the circle with star indicates Point Lookout, Queensland where surveys of the same population were conducted. Whales pass by Sydney and Brisbane then travel further north to northern Queensland. The solid black line is indicative of the general northward migration route.

Each austral winter since 1997, the study has counted humpback whales migrating north through the waters off Cape Solander between May and July. In addition, the CSWMS has recorded other marine species that were identified reliably. Since the commencement of the study, over 20,000 citizen science hours have been dedicated to the counts, with one observer present over the entire study period (1997-2017). Throughout the monitoring programme, new observers were trained with the overview of the single lead observer, and this has resulted in a standardised counting methodology persisting for the duration of the study.

The CSWMS was initiated by the lead observer (WR) who has regularly recorded whale numbers from Cape Solander since 1995, two years prior to the official commencement of the study. Project funding has been maintained by the New South Wales National Parks and Wildlife Service (NSWNPWS) who recognised the potential of shore-based observations to help provide information on humpback whale population trends (Nicholls et al. 2000). Information collected from this study has contributed to State conservation policies, such as contributing data to a review of the "approach distance" legislation in State waters under the New South Wales Government *Biodiversity Conservation Regulation 2017*.

Interactions between scientists and citizen science projects are integral to long-term monitoring as it ensures data are collected, maintained and analysed with consistent and appropriate methods. This is particularly important for the CSWMS as there has not been an analysis of trends in whale numbers since 1999. Using this long-term dataset collected by citizen scientists, we sought to assess the humpback whale population trend as it continues to recover post-exploitation. In addition, we present incidental observations of other marine species sighted during these observations off Sydney, Australia. This work outlines realised benefits of citizen science when used for long-term wildlife monitoring and details how this cost-effective collection of data can be used to supplement the conservation and management of marine wildlife.

Study Area

Humpback whales were recorded from Cape Solander, Sydney, Australia (Figure 4.2a and b). All observations were collected from a purpose-built whale observation platform located 30 metres above sea level. The platform has a 170 degree north to south view of the humpback whale migratory corridor. This is also the location for ship entry into Australia's largest

container port, Botany Bay, which had over 1,700 shipping movements in 2016/17 (Port Authority of New South Wales 2017).

4.2 Method

Observers (> 2) scanned the area from first light (0600) until dark (1700) each day (weather permitting) using unaided vision and 7x50 binoculars (with compass and reticules). Most of observations were conducted in good weather conditions i.e., good visibility (>1km) and a Beaufort of <5. Weather conditions were not consistently recorded each day but included rain, sea state, Beaufort and visibility. Observers used a Waverider buoy (Hemer et al. 2007) located 3.5km east of the platform as a marking point to count individuals or pods (two or more whales) as they swam northward. Once individuals or pods were sighted, they were tracked (focal follow method; Altmann 1974) until they passed the Waverider buoy and recorded on data sheets which included information on the time at which they passed, the number of whales in a pod (if applicable), behaviour such as swimming, and approximate distance to the observation platform. Once observers recorded this information, they ceased tracking whales or pods north of the Waverider buoy, but focused south again to search for new whales entering the study area. The counting method was then repeated. Observers also monitored whale movements north of the study area to ensure whales that had been counted did not change direction and reappear south of the Waverider buoy.

Initial observations each day involved observers focusing north of the Waverider buoy to record any whales that had passed through the study area before the commencement of whale counting (before 0600). Once observers had recorded all these whales, they focused their attention to the south.

Whale observations took place during the northward migration only. During this time, all whales were heading north and therefore each newly-observed whale or pod was considered unique. When recording whale numbers, observers carefully observed pods in order to ensure they counted the correct number of individuals. If observers were unsure about the number of whales in a pod, they recorded a minimum number. During peak times, observers were often allocated a pod to track until they were confident of their count. Other whale species and marine wildlife seen in the area were also recorded. At the end of each day, whale numbers were totalled and recorded in the official CSWMS database (paper records and an excel workbook).

Data analysis

Total whale numbers were recorded at the end of each season. Observer effort across all years was limited to number of sighting days. Effort per hour was only recorded in recent years (2013-2017). To estimate the change in whale numbers over the study period, a generalized linear model (GLM) with Gaussian errors was fitted to the observed whale counts per year with a log link function. We log-transformed number of sighting days in each year as an offset to account for variation in sighting effort over the time-series.

A generalized additive mixed model (GAMM) was fitted to account for the effect of weather conditions on whale counts. Due to inconsistences in weather recording by the observers, we used historical weather records of rainfall, wind speed and wind direction obtained from the closest weather station, Sydney Airport (obtained from the Australian Bureau of Meteorology). Weather records recorded every three hours were averaged over daylight hours of observations (0600-1800). Rainfall was excluded from the model as it was dominated by zero values (days with no rainfall). The GAMM was fit, using the mgcv R-package (Wood 2011), to the daily whale counts using a two-dimensional spline of the daily averages of wind speed and direction. We assumed Gamma-distributed errors and included a random intercept for year. All data collected were analysed using the statistical software package R *v. 3.5.0* (R Core Team 2017).

4.3 Results

Shore-based observations of humpback whales were collected from 1997-2017 from Cape Solander, Sydney. Observer effort was lower during the initial years of the study from 1997-1999 (Supplementary table 4.7.1). In the first year of the study (1997), data were recorded from the 5 June to the 2 July and in 1998 and 1999, data were collected from 1 June- 4 July. From 2000-2017 the collection dates were standardised and commenced 24 May and terminated 31 July. The highest count ever recorded on a single day during the entire study period (1997-2017) was 224 individuals observed on 26 June 2017 (Supplementary table 4.7.1). The highest number of whales recorded over a season also occurred in 2017 with 4,813 whales counted. Observations of the easily identifiable all white humpback whale 'Migaloo' occurred in 1996 (prior to the study), 2004, 2005, and 2014.

Statistical modelling

The GLM-estimated exponential growth rate was 0.099 (95% CI = 0.079-0.119 with; Figure 4.3). Variability in the annual counts was relatively low i.e., within the 95% CI, except 2003, which implies counts were conducted rigorously. This equates to approximately 10% per annum growth rate based on 20 years of observations at Cape Solander. In addition, we found the highest number of whales observed occurred when wind speed was <25-30km/hour and wind direction was between the south (180 degrees) and north west (~325 degrees; Figure 4.4). There tended to be less whales sighted in high wind conditions (>40km/h).
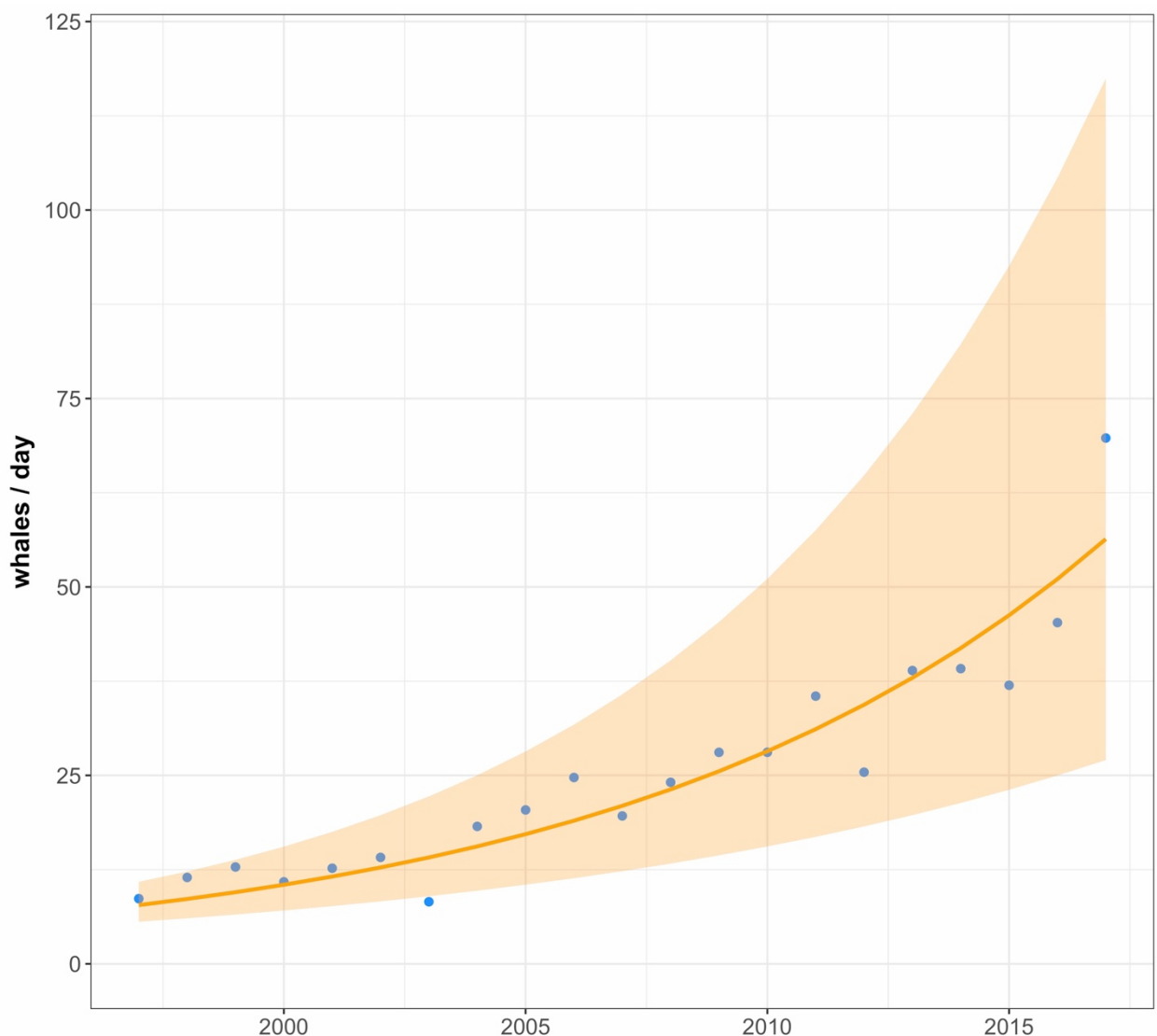
Figure 4.3: Estimated exponential growth rate (orange line) of annual humpback whales numbers per day (blue points) from sightings off Sydney over the last 20 years. The 95% confidence interval is depicted by the orange-shaded area.

s(wind_spd,winddir,16.03)
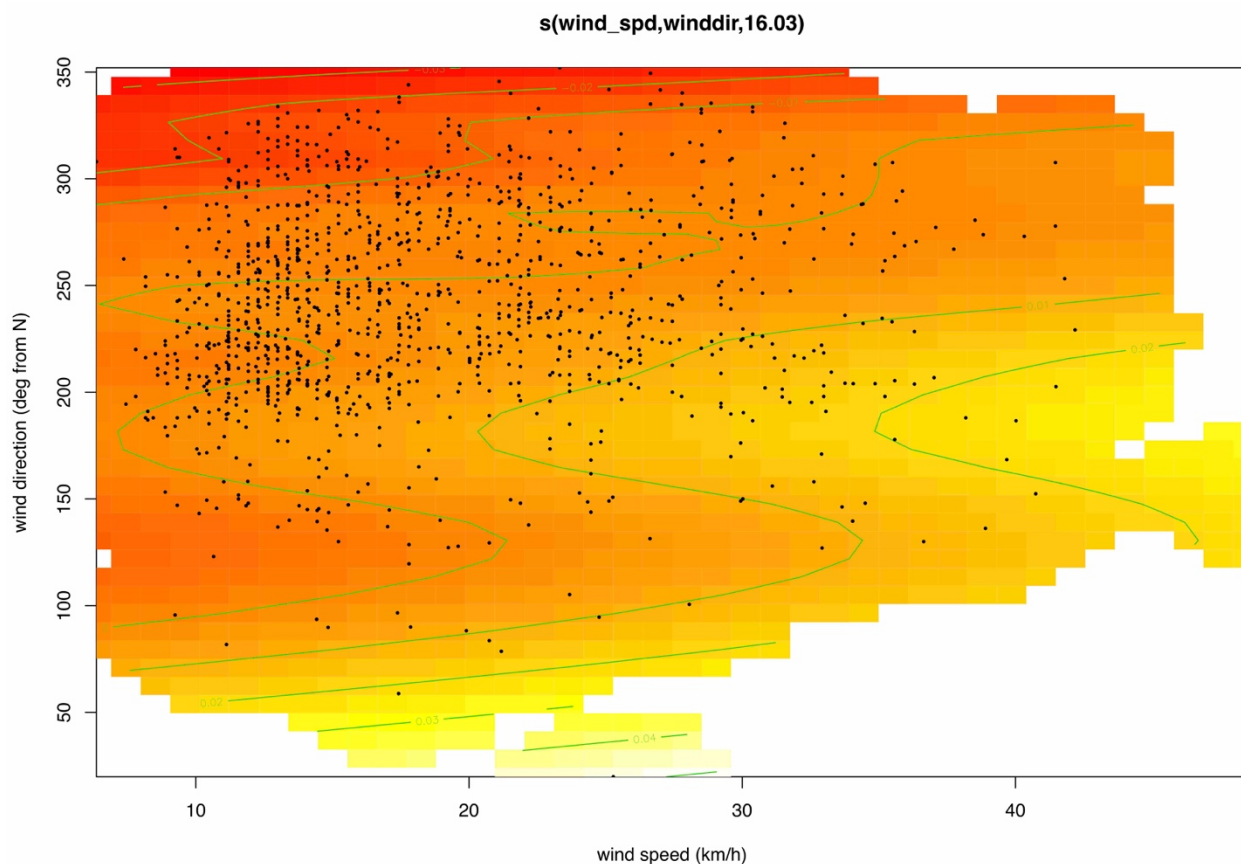
Figure 4.4: Weather effects on whale sightings. Observers saw more whales (indicated by black dots) when wind speed was less than 30km/hr and wind direction was from south (180) to north-west (315).

Observer effort

Observer effort varied over the years but a single observer (WR) was present throughout the entire study (1997-2017). Detailed records of observer effort (per hour) were only recorded for the last five years (2013-2017; Supplementary table 4.7.2). During this period, 16-19 observers contributed to the study. Observer effort (hours per day) ranged from 5.5 hours (2016, n=381.1hrs, ±SE 0.17) to 6.2 hours (2014: n= 428.5 hrs, ±SE 0.14 and 2017: n= 430.4hrs, ±SE 0.16).

Incidental observations of other species

In some years observers recorded other cetacean species (Table 4.1). Other marine vertebrates such as green sea turtles (*Chelonia mydas*) and fur seals (*Arctocephalus spp*) were occasionally recorded.

Year	Dwarf minke whale (*Balaenoptera acutorostrata)*	Southern right whale (*Eubalaena australis*)	Blue Whale (*Balaenoptera musculus*)	Killer whale (*Orcinus orca*)	False Killer (*Pseudorca crassidens*)	Dolphins (*Tursiops truncates, T. aduncus and Delphinus delphis*)
1997	3					*
1998	2	2				*
1999	5					99
2000	6					533
2001	24	2				337
2002	7	4				611
2003	28	5				1162
2004	30	4				785
2005	14					557
2006	*					*
2007	*					*
2008	*					*
2009	1	3				1052
2010	10	6				707
2011	4	5				400
2012	17	4				1275
2013	32	2	1			1171
2014	26	5		2		32
2015	37			15 +		664
2016	17	1			3	*
2017	17	2				*

Table 4.1: Incidental observations of other marine species during the annual Cape Solander Whale Migration Study. Number of sightings reported. In addition to humpback whale sightings, this study provided an opportunity to record a variety of multi species observations offshore of Sydney, Australia. *Indicates species where observations likely took place each year but were not recorded.

4.4 Discussion

The CSWMS is a long-term citizen science based project focused on counting east Australian humpback whales each austral winter off Sydney, Australia. We used information gathered from the CSWMS and environmental records to estimate the recovery of humpback whales off Sydney over the last 20 years. We estimated a 10% per annum growth rate in humpback whales and found specific environmental conditions (e.g., wind <30km/hour, wind direction S-NW) were most favourable for seeing whales. The low variability of the annual mean counts of whales per day (within the 95% CI), except in 2003, which had a decrease in whale numbers, suggests that this methodology is rigorous enough to detect changes over the long term.

The 10% per annum growth in humpback whale numbers over the last 20 years at Cape Solander is similar to the overall increase in the east Australian humpback whale population documented by systematic land-based surveys in Queensland (Noad et al. 2016). The first land-based surveys of this population (also only focused on northward migrating humpback whales) were started in the late 1970's by two independent groups located at Point Lookout, Stradbroke Island, Queensland (Figure 2c) (Brown et al. 2003; Bryden 1985; Paterson and Paterson 1984). The topography of that location funnels a large proportion (96%) of the population within 10km of the coast (Noad et al. 2008). Early estimates of this population from this location were 403 ± 320 (no CI) in 1980 (Bryden 1985) and 1,400 individuals between 1981-1987 with 10% estimated annual growth (95% of 6% and 13%) (Paterson and Paterson 1989). Subsequently whale abundance from this site was estimated for years 2004: 7,090 ± 660 (95% CI) (Noad et al. 2011b), 2007: 9,683 whales (95% CI = 8,556 – 10,959) (Noad et al. 2008) and 2010 (14,522 whales, 95% CI = 12,777 – 16,504) (Noad et al. 2011a). Most recent estimates suggests the population is recovered to 58 - 98% of its historic maximum, documenting a long-term growth rate of 11% per annum (95% CI = 10.6%-11.3%) and an absolute abundance for 2015 of 24,545 whales (95% CI = 21,631-27,851) (Noad et al. 2016).

The proportion of the northerly migrating population that pass through the observation area of the CSWMS is unknown. An early study conducted at Cape Solander (1998-1999) observed a higher proportion of individuals within 750m-2.5km of the coastline, with fewer whales detected further out to sea (>4.5km) (Nicholls et al. 2000). This is consistent with whales avoiding the strong southward-flowing East Australia Current (EAC) as they migrate north. This southward current can flow at speeds up to 4 knots, separating from the coast between 30°S and 34°S (Cape Solander 34° 01' S), flowing eastward across the Tasman sea, creating a

southward-moving eddy field off the coast off Sydney (Everett J. D. et al. 2012; Suthers et al. 2011). Detectability and the number of whales passing through the area is likely to vary between years, possibly due to natural variation in environmental conditions, e.g., weather. For example, our GAMM results indicated decreased wind speed and certain wind direction yielded higher sighting rates. Similarly, increased wind speed was found to reduce whale sightings at the start of the study (1997-1999) (Nicholls et al. 2000). In addition, the position and strength of ocean currents may also influence whale movements, e.g., the EAC (Cetina-Heredia et al. 2014). Unlike the Queensland-based surveys, there have never been aerial surveys conducted to determine what proportion of individuals pass through the observation area. However, unless there is inter-annual variability in the position of the migratory corridor, relative comparisons should be robust and the similarity of the two growth rates lends support to this assumption.

The recovery of this population has led to discussions to modify this population's Vulnerable status under Australian Federal protection (EPBC Act,1999) (Bejder et al. 2015). Increases in whale numbers will likely have implications for management authorities, especially for managing interactions with anthropogenic activities e.g., commercial fisheries and the increased risk of entanglement (Pirotta et al. 2016). Continued monitoring is also necessary to monitor the effect of global climate change e.g., loss of Antarctic krill habitat due to receding sea ice may have implications for krill availability, which are a major food source for this population (Nicol et al. 2008). Large-scale monitoring of the same population at multiple sites provides an opportunity to increase our understanding of the population throughout its range by enabling scientists to compare findings (Robinson et al. 2014). For example, similar growth estimates between this study and the Queensland land-based surveys provide increased confidence in both estimates and therefore certainty in the recovery of the population and support for potential delisting (Noad et al. 2016). In addition, there are many co-benefits of citizen science arising from a project like this (Burgess et al. 2017). Citizen science is a powerful education tool for bringing awareness to conservation needs via an increased scientific understanding; the CSWMS is visited by numerous sightseers daily throughout the migration, with *in situ* educational material supplemented by the knowledge and passion of the observers at the platform (McKinley et al. 2017). The CSWMS has enabled these observers to interact with scientists to discuss methods, data management, and the wider implications of the study for conservation. Furthermore, citizen science can help facilitate science communication,

making science accessible to a general audience and encouraging public action (McKinley et al. 2017).

Citizen science, like the CSWMS, act as "complementary tools to monitor cetacean biodiversity" (Lodi and Tardin 2018). There have been a number of marine wildlife biodiversity studies conducted by scientists off Sydney (Gulesserian et al. 2011; Harcourt et al. 2014; Pirotta et al. 2016; Pirotta et al. 2017), but the intensive nature of the CSWMS provides a unique opportunity to document rarely-seen marine species. For example, observations of southern right whales most likely from the Australian southeast stock, provided an opportunity for photo identification of individuals from this small and remnant population (Carroll et al. 2011). This observation contributes to our understanding of individual southern right whale movements beyond their usual breeding grounds off South and West Australia (Department of Environment 2012). Observations of less commonly sighted cetaceans also contribute to species presence information for endangered (e.g., blue whales *Balaenoptera musculus*) and data deficient species (e.g., killer *Orcinus orca* and false killer whales *Pseudorca crassidens*) in Australian waters. Evidence of mothers with calves (humpback and dwarf minke whales) in the migratory corridor provided insight into the occurrence of calving before whales reached their northern Queensland breeding grounds. Moreover, the CSWMS has assisted investigations of compliance with whale-watching regulations (Kessler and Harcourt 2013; Kessler et al. 2014). Information obtained from studies like this can be leveraged to help inform local, State and Federal governments as many of these species are listed 'migratory' under the *Environment Protection and Biodiversity Conservation Act 1999* and 'data deficient' under International Union for Conservation of Nature Red List of Threatened Species (IUCN Red List of Threatened Species 2018). Information gathered from this study can also be used to assist with cetacean management and environmental protection (McKinley et al. 2017).

The ability to observe northward migrating humpback whales within close proximity to shore was a contributing factor to the longevity of this study. The convenience and accessibility of shore-based observations minimizes the logistical challenges of data collection (i.e., the study site was not remote, was wheelchair accessible, and did not require a vessel). Shore-based observations have also supported similar long-term citizen science studies focused on cetaceans (e.g., Embling et al. 2015; Tonachella et al. 2012). The consistent presence of a dedicated observer from the onset of this work has been an important driver in its persistence, aided by support from the managing authority, the NSWNPWS. An apprenticeship model of

observer training has ensured a standardised method of data collection which provides greater confidence in the data recorded. The simple methodology used by CSWMS reduces observer error while ensuring that the programme is not limited to those with extensive training or field experience. Limitations with this study are largely known and accounted for: factors such as bad weather (e.g., rain, wind, poor visibility, sun glare) and limited detection out to sea e.g., >4.5km (Nicholls et al. 2000). The main constraint is that while this type of observational data can measure trends in abundance, it does not provide information that can be used to understand cause of changes in population growth due to factors such as breeding success, survival, immigration, and emigration (Clutton-Brock and Sheldon 2010).

Future directions for citizen science based projects

The use of citizen science is becoming more frequent in assisting traditional science (Bonney et al. 2014; Cigliano and Ballard 2017; Cooper 2017; Silvertown 2009). Participants are often passionate about the study species, in this case humpback whales, and are willing to dedicate a substantial amount of their time to the programme. In this study, observers had a high level of community engagement due to the accessibility of the whale observation platform within an urban National Park. Observers spoke regularly with the public, informing people about the research which reinforced the value of their work. Media interest created by the project encouraged observers to engage with journalists. Observers became active on social media platforms such as Twitter, tweeting daily whale numbers and expanding the reach of the study. In addition, observers actively supported other scientific programs conducted in the study area e.g., stock identification of southern right whales, vessel interactions with whales, mitigation of fishing gear entanglements and whale drone research (Gulesserian et al. 2011; Harcourt et al. 2014; Pirotta et al. 2016; Pirotta et al. 2017). Annual support and study coordination such as pre-season meetings, post season celebrations and maintenance of the observation database was provided annually by NSWNPWS personnel. This helped foster and maintain a strong network of observers with a keen interest in whales and whale watching. Like others, we strongly recommend all efforts be made to publish data collected from citizen science based projects to reinforce the value of volunteer efforts (Theobald et al. 2015).

Conclusions

The dedication of observers in the CSWMS has made this one of Australia's longest running whale citizen science based studies. This study demonstrates the benefits of citizen science in

modern long-term data collection and multispecies observations to provide an assessment of cetacean presence off one of Australia's largest cities.

4.5 Supplementary material

Year	Total observation days	Total whales counted	Peak daily count	Date of peak
1997	17	147	27	13-Jun
1998	28	298	29	29-Jun
1999	23	424	31	21-Jun
2000	53	566	30	8-Jul
2001	61	723	48	23-Jun
2002	68	932	46	23-Jun
2003	69	519	23	16-Jun
2004	66	1,094	52	27-Jun
2005	67	1,368	68	5-Jul
2006	66	1,607	61	9-Jul
2007	66	1,295	74	28-Jun
2008	63	1,493	73	25-Jun
2009	69	1,908	89	5-Jul
2010	65	1,824	68	14-Jul
2011	62	2,202	102	6-Jul
2012	68	1,729	77	20-Jun 22-Jun 29-Jun
2013	68	2,646	101	6-Jul 8-Jul
2014	67	2,624	103	23-Jun
2015	68	2,513	107	28-Jun
2016	67	3,033	116	13-Jun
2017	69	**4,813**	**224**	26-Jun

Supplementary table 4.7.1: Summary total observation days per year, total whales observed each season and peak days with highest number of whales observed.

Year	Total observation hours	Mean hours/year	Mean no. observer hours/day	Stand Dev	SE
2013	2,218.5	401.6	5.8	1.428593387	0.171982434
2014	2,282.7	428.5	6.2	1.170182434	0.140873411
2015	2,157.0	391.8	5.7	1.443864787	0.173820894
2016	1,699.5	381.1	5.5	1.445360329	0.174000936
2017	2,028.7	430.4	6.2	1.296296135	0.156055716

Supplementary table 4.7.2: Observer effort over the last five years (2013-2017).

Section three:

Conservation technologies

Chapter Five

An economical custom-built drone for assessing whale health

Abstract

Drones or unmanned Aerial Vehicles' (UAVs) have huge potential to improve the safety and efficiency of sample collection from wild animals under logistically challenging circumstances. Here we present a method for surveying population health that uses UAVs to sample respiratory vapour, 'whale blow', exhaled by free-swimming humpback whales (*Megaptera novaeangliae*), and couple this with amplification and sequencing of respiratory tract microbiota. We developed a low-cost multirotor UAV incorporating a sterile petri dish with a remotely operated 'flip lid' to sample whale blow with minimal disturbance to the whales. This design addressed several sampling challenges: accessibility; safety; cost, and critically, minimized the collection of atmospheric and seawater microbiota and other potential sources of sample contamination. We collected 59 samples of blow from northward-migrating humpback whales off Sydney, Australia and used high throughput sequencing of bacterial ribosomal gene markers to identify putative respiratory tract microbiota. Model-based comparisons with seawater and drone-captured air demonstrated that our system minimized external sources of contamination and successfully captured sufficient material to identify whale blow-specific microbial taxa. Whale-specific taxa included species and genera previously associated with the respiratory tracts or oral cavities of mammals (e.g., *Pseudomonas*, *Clostridia*, *Cardiobacterium*), as well as species previously isolated from dolphin or killer whale blowholes (*Corynebacteria*, others). Many examples of exogenous marine species were identified, including *Tenacibaculum* and *Psychrobacter* spp. that have been associated with the skin microbiota of marine mammals and fish and may include pathogens. This information provides a baseline of respiratory tract microbiota profiles of contemporary whale health. Customized UAVs are a promising new tool for marine megafauna research and may have broad application in cost-effective monitoring and management of whale populations worldwide.

5.1 Introduction

Conservation biology is entering a new era of innovation, with unprecedented growth across a range of techniques, from genetics and genomics to telemetry and remote sensing (Schierwater et al., 2013; Hussey et al., 2015). Rapid advances in the technology underpinning Unmanned Aerial Vehicles (UAVs also known as Unmanned Aircraft Systems or drones), are driving new and innovative environmental applications (Koh and Wich, 2012; Anderson and Gaston, 2013; Christie et al., 2016; Smith et al., 2016; Duffy et al., 2017). The application of UAVs in conservation science makes it possible to collect information from dangerous and inaccessible environments and answer research questions that were previously limited to the hypothetical (Harvey et al., 2016). UAVs also provide an alternative, safer, quieter and often cost-effective option for monitoring fauna and flora, from individuals and populations to entire ecosystems, and in so doing are replacing expensive manned systems such as helicopters and fixed-wing aircraft (Christiansen et al., 2016; Christie et al., 2016). UAV applications in wildlife research now encompass almost all environments, from arid deserts, through rainforests, oceans to polar regions (Linchant et al., 2013; Durban et al., 2015; Goebel et al., 2015; Linchant et al., 2015; Duffy et al., 2017).

UAVs are transforming the way scientists monitor and conserve wildlife (Gonzalez et al., 2016). In the terrestrial world, UAVs have been used for a wide variety of conservation applications (van Gemert et al., 2015; Gonzalez et al., 2016). Some examples include, counting elephants (*Loxodonta africana*) (Linchant et al., 2013; Vermeulen et al., 2013), UAV surveillance (anti-poaching tools) for elephants and rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) (Marks, 2014; Mulero-Pázmány et al., 2014; Hahn et al., 2017), locating chimpanzee nests (*Pan troglodytes*) (Van Andel et al., 2015) and mapping Sumatran orangutan (*Pongo abelii*) habitat, distribution and density (Wich et al., 2015; Szantoi et al., 2017). UAV applications now extend to the polar regions where they have been used to monitor and estimate abundance of penguin populations (gentoo, *Pygoscelis papua*, and chinstrap, *Pygoscelis antarctica*) and estimate size and condition of leopard seals (*Hydrurga leptonyx*) (Goebel et al., 2015; Ratcliffe et al., 2015). In the marine environment, UAVs are revolutionizing the way marine species can be studied due to their small size, apparent minimal disturbance of wildlife and improved safety for both operators and animals (Nowacek et al., 2016; Fiori et al., 2017). UAVs have been utilised for a wide variety of applications including aerial surveys, monitoring, habitat use, abundance estimates, photogrammetry and biological sampling e.g., whale 'blow' (Hogg et al., 2009;

Acevedo-Whitehouse et al., 2010; Hodgson et al., 2013; Durban et al., 2015; Pomeroy et al., 2016; Schofield et al., 2017).

There are widespread concerns about the health of marine mammal populations in the face of global anthropogenic stressors (Gulland and Hall, 2007). Yet health assessments typically involves collecting samples from stranded animals, which are often biased as these animals are most likely to be health-compromised (Geraci and Lounsbury, 2005). Sampling exhaled breath or 'blow' from wild whales may therefore provide a more representative assessment of the health status of individuals because samples can be randomly taken from the population. From a blow single sample, scientists may be able to collect respiratory bacteria, lipids, proteins, DNA and hormones (Hogg et al., 2005; Hogg et al., 2009; Schroeder et al., 2009; Acevedo-Whitehouse et al., 2010; Hunt et al., 2013; Hunt et al., 2014; Thompson et al., 2014; Burgess et al., 2016; De Mello and De Oliveira, 2016; Raverty et al., 2017). This information is important for whale conservation, as it can be collected overtime to help monitor the recovery of whale populations post-whaling. Early approaches to sampling whale blow involved passing a cotton gauze or nylon stocking on the end of a carbon fiber pole through the blow when the animal surfaced (Hogg et al., 2009; Hunt et al., 2014). Recent advancements on this method has seen the use of a pole with a number of petri dishes with lids to sample wild killer whales (Raverty et al., 2017). However, this method requires extremely close vessel approaches to whales (Hogg et al., 2009). Given the large size, mass and power of whales, this approach involves high risk to both researchers and to the whale itself. Even under ideal circumstances this method is likely to disturb to the animal, potentially compromising the validity of some of the measures such as stress hormones which elevate rapidly (Harcourt et al., 2010). Accordingly, alternative approaches have long been sought. Acevedo-Whitehouse et al. (2010) deployed a single-rotor UAV (a remote-controlled helicopter) to sample whale blow. Their study demonstrated the feasibility of the approach but loss of samples from the UAV as it careers through the sea air proved a potential issue as did contamination from airborne particulate not expired by the whale.

Here we describe a purpose-built UAV designed to sample whale blow in the field with minimal contamination. Our goal was to provide a snapshot of whale health. We specifically targeted northward migrating humpback whales (*Megaptera novaeangliae*) off the East coast of Sydney, Australia for the collection of baseline microbiota information. The UAV used in our study has a unique combination of features that represent a significant advance over existing UAVs. It is

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fast, highly manoeuvrable, durable, waterproof, low-cost (< $USD 1,000) and provides flexible payload mounting options. The UAV is scaled to the sampling gear (in this case a 100mm Petri dish), which is held in a mechanism that allows the dish to be opened/closed during flight – minimizing sample contamination or loss.

5.2 Method

Study site and species

All flights were conducted offshore Sydney, Australia (Figure 5.1). Each year from May-November, migratory Group V (Stock E1) humpback whales migrate past Sydney, as they swim from high latitude feeding areas in Antarctica to low latitude breeding waters off Queensland (Chittleborough, 1965). All sampling took place in coastal waters <3 nautical miles from Sydney between 30 May 2017 and 27 June 2017.
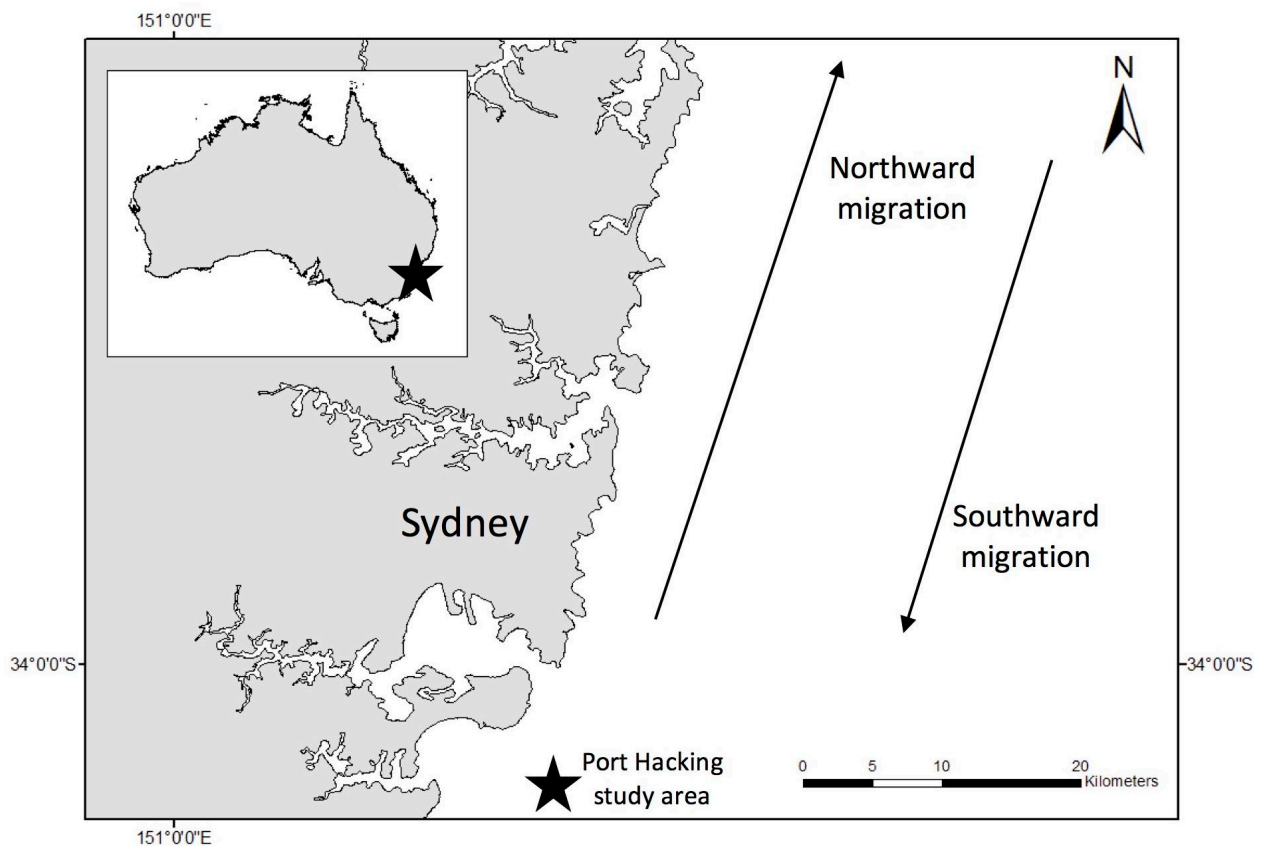

Figure 5.1. Study site (indicated by black star on insert). All samples were collected in coastal waters (<3nm) off Sydney, Australia. Blow samples were collected only from northward migrating East coast Australian humpback whales. Water samples were collected over a number of years from Port Hacking (indicated by star outside of insert).

UAV design

The UAV is a 4-motor electric multirotor (quadcopter) 500mm across (motor to motor, diagonally) (Figure 5.2a). It has a relatively high power to weight ratio making it fast, maneuverable, resistant to strong wind gusts and relatively quiet while hovering. It carries the bare minimum of hardware and is operated in 'manual mode' (no GPS or autolevelling assistance) with a heavy reliance of the onboard video feed for control, navigation, and sampling operations. The airframe structure of the UAV is a 'sandwich' style construction cut from carbon fibre plate, with a top shell moulded from impact-resistant polycarbonate. This seals against the airframe to create a waterproof compartment which houses the power distribution, flight control, motor control, radio control transceiver and video transmitter components. The float booms/legs were cut from expanded polypropylene (EPP) – a closed-cell foam, chosen for high strength, resistance to bending loads and excellent water resistance. A clear acrylic tube at the front of the aircraft houses a forward facing, tilting camera that provides a real-time position reference to the pilot (First Person View). The resulting composite structure is light, stiff, strong and waterproof. Buoyancy is provided by the two watertight compartments and EPP foam floats under the arms. In the event of a crash or forced landing over water, the UAV floats in an upright position so it can be recovered or take off again. Two reinforced mounting areas on the top shell accept payloads of around 100g. For this configuration, the blow-sampling apparatus was mounted at the front. This is a hinged frame which opens to 180 degrees and holds a 100mm diameter petri dish with suction cups. A servo motor opens and closes the dish remotely, during flight. Airflow testing using smoke indicated the best position for the sampling dish relative to the propellers. A forward-looking waterproof video camera (GoPro® Hero Session™) is positioned at the rear and logs video to an internal memory card. The dish is in the frame of the recorded video, so the footage can be used to confirm the source of the sampled material.

Sampling method

This study was approved by the Macquarie University Animal Ethics Committee, and carried out in accordance with the Animal Research Authority (2016/010). This research was permitted by New South Wales National Parks and Wildlife Services (NSWNPWS) to fly UAVs over whales in New South Wales coastal waters (permit number SL101743). To adhere to Australian legislative requirements, the UAVs (including backup UAV) were registered with the Civil Aviation Safety Authority (CASA) and operated by a CASA certified operator (Heliguy Pty.Ltd.). All flights were conducted in good weather (no rain, Beaufort < 3), from small research vessels, where the UAV

was launched and landed on a launch pad at the bow or stern of the boat. A closed, sterile petri dish with nutrient agar covering the base of the petri dish was secured using eight suction cups affixed on the UAV before each flight.

Members of the team scanned the area for humpback whales. Once an individual was selected, the vessel was driven maintaining a constant speed and distance from the whale (> 200 m). Once the respiratory rhythm of an individual was determined (downtime length in minutes), the UAV was launched to coincide with the individual surfacing. The UAV pilot was directed by spotters on the vessel and positioned the UAV with the aid of the live feed from the forward-facing camera. To minimize sample contamination, the petri dish remained closed until just before the whale surfaced, when the dish remotely opened as the UAV accelerated towards and through the densest part of the whale blow, collecting the maximum amount of sample in the dish and lid (Figure 5.2b and 5.2c). The petri dish was immediately closed and the UAV was returned to the vessel. The petri dish containing the sample was removed from the UAV and Parafilm® was wrapped around the closed petri dish to secure the sample. All samples were temporarily stored in a cooler box on ice until further processing in the laboratory at the end of each day.

Figure 5.2. (A) Purpose-built UAV designed to sample whale blow. The UAV consists of a sandwich style carbon fibre body. White foam floats support the UAV during take-off and landings and provide floatation in water. The yellow shell houses all electrical equipment. A GoPro® hero session is mounted at the back of the yellow shell to record flights. A hinge mechanism with disposable petri dish is located in the centre of the yellow shell. This can be remotely operated to minimize sample contamination in the field. The clear round tube at the front of the UAV houses the first-person camera to assist with sampling. (B) UAV sampling whale blow. This photo was taken just as the UAV had passed through the visible blow (plume of spray). The petri dish is still in the open position. Sample was collected on both the lid and bottom (nutrient agar filled) side. The petri dish was shut immediately after collection to minimize sample contamination and the drone was flown back to the research vessel >200 meters away. (C) Screenshot from the UAVs on-board GoPro® camera mid whale sample collection. This footage shows the petri dish at the bottom of the picture. The whale is located on the right-hand side. The petri dish is completely extended (open) with blow droplets visible on both sides of the dish and GoPro® lens.

Attempts were made to sample a different whale each flight. Individuals within a pod were chosen based upon unique markings (e.g., white flanks/patterns/scarring/barnacle arrangements). To ensure the same individual was not sampled twice, a live video feed was used to target individuals. Cross contamination among whales was avoided by not triggering the opening of the flip lid until only the targeted whale respired. Footage collected from the GoPro® throughout each flight was used to validate sample collection and eliminate repeated sampling of the same individuals by post-hoc identification. The behavioral response of whales was recorded for each pass using by scoring system of one to three (one: 'no response', two: 'minor response' minor surface activity such as logging, spy hopping and three: 'severe/elevated? response' e.g., breaching, peduncle throw or chin slap).

Air and seawater samples

To enable direct comparison of UAV-captured air and whale blow samples with bacteria inhabiting the adjacent seawater, the data were combined with 16S sequence libraries prepared from 26 surface seawater samples. This represents a complete annual cycle, collected from the National Time Series Station known as Port Hacking 100 (PH100). All UAV-captured samples were collected within 20 km of PH100.

Laboratory processing of samples

Initial processing of samples occurred in two stages. First, in an Ultra Violet-sanitised class II biosafety hood, the top of the petri dish lid (non-agar) side was swabbed using a dry sterile cotton tip and then placed in a sterile 1.5 ml tube and stored in the freezer at -30°C. Secondly, the petri dish (both the lid and nutrient base) was placed in an incubator at 37°C after the lid was swabbed, simulating average mammalian body temperature 36-37 °C (Whittow, 1987; Cuyler et al., 1992). Plates were observed daily for colony growth. If growth occurred, colonies were counted and a representative number of colonies were picked from each plate, resuspended in 100µl of sterile water, vortexed for 10s and immediately frozen at -30˚C until further processing. Plates were then stored in the fridge for future reference if needed.

Bacterial DNA extraction

DNA extractions were conducted using the *Quick*-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, California, USA) with minor modifications to the manufacturer's instructions. Each swab was transferred to a tube containing 1.2g of ZR BashingBeads™ (equivalent to ~half of the portion supplied for each extraction). The original storage tube was rinsed with lysis

solution (750 μl) to ensure the complete transfer of material into the extraction tube. The swab was then bead-beaten on a Vortex-Genie® 2 (Mo Bio Laboratories/QIAGEN, California, USA) for 20 minutes at room temperature. All other steps were followed according to the manufacturer's instructions, with the exception that two successive final elutions were carried out, each with 20 μl of sterile DNA elution buffer.

Amplification and sequencing

Amplicons targeting the bacterial 16S rRNA gene (27F–519R; (Lane et al., 1985; Lane, 1991) were generated and sequenced for each sample at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) using 250 bp paired end illumina sequencing according to established protocols (http://www.bioplatforms.com/wp-content/uploads/base_illumina_16s_amplicon_methods.pdf).

Amplicons generated from drone-captured air and whale blow were combined with 27F–519R sequences generated from 26 surface (2 m and 10 m depth) seawater samples collected over a complete annual cycle from the nearby National Reference Station (PH100) time series (Dec 2014-Mar 2016). Monthly microbial sampling has been conducted at the Port Hacking100 reference station since 2009 (Seymour et al., 2012). All UAV-captured whale and air samples were collected within 20 km upstream of this reference station, within 1-3km from shore. We reasoned that this dataset, which was sampled and sequenced using standardised protocols at the same sequencing centre, would provide a comprehensive and unbiased assessment of bacterial species characteristic of seawater in this region, which could be excluded as potential contaminants from the whale blow samples. Whale, air and seawater samples analysed in this study are detailed in Appendix tables 1 and 2.

Sequence Operational Taxonomic Units (OTUs) tables were prepared after (Bissett et al., 2016). Briefly, paired-end reads were filtered using Trimmomatic (ILLUMINACLIP: NexteraPE-PE.fa:2:30:10 SLIDINGWINDOW:4:15 MINLEN:76) (Bolger et al., 2014) then merged using PEAR (Zhang et al., 2014). The combined amplicon data were clustered into OTUs at 97% sequence similarity using an open reference OTU picking pipeline in USEARCH 64 bit v8.1.1756 (Edgar, 2010), which included denovo chimera detection. Clusters with < 4 sequences were removed, and reads were mapped to representative OTU sequences using USEARCH (97% ID) to calculate read abundances. From an initial pool of 10.5 million paired-end reads, a total of 7.62 million filtered, merged sequences, with chimeras removed, were added to the OTU table. OTU tables were sub-sampled to a constant sampling depth of 10,000 sequences using rrarefy in vegan

(Oksanen, 2017). All subsequent analyses were conducted on sub-sampled OTU tables. Sequences generated over the course of this project are deposited in the European Nucleotide Archive under project PRJEB23634. All seawater sequence data are deposited in the NCBI Sequence Read Archive PRJNA385736.

Data Analyses

Hierarchal clusters of OTU abundance profiles generated from seawater, drone-captured air and whale blow were compared using the simprof test following square-root transformation and conversion to a Bray-Curtis dissimilatory matrix in the r package clustsig (Whitaker and Christman, 2014). Data from samples that were near misses, which would reflect a mixture of air and whale blow microbiota, were set aside from the subsequent statistical analyses. The community structure dissimilarity between samples was observed with non-metric multidimensional scaling. Significant differences in communities sampled in seawater, UAV-captured air or whale blow samples were defined using generalized linear models within mvabund (Wang et al., 2012). Briefly, a negative binomial model was fit to the OTU abundance data and the sample grouping was analyzed using Analysis of Variance (ANOVA). OTUs that were significantly overrepresented in seawater, drone-captured air or specific for whale blow samples were defined using ANOVA with the 'p.uni="adjusted"' option. OTUs were classified against the Silva 123 release database (Quast et al., 2013) using mothur 'classify.seqs' with default parameters (v1.36.1, Schloss, 2009).

Identifying bacteria isolated from agar plates

Bacterial 16S rRNA genes were directly amplified from cell suspensions obtained from colony picks using conserved primers 27F and 519R (Lane et al., 1985; Lane, 1991). PCR amplifications consisted of 1.0 µl of template and cycle specific for 16S consisted of 95°C for 10 min, 94°C for 30 s, 55°C for 10 s, 72°C for 45 s and 72°C for 10min, and Taq DNA Polymerase (Qiagen). Amplified DNA was prepared for Sanger sequencing using Agencourt® AMPure® XP beads (Beckman Coulter). Sequences were trimmed to q20, and classified against the Silva Database (version 123).

5.3 Results

A total of 74 flights were conducted over four days of sampling. Each pod was considered independent as all whales were on their annual northern migration (Pirotta et al., 2016).

Overall, 59 successful samples were collected from at least 48 different whales (11 whales were sampled but not identified via video due to occasional failure of the GoPro® camera e.g., low battery or maximum storage capacity reached). Sample volume varied between 50-150 µl of exhaled breath. The average opening time of the flip lid was 4 seconds (min 2 s, max 6 s). The UAV had a maximum flight time (battery time) of 15 minutes and sampling attempts on average were 4min 28 seconds long (range: 27 s to 7 mins). The majority of flight time was used to search for the whale's next surfacing position. The time that the UAV was in close proximity to a whale (UAV approximately within 5 m horizontal distance) varied but was on average 53 s (range: 2 s to 2.36 min or 141 s). The most number of samples collected in one day was 38. In all cases, there was no behavioral response to the drone (level 1, n= 48). Twice there were strong social interactions that occurred prior to the drone approaching the whales (one tail slap, one breach) but sampling was continued on the group in each case and samples collected successfully.

Next Generation Sequencing Results

A total of 7.62 million filtered bacterial 16S ribosomal gene sequences were produced from 59 UAV-captured whale blow and six air samples. These were combined with 0.91m sequences generated from 26 seawater samples to generate bacterial OTU abundance profiles. Distance-based clustering of blow, air or seawater bacterial community profiles defined at least three significant clusters (simprof, P<0.05), encompassing one group exclusively composed of seawater, one group exclusively composed of whale blow samples and a third group which clustered the six air samples along with 11 whale-blow samples (Figure 5.3A). Whale blow samples in this group may correspond to UAV sorties that missed, or narrowly missed, capturing whale blow material and were highly correlated with low capture scores based on a visual score of the amount of whale material recovered (Appendix Table 1).
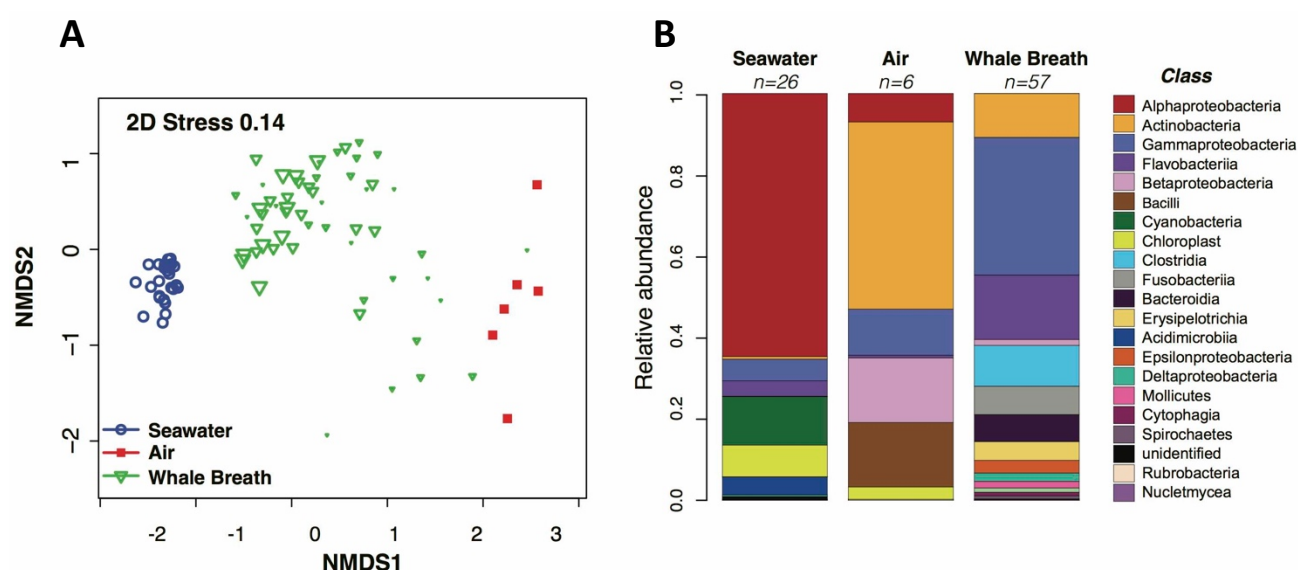
Figure 5.3. Similarity analysis of Operational Taxonomic Units (OTUs) abundance profiles and comparison of bacterial Classes identified in sampled whale blow, air and seawater. (A) non-metric multidimensional scaling plot of bacterial OTU abundance profiles. The size of each whale plotting character is scaled to a visual score of the amount of whale blow captured on the petri dish (e.g., bigger the triangle, greater amount of sample). OTUs were defined at 97% nucleotide identity. (B) Relative abundance of taxonomic classes identified as whale-, air- or seawater-specific in each sample type.

Bacterial OTUs correlated with seawater, whale blow or air samples were identified using Analysis of Variance (ANOVA) based on generalized linear models fit to the data (Wang et al., 2012). OTU diversity and abundance profiles for air and whale blow were significantly different (p < 0.05) from each other and bear little similarity with communities characteristic of the adjacent seawater. At the Class level whale blow bacteria were dominated by Gammaprotobacteria, Flavobacteriia, *Clostridia* and *Fusobacteria*, in contrast to seawater communities, where species composition reflected values typical for sub-tropical waters of the Tasman Sea, i.e., ~60% Alphaproteobacteria, 15% *Cyanobacteria* and smaller proportions of Gammaproteobacteria and Flavobacteriia (Figure 5.3B; (Seymour et al., 2012)).

Overall, whale blow samples displayed the greatest OTU diversity, followed by seawater and air (Figure 5.4). Model-based multivariate analyses identified 198 OTUs that were seawater-specific and 35 OTUs that were significantly correlated with air samples (ANOVA, P<0.1; Supplementary Tables 3,4 [2]online). Successfully collected whale blow samples contained a small proportion seawater- and air-specific OTUs, contributing on average 15.7 ($\pm$10.8) % and 11.5($\pm$4.4) %, respectively, of total sequences. The proportion of air-specific and seawater OTUs in near-miss samples was significantly higher (41.0% and 24.1%, respectively). Subtraction of seawater and air specific OTUs from the total enabled us to define 129 OTUs that were highly specific to whale samples (ANOVA, P<0.05, Figure 5.5, Supplementary table 5, online). Abundant bacterial species identified as whale-blow-specific include multiple OTUs belonging to the genera Cardiobacteriaceae and species *Tenacibaculum*, followed by OTUs related to *Pseudomonas* sp. Strain wp33, *Leptotrichia* sp. and *Corynebacteria* spp. While these analyses identified which OTUs were highly specific for whale, air and seawater, an addition set of whale-related OTUs could be identified in the remaining non-significant OTUs. We used the following criteria: present in greater than five whales and >100 sequences, to add an additional 145 OTUs that were highly specific to whales but found only in a small proportion of the sampled whale population (5-17 individuals, out of a total of 57) (Supplementary Table 6, online). Many of the OTUs in this group are closely related to whale-specific OTUs at the genus and species levels, e.g., *Cardiobacteriaceae*, *Tenacibaculum* and *Fusibacter* strains. However, potential respiratory pathogens were also detected, such as Balneatrix (Gammaproteobacteria), and a range of Gram positive Clostridia and Bacilli, such as *Staphylococcus* and *Streptococcus*. In the context of monitoring whale respiratory health, potential pathogens may be present in a subset of the population only. OTUs in this whale-associated group were present in low abundance, and on average constituted 13($\pm$5.7) % of the total sequences detected in each whale sample.

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[2] Due to their size, supplementary tables 3, 4, 5, 6 have not been reproduced in this thesis and are available online:
https://www.frontiersin.org/articles/10.3389/fmars.2017.00425/full#supplementary-material
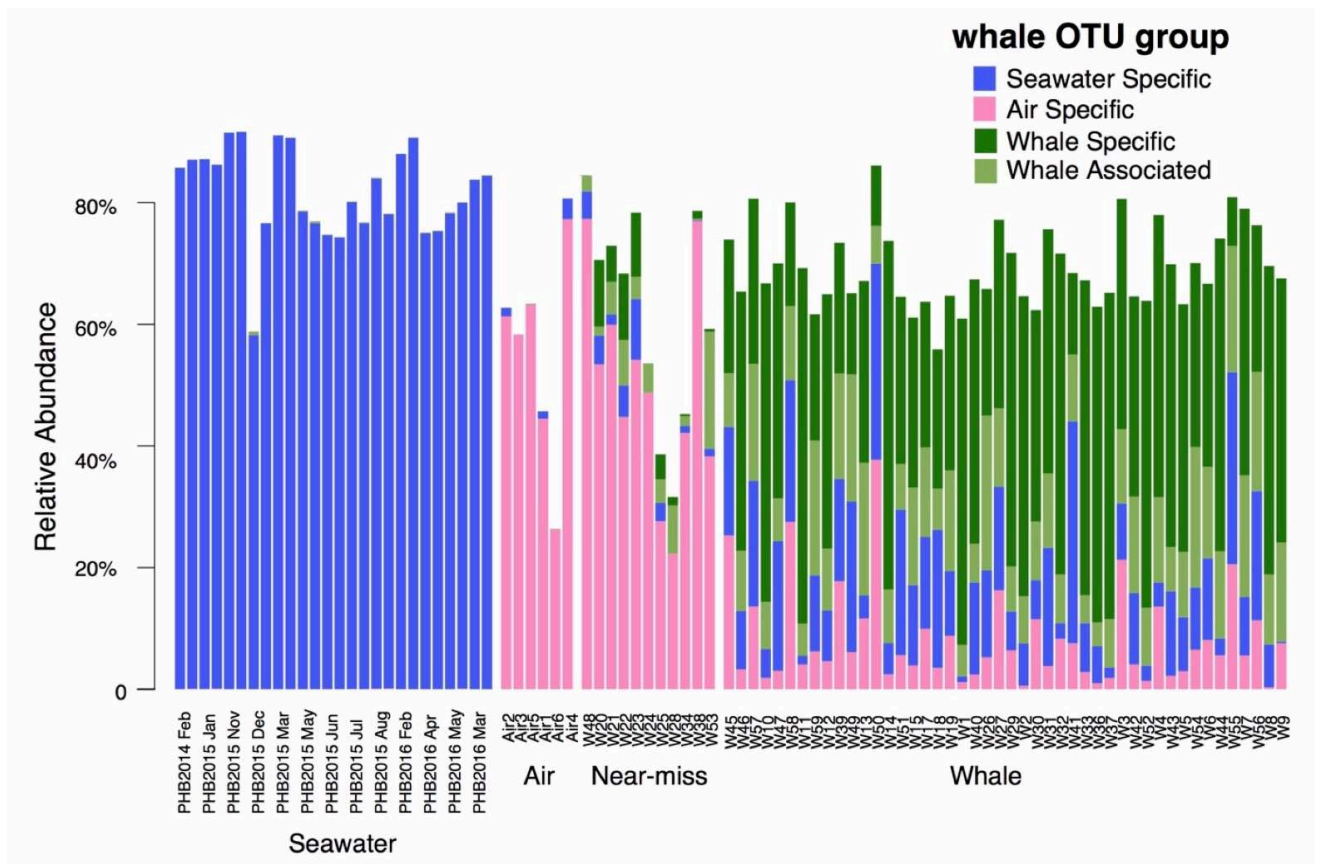
Figure 5.4. Proportion of sequences specific for whale, seawater and air in each sample.

Statistical analyses of OTU abundance identified individual OTUs that were highly specific for each sample type. An additional group of 125 whale-associated OTUs that were present in 5-17 individuals, absent from seawater and air and displayed > 100 sequences in the rarefied dataset are also included. OTUs with low abundance (< 9) and those with no significant association (ANOVA P>0.1) were omitted.
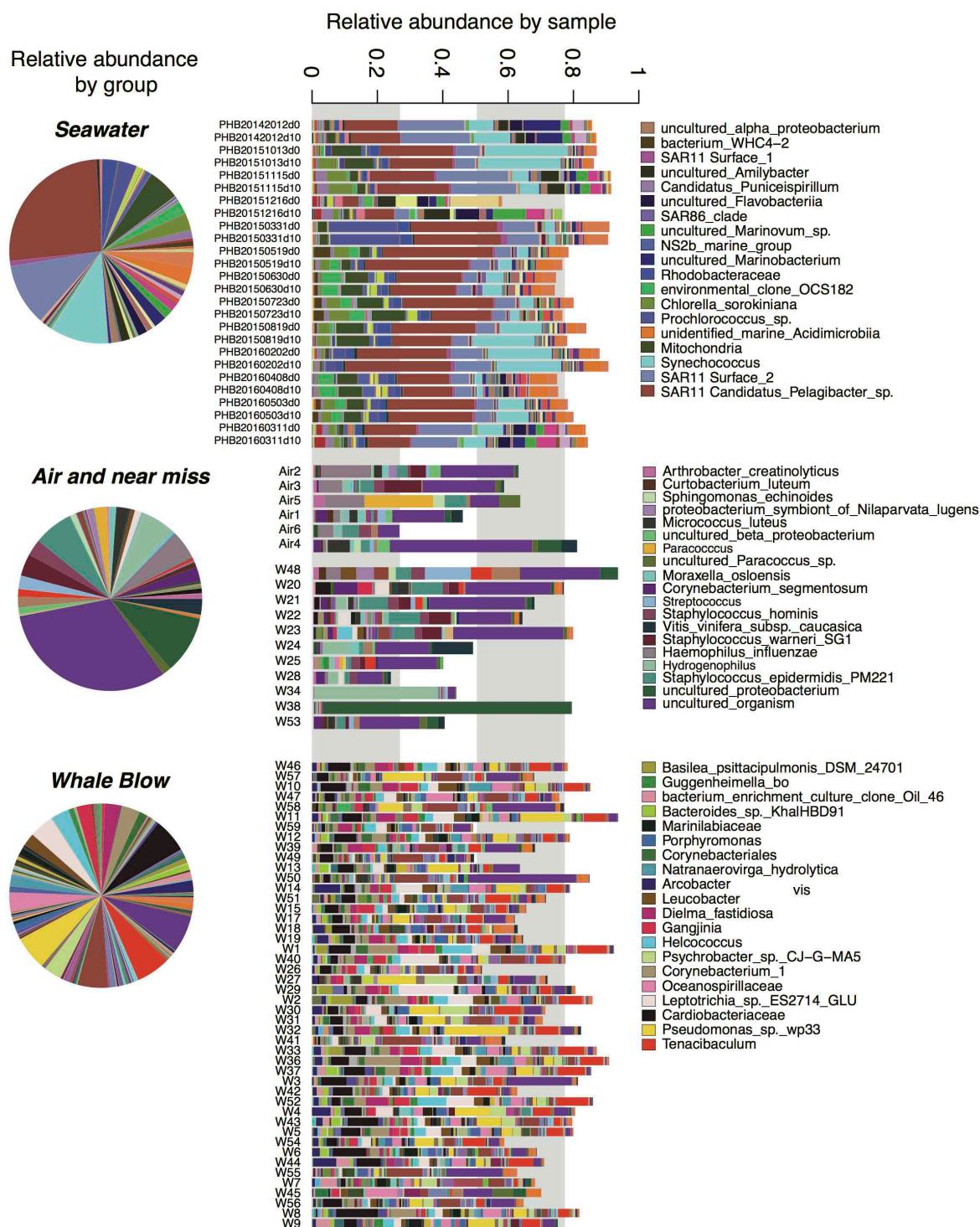
Figure 5.5. Relative abundance of bacterial taxa identified in seawater, UAV captured air, and whale blow. OTUs with abundance < 9 across the entire dataset were omitted for clarity. Relative abundances are presented for each group (i.e., seawater, air plus 'near-miss' samples and whales, as well as for each sample. Taxa names correspond to the highest taxonomic level identification, full taxonomies are present in supplementary tables (3,4,5, and 6 online) only the top taxa by abundance are shown in the legend.

Comparison with culture-dependent identification of whale blow microbiota

Bacterial growth was observed on 48 UAV-mounted agar plates exposed to whale blow. Unexposed control plates displayed no bacterial growth. Sequencing of rRNA genes amplified from single colonies identified 18 different bacteria taxa isolated from 19 different whales (Appendix Table 3). Overall, the most common bacteria identified at the phylum level included Proteobacteria (n= 7), Firmicutes (n= 7) and Actinobacteria (n= 4). Two samples were identified to the family level, Brucellaceae (n=1) and Microbacteriaceae (n=1). At the genus level, *Micrococcus* (n= 3), *Acidovorax* (n= 3), *Bacillus* (n=3), *Enterobacteriaceae* (n= 2), *Paenibacillus* (n=2), *Streptococcus* (n= 2), and *Staphylococcus* (n=2) were most common. Seven whales had more than one bacterium identified. *Staphylococcus* was identified in both an individual sampled via our UAV and from the blow of the stranded juvenile humpback whale.

5.4 Discussion

UAVs are rapidly transforming the way scientists collect information on their study species (Christie et al., 2016; Lowman and Voirin, 2016; Nowacek et al., 2016; Duffy et al., 2017). In whale research, UAVs have enabled sampling methods to be refined and have eliminated the need for close vessel approaches. To our knowledge, this study is the first to successfully demonstrate the use of a purpose-built UAV designed to sample humpback whale blow in Southern Hemisphere waters. The minimal behavioral disturbance observed suggests this method is an excellent, low-impact alternative to pole sampling methods for large, migrating whales. Humpback whales may have been aware of the UAV and did not react or, mostly likely, were not even aware of the UAV's presence. Underwater noise generated from the UAV was likely to be very low level at the heights flown (<10m), as it is smaller, lighter and has a lower disc loading than comparable off-the-shelf UAVs shown to transmit minimal noise transmission underwater (e.g., SwellPro Splashdrone and the DJI Inspire 1 Pro) (Christiansen et al., 2016). The combination of the waterproof design and the remotely operated flip lid petri dish designed to minimize airborne contamination, is a significant improvement over existing UAV types.

Our results demonstrate that whale blow can be effectively sampled while minimizing species associated with likely sources of contamination, i.e., air and seawater, to define microbes specifically associated with whales. Amplification of DNA extracted from UAV-captured air

highlights the sensitivity of PCR-based approaches for detecting microbiota, even from low amounts of extracted DNA, while also demonstrating the sensitivity of this approach to contamination from external sources. The development of a flip-lid sampling system using sterile petri-dishes enabled us to effectively reduce contamination from typical seawater bacteria, which may exist in aerosols above the sea surface. While the presence of abundant seawater species (Alphaproteobacteria SAR11 and cyanobacteria) in air and whale blow samples is not surprising, the source of some major species detected in air samples is less clear. Some of the most abundant species detected in air samples, *Propionobacteria*, *Arthrobacter* and *Staphylococcus*, are common commensal organisms of mammalian (human) skin and nasal cavities (Human Microbiome Project Consortium 2012; Prussin and Marr, 2015). A potential source of some non-marine material may have been contamination during the DNA extraction or amplification procedure, especially when the amount of captured material was low (i.e., for air or near-miss samples). In the context of developing indicators of whale health the presence or absence of species that are common in humans should be interpreted cautiously. Nevertheless, in the UAV-sampled blow where a sufficient amount of material was collected, our analyses indicate that ~70% of the total sequences were specific to whales, a group of whale associated sequences accounted for a further ~12% and the remainder could be confidently identified as seawater- or air-specific.

To our knowledge this is the first study to utilise a long-term seawater dataset to identify and subtract seawater bacteria from community profiles of field-captured mammalian samples. The seawater data provided a comprehensive, temporal assessment of the composition of microbial communities present in sea water off Sydney. Critically, a much larger quantity of seawater was collected (2L) and analysed in comparison to the whale samples. This method minimised the impact of external sources of contamination and allowed for the greater coverage of the seawater community diversity. We used this resource to filter out all sequences characteristic of seawater to produce a whale blow dataset that could be used as a diagnostic for whale health. The distinct differences observed between statistically-defined bacteria in whale, sea water and air samples indicates that this method was effective for collecting whale microbiota with minimal contamination.

The successful collection of bacterial DNA in this study provides baseline information of microbiota found in migrating humpback whale blow. Due to the infancy of sampling whale breath as an assessment of whale health (Acevedo-Whitehouse et al., 2010; Hunt et al., 2013),

it is not clear as to the type of microflora/bacteria species that are considered 'normal' for northward migrating humpback whales off Sydney. Despite this, there are similarities in our collection of bacterial genera from the few studies that have collected blow for the assessment of microbiota (Acevedo-Whitehouse et al., 2010; Denisenko et al., 2012; Hunt et al., 2013). For example, *Streptococcus* and *Staphylococcus* genera were detected in our samples and have been detected in the blow of blue whales (*Balaenoptera musculus*), grey whales (*Eschrichtius robustus*) and Southern resident killer whales (Acevedo-Whitehouse et al., 2010; Denisenko et al., 2012; Hunt et al., 2013; Raverty et al., 2017). Bacteria from the *Streptococcus* genus is common in mucous membranes of animals (and humans) and is known to be found in the upper respiratory tract (Krzyściak et al., 2013). *Streptococcus* bacteria has previously been responsible for pneumonia causing death in cetaceans (Acevedo-Whitehouse et al., 2010)). *Bacillus sp*. was also identified via blow collection from western North Pacific grey whales and Southern resident killer whales (Denisenko et al., 2012; Hunt et al., 2013; Raverty et al., 2017).

Next generation sequencing identified *Cardiobacteriaceae* (family) and *Tenacibaculum* (genus) to be the most abundant bacterial rRNA genes in whale blow. *Cardiobacteriaceae* has previously been isolated as a dominant taxa in the respiratory system of 'healthy' captive bottlenose dolphins (*Tursiops aduncus* and*, T. truncates*) and free-ranging species (*T. truncates*) (Johnson et al., 2009; Lima et al., 2012). These findings may indicate that these genes are part of the normal microflora of dolphins, whilst presence in whales until now was unknown. *Cardiobacteriaceae* are abundant on humpback whale skin (*Gammaproteobacteria* genus), as is *Tenacibaculum* (Apprill et al., 2011; Apprill et al., 2014). It may be possible that bacteria found on whale skin also occur within the respiratory tract or epithelial cells. *Tenacibaculum* has been associated with the microbiome of other marine species such as southern bluefin tuna (*Thunnus maccoyii castelnau*) (Valdenegro-Vega et al., 2013), while *Psychrobacter* is part of the thresher shark and rainbow trout skin microbiome (Lowrey et al., 2015; Doane et al., 2017).

The collection of bacterial microbiota is as an indicator of cetacean health is growing (Hogg et al., 2009; Schroeder et al., 2009; Acevedo-Whitehouse et al., 2010; Lima et al., 2012; Hunt et al., 2013; Nelson et al., 2015; Raverty et al., 2017). We were able to sample a number of individuals from a single population over a very short time frame. The use of the waterproof GoPro® camera made identification of different individuals reliable and therefore reduced repeated sampling. Our remotely operated 'flip dish' design proved effective at reducing possible contamination from the pilot/research team (e.g., breath, touch, clothing) and vessel

vapour/fumes. The placement of Parafilm® around the dish after sampling ensured that the sample remained unexposed until back in the laboratory for processing. Recently published work by Burgess et al. (2016) found polystyrene dishes (petri dish) to be the most effective surface for sampling whale blow in comparison to other sampling materials like veil nylon and nitex nylon mesh. In addition, the use of ice chilling of our samples for temporary storage was also consistent with Burgess *et al.* (2016), which found storage in a cooler box with ice packs was appropriate for preserving samples (at least for hormones) for daylong fieldwork at sea (<6h). Our samples only collected a fine mist (we estimated between 50-150 µL per sample, similar to amounts collected by Hogg et al. (2009), and so we were unable to directly pipette samples but we found that swabbing the non-agar lid of the petri dishes to be effective. Variability in blow sample volumes appear to be a common issue (Hogg et al., 2009; Acevedo-Whitehouse et al., 2010) and therefore the need for repeated sampling is recommended. Sample success increased with effort/experience and we recommend effort be made early in any study to improve pilot skill, sample collection, quality, and quantity.

While overall highly successful, UAVs still require a high level of skill and effort. Predicting when the whale is about to surface, positioning the UAV and opening the petri dish in time remains challenging. This may be complicated when a whale comes to the surface to breath but does not respire forcefully. When this happens, the plate is exposed to the air and so the drone must return to the boat so the petri dish can be exchanged, our miss/near-miss rate was 11/59= 20%. Second, not using an off-the-shelf product requires a high level of UAV competence both to fly and to fix problems as they arise. Third, the flight time for this UAV is 15 minutes, restricting the number of opportunities for sampling before the UAV must return to the vessel in order to replace the battery. Flight time will increase as battery technology progresses (Nowacek et al., 2016).

Our dataset details the diversity and abundance of the microbiota found in a migrating whale population which provides the baseline to identify pathogenic species. Ultimately, the isolation of pathogens from healthy or diseased animals will be an important step towards understanding the causes of disease and the factors that contribute to virulence. Culture-dependent techniques remain a viable option for the surveillance of pathogens in populations. In this study, nutrient agar was an effective way of culturing a subset of whale blow microbiota, including species commonly associated with respiratory disease in mammals. The use of both sides of the petri dish effectively doubled the chance of obtaining bacterial samples. While next

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generation sequencing has the capacity to probe the diversity of whale blow microbiota, at present, the isolation and identification bacteria from agar plates can be achieved within 3-5 days, compared to a practical timeframe of weeks for illumina sequencing. Selective media could be used to target potential pathogens in conjunction with opportunistic sampling of diseased or distressed animals.

5.5 Conclusion

Our purpose-built UAV proved highly successful in sampling whale blow for microbial community analysis. It is cost-effective, has low risk of contamination and greatly reduces disturbance of whales. Future applications include other free-ranging whale species (e.g., southern right whales, *Eubalaena australis*), as well as sampling smaller cetaceans (e.g., dolphins). Our UAV is useful addition to the conservation scientist's tool box, enabling collection of health information and therefore the ability to monitor changes in individual health as populations recover and to provide an early warning system for potential future changes.

Chapter Six

Virological Sampling of Inaccessible Wildlife with Drones

This chapter has been published:

Abstract

There is growing interest in characterizing the viromes of diverse mammalian species, particularly in the context of disease emergence. However, little is known about virome diversity in aquatic mammals, in part due to difficulties in sampling. We characterized the virome of the exhaled breath (or blow) of the Eastern Australian humpback whale (*Megaptera novaeangliae*). To achieve an unbiased survey of virome diversity a meta-transcriptomic analysis was performed on 19 pooled whale blow samples collected via a purpose-built Unmanned Aerial Vehicle (UAV, or drone) approximately 3km off the coast of Sydney, Australia during the 2017 winter annual northward migration from Antarctica to northern Australia. To our knowledge, this is the first time that UAVs have been used to sample viruses. Despite the relatively small number of animals surveyed in this initial study, we identified six novel virus species from five viral families. This work demonstrates the potential of UAVs in studies of virus disease, diversity, and evolution.

6.1 Introduction

There is a growing interest in understanding the diversity, evolution and disease associations of viruses in natural populations (Geoghegan et al. 2017). Although sampling of many terrestrial species is relatively straightforward, there may be serious logistical challenges for animals that live in inaccessible habitats. Marine environments are one such habitat (Suttle 2005, Culley et al. 2006, Bogomolni et al. 2008). It has recently been shown that wild whale populations can be sampled using Unmanned Aerial Vehicles (UAVs) (Pirotta et al. 2017, Apprill et al. 2017). UAVs are rapidly transforming wildlife science, allowing sampling from dangerous and inaccessible environments to address questions previously only approached by theory. Here, we show how UAVs can be used to sample viruses. This approach may ultimately enable a better understanding of the patterns and drivers of disease emergence in wild populations.

There is evidence that marine mammal health is deteriorating as anthropogenic stressors on the world's oceans increase (Gulland et al. 2007). However, contemporary assessments of marine mammal health are strongly biased towards animals whose health is already compromised, such as stranded animals, which in part reflects the difficulties in sampling aquatic environments. Sampling from free-ranging marine mammals is therefore critical to assess whether healthy animal populations are potential reservoirs of viruses and other transmittable agents.

6.2 Method

Following the use of UAV technology for sampling, we employed a meta-transcriptomic approach (Li et al. 2015, Shi et al. 2016) to help characterize the virome of an important marine mammal, the Eastern Australian humpback whale (*Megaptera novaeangliae*), which serves as a model for work in this area. Recent analyses of whale breath, or 'blow', have revealed an extraordinary diversity and abundance of microbiota. Importantly, the microbial communities observed were divergent from those present in the surrounding seawater such that they could be considered as distinctly whale blow-associated (Pirotta et al. 2017, Apprill et al. 2017). To date, however, these studies have not included virus sampling, and little is known about the diversity of the whale virome and whether this differs fundamentally from that seen in terrestrial mammals.

We collected whale blow samples from 19 humpbacks during the 2017 annual northward migration from Antarctica to northern Australia (Figure 6.1a). To adhere to all Australian legislative requirements, our UAVs were registered with the Civil Aviation Safety Authority (CASA) and operated by a CASA certified remote pilot. All flights were conducted in good weather (no rain, Beaufort < 3), from a small research vessel, where the UAV was launched and landed on a launch pad at the stern of the boat. A closed, sterile petri dish was placed on eight suction cups on the UAV before each flight.

Members of the team visually scanned the area for humpback whales. Once an individual or pod was chosen, the vessel was driven at a constant speed and distance from the whale. Once the respiratory rhythm was determined (i.e., downtime length), the UAV was launched to coincide with surfacing. The UAV pilot was directed by spotters on the vessel and positioned the UAV with the aid of the live feed from a forward-facing camera. To minimize sample contamination, the petri dish remained closed until immediately before the whale surfaced. The dish was remotely opened as the UAV accelerated towards and through the densest part of the whale blow, collecting the maximum amount of sample in the dish and lid. The petri dish was immediately closed and the UAV was returned to the vessel. The petri dish containing the sample was removed from the UAV and secured with Parafilm®. All samples were stored immediately in a portable -80°C freezer. A different whale was sampled each flight. Different individuals within a pod were chosen based upon unique distinctive markings (e.g., white flanks and barnacle arrangements).

RNA was extracted using RNeasy Plus Universal mini kit (Qiagen). Due to low RNA concentration, all 19 samples were pooled and concentrated using a NucleoSpin RNA Clean-up XS kit (Macherey-Nagel). A single library was produced for RNA sequencing using the Low Input SMARTer Stranded Total RNA Sample Prep Kit with Mammalian rRNA depletion (Clontech), with 1ng of the pooled whale blow RNA as input. Paired-end (100 bp) sequencing of the RNA library was performed on the HiSeq 2500 platform (Illumina) at the Australian Genome Research Facility.

RNA sequencing of the rRNA-depleted library resulted in 19,389,378 paired reads (100 nt in length) that were assembled *de novo* into 107,681 contigs. Sequencing reads were first quality trimmed then assembled using Trinity (Haas et al. 2013). The assembled transcriptome was annotated based on similarity searches against the NCBI nucleotide (nt) and non-redundant protein (nr) databases using BLASTn and Diamond (BLASTX) (Buchfink et al. 2014), respectively,

and an e-value threshold of 1×10^{-5}. Transcript abundance was estimated using RSEM (Li 2011) implemented within Trinity.

6.3 Results

Our transcriptome data revealed that the humpback whale blow contains a wide diversity of DNA and RNA viruses (that we refer to 'whale blow-associated' viruses). BLAST analysis revealed the relative abundance of taxonomic classes present in the non-rRNA transcriptome data, of which bacteria occupied ~45%, while ciliates were the second-most abundant source at ~29%. Importantly, baleen whale species contributed 0.9% of the transcriptome data and were the most abundant source of mammalian RNA, indicating our sample is indeed whale-associated. Viruses occupied ~0.01% of the non-rRNA transcriptome, which falls within the range of other meta-transcriptome studies of vertebrates (Shi et al. 2016). Despite this relatively low abundance, the viral contigs observed fell into 42 classified viral families, including 29 families of bacteriophage (Figure 6.1b). The most relatively abundant bacteriophage included the *Siphoviridae* (18.4% of all viruses) and the *Myoviridae* (15.2% of all viruses). Among the most abundant viral families that are known to infect eukaryotes were small single-stranded (ss) DNA viruses, specifically the *Circoviridae* (and *Circoviridae*-like viruses) (6.5% of all viruses), as well as members of the *Parvoviridae* (2.4%) and an RNA virus family, the *Tombusviridae* (0.9%).
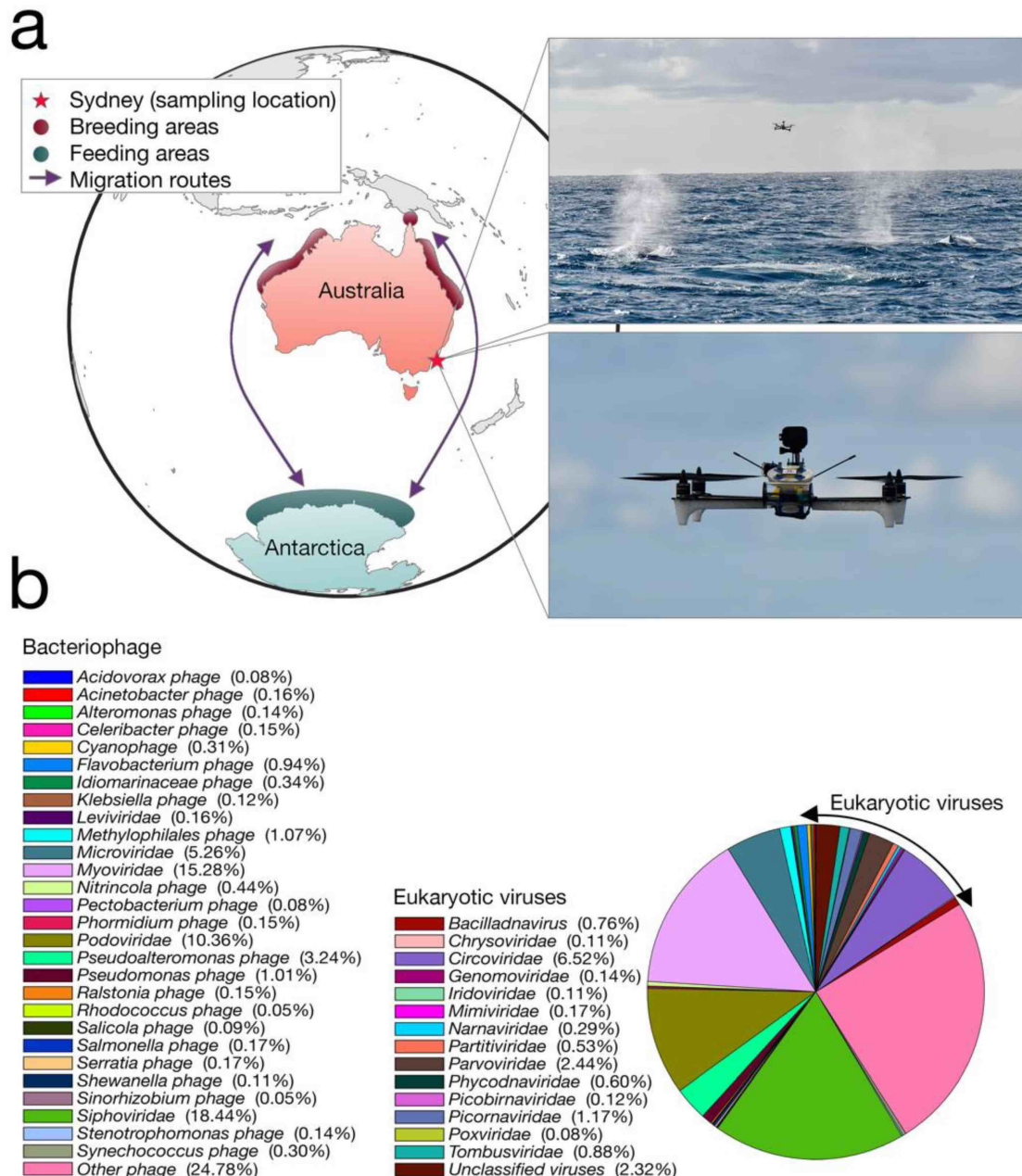
Figure 6.1. (a) Map showing the humpback whale sampling location (red star), approximately 3km off the coast of Sydney, New South Wales, Australia. Purple arrows indicate the typical seasonal migratory routes of the humpback whale from their likely feeding ground in Antarctica (dark green) to their breeding areas around northern Australia (dark red). Photographs demonstrate the UAV in action. (b) Relative abundance of viruses and their taxonomic families. Taxonomy was based on both protein and nucleotide BLAST search results, taking the best e-value for each (for those with identical e-values we used the taxa with the closest percentage identity). This included 42 viral families, including 29 families of bacteriophage. Percentages indicate relative abundance of all viruses in the sequence library.

We next inferred the evolutionary relationships of the viruses contained in whale blow with their closest phylogenetic relatives. Translated open reading frame segments were combined with protein sequences obtained from GenBank, using the top search results from BLAST (see Table 6.1 for more details of the sequences analyzed). Sequences were aligned using MAFFT v.3.4 (Katoh et al. 2002), employing the E-INS-I algorithm with poorly-aligned regions removed using trimAl v.1.2 (Capella-Gutiérrez et al. 2009). To estimate phylogenetic trees for the virus data sets we selected the optimal amino acid substitution model identified using the Bayesian Information Criterion as implemented in Modelgenerator v0.85 (Keane et al. 2006) and analyzed the data using the maximum likelihood approach available in PhyML v3.1 (Guindon et al. 2009) with 1000 bootstrap replicates. Phylogenetic trees were annotated with FigTree v.1.4.2.

Of the most abundant eukaryotic viruses, two novel (as determined by phylogenetic analysis) circular Rep-encoding ssDNA viruses (CRESS-DNA viruses) *Circoviridae*-like viruses were identified, denoted here as humpback whale blow-associated circo-like virus 1 and 2 (Table 6.1; Figure 6.2). Related viruses have previously been identified in many aquatic systems, for which marine invertebrates, particularly crustaceans, are thought to be a primary host (Rosario et al. 2015). Humpback whale blow-associated circo-like virus-1 exhibited 51% amino acid identity to replication-associated protein (Rep) of its closest genetic relative, sewage-associated circular DNA virus-29, and 46% amino acid identity to the Rep of Lake Sarah-associated circular virus-32. Humpback whale blow-associated circo-like virus-2 shared 46% amino acid identity to the Rep of McMurdo Ice Shelf virus-5, isolated from a freshwater pond in Antarctica (Zawar-Reza et al. 2014). As these ssDNA viruses appear to be major virome components in many aquatic environments (Rosario et al. 2015), they are likely associated with aquatic ecosystems in general.

Another relatively abundant viral contig was a partial genome of a novel densovirus (family *Parvoviridae*). The most similar amino acid sequence to this new virus, denoted here as humpback whale blow-associated denso-like virus, was a densovirus isolated from a *Periplaneta fuliginosa* (i.e., a cockroach), sharing only 47% sequence similarity to the non-structural protein (Table 6.1; Figure 6.2). Similarly, a novel tombus-like viral partial genome, falling into the *Tombusviridae*, was identified and was closely related to Changjiang tombus-like virus-9 isolated from crayfish, with 41% sequence similarity to the RdRp. We denote this virus humpback whale blow-associated tombus-like virus (Table 6.1; Figure 6.2).

Virus family	Virus species	Contig length (nt)	% Relative abundance in library	% Amino acid identify	Closest match (GenBank accession number)
Circoviridae	Humpback whale blow-associated circo-like virus 1	702	0.000115%	51%	Sewage-associated circular DNA virus-29 (YP_009117067)
Circoviridae	Humpback whale blow-associated circo-like virus 2	909	0.000164%	46%	McMurdo Ice Shelf pond-associated circular DNA virus-5 (YP_009047137)
Parvoviridae	Humpback whale blow-associated denso-like virus	315	0.000143%	47%	*Periplaneta fuliginosa* densovirus (NP_051022.1)
Tombusviridae	Humpback whale blow-associated tombus-like virus	279	0.000164%	41%	Changjiang tombus-like virus-9 (YP_009337417.1)
Picornaviridae	Humpback whale blow-associated picornavirus	255	(N/A – assembled contigs from raw reads)	61%	Quail picornavirus (NC_016403)
Astroviridae	Humpback whale blow-associated astrovirus	130	(N/A – assembled contigs from raw reads)	76%	Porcine astrovirus 5 (YP_009010969)

Table 6.1. Amino acid identity, contig length and relative frequency of the viruses identified in this study. All sequence reads generated in this project are available in the NCBI Short Read Archive (SRA) under accession number SRP149185 and virus sequences have been deposited in GenBank (accession numbers pending).
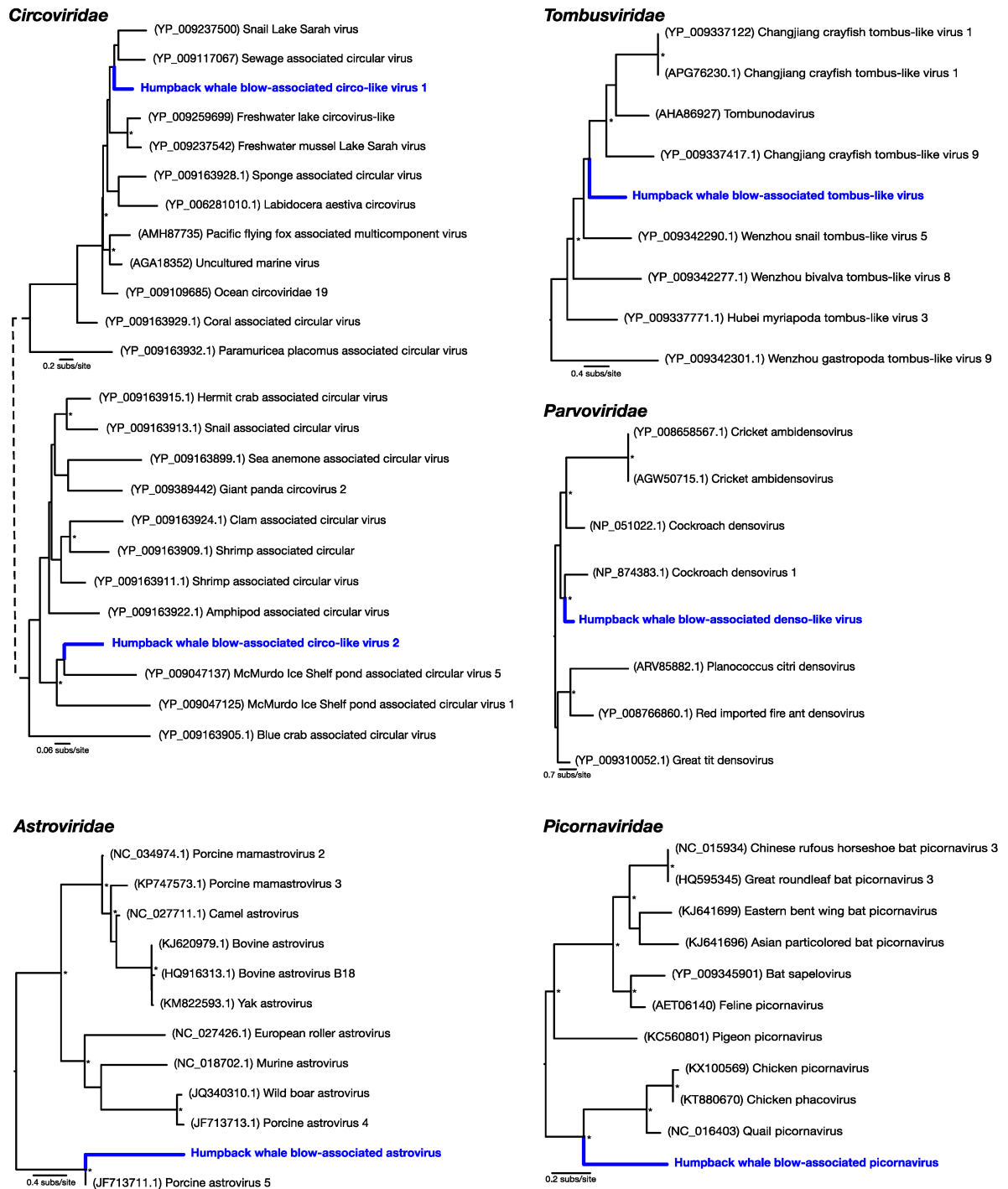
Figure 6.2. Phylogenetic relationships of the viruses discovered from assembled contigs along with their closest genetic relatives obtained from GenBank (accession numbers in parentheses). The families described here are: *Circoviridae*-like, *Parvoviridae*, *Tombusviridae*, *Picornaviridae* and *Astroviridae*. The maximum likelihood phylogenetic trees show the topological position of the newly discovered viruses (blue). Asterisks indicate branch support >70%, based on 1,000 bootstrap replicates. All branches are scaled per the number of amino acid substitutions per site. Trees were mid-point rooted for clarity only.

To reveal viruses at very low relative abundance a Diamond BLAST (Buchfink et al. 2014) analysis was performed against the raw 100bp sequencing reads. This process identified several sequencing reads that matched viruses, later assembled into short contigs, that comprised two potentially new RNA viruses from the *Picornaviridae* and the *Astroviridae*. Humpback whale blow-associated picornavirus shared 61% amino acid similarity to the RdRp of the most closely related *Coturnix coturnix* (quail) picornavirus (Table 6.1; Figure 6.2). Similarly, humpback whale blow-associated astrovirus shared 76% amino acid identity with the non-structural protein 1a of porcine astrovirus-5 (Figure 6.2). Both picornaviruses and astroviruses are single-stranded, positive-sense RNA viruses with small icosahedral capsids and no external envelope which may aid their preservation in harsh marine environments, and viruses from these families are commonly found in aquatic vertebrates (Shi et al. 2016). As only short fragments of these viruses genomes were identified in our data set, their phylogenetic position requires confirmation. This is likely due to the low quantity of RNA isolated from the whale blow samples and the pooling of individual samples. However, that both these viruses were most closely related to other vertebrate viruses suggested that they are likely whale-associated rather than sampled from the surrounding seawater.

6.4 Discussion

Little is known about the transmission of whale viruses. Analyses of whale influenza viruses suggest that they likely originated from gulls and that feeding activities of gulls and whales often place them in close contact, such that oral-fecal transmission through seawater is a likely route (Hinshaw et al. 1986) and which might explain our observation of viruses associated with aquatic ecosystems. In addition, given the vast aerosol produced by whales, and their close contact within migrating pods as well as at feeding and breeding grounds, respiratory transmission may also play an important role in the movement of viruses in whales. Further sampling of the sea water virome is required to understand the enormous potential diversity that comprises the aquatic virosphere.

In summary, we show that drone-based virological surveys of previously inaccessible wildlife populations has the potential to help reveal the diversity of the virosphere, facilitating the detection of viruses infecting wildlife, and aiding evaluation of their pathogenic and zoonotic potential.

Chapter Seven

7.1 General Discussion

Conservation biology provides principles and tools for preserving biological diversity (Soulé 1985). The global expanse and diverse habitats of cetaceans means species are at risk from a multitude of threats which could make conserving them challenging. Many species and populations have been severely depleted through whaling activities, and while some have recovered well, others have not, and are at best stable or even declining. New and emerging stressors are further threatening many populations including those cetaceans that have never been exploited. Compounding these varying trends, while there is much information about some species, there is very little about others, leading to knowledge gaps and limiting our ability to effectively conserve. In my thesis, I have attempted to fill some of these knowledge gaps through an examination of conservation threats to cetaceans, an exploration of broader, theoretically sound methods for mitigation, and through the development and refinement of new technologies that could be used in support of cetacean conservation. We live in a rapidly changing world and one in which resources for conservation are limited, thus developing cost effective and new means of monitoring populations and population health is critical for their conservation. In addition, information obtained from new research will contribute to monitoring efforts for cetaceans to better understand information about species over time to help inform future conservation management. Here, I summarise the preceding chapters and discuss how each are related and contribute to the overall theme of cetacean conservation. I also provide comment on future research directions. To help clarify the organization of my thesis, I have created a diagram to demonstrate the linkages between the chapters (Figure 7.1).

Aims of thesis:
1. Examine conservation threats to cetaceans
2. Explore alternative methods for mitigation
3. Develop and refine new technologies for conservation
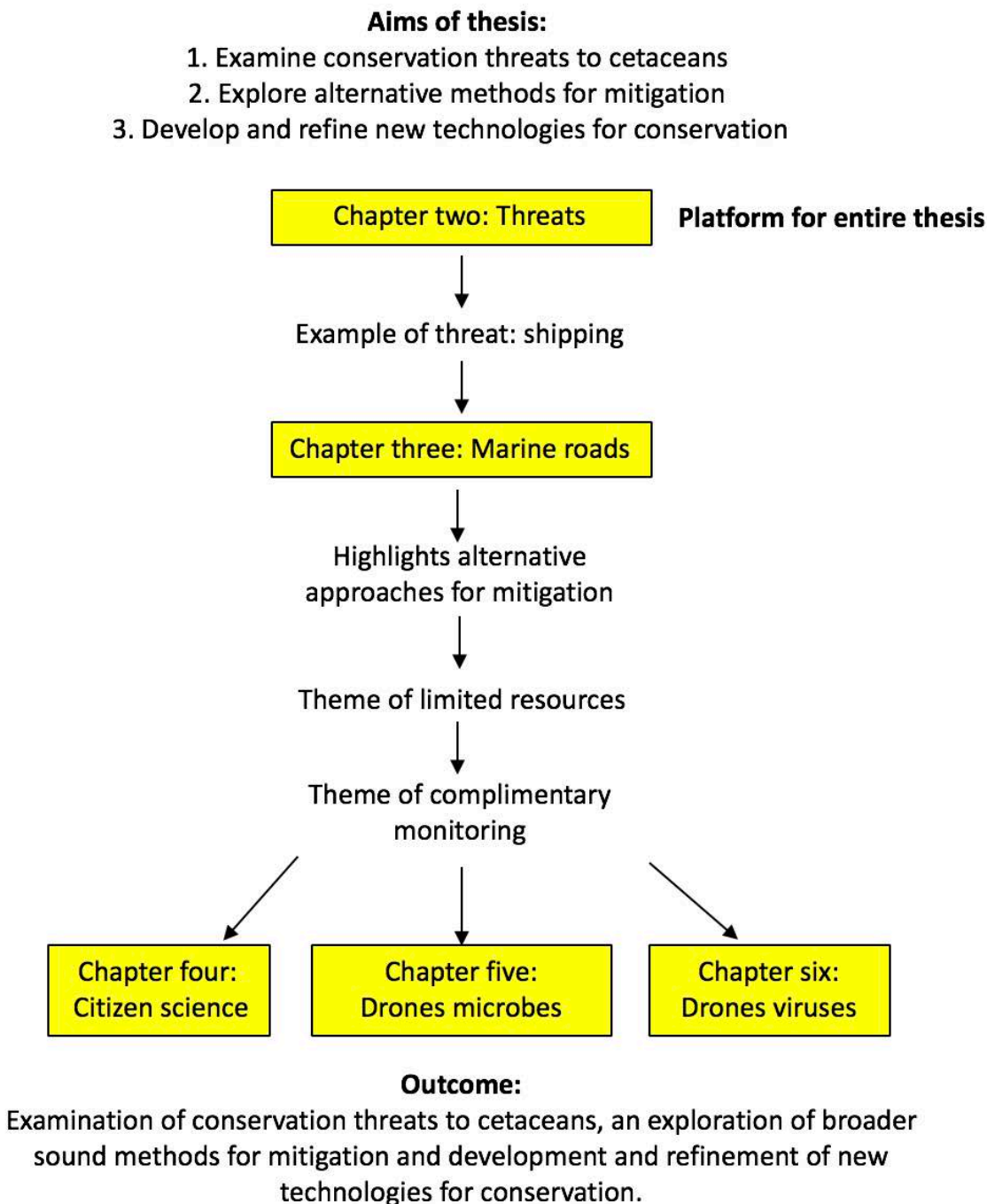
Figure 7.1: Illustration of my thesis organization. Themes explored in chapter two e.g., the identification of cetacean threats and conservation priorities, provide the basis for the thesis. From this, chapter three explores an example of an identified threat, shipping, to which I apply a terrestrial road ecology framework to enhance approaches to mitigation. Continuing this theme - but in a management regime with limited conservation resources - chapters four, five, and six provide examples of complimentary methods to inform conservation actions for

cetaceans i.e., citizen science and the development and refinement of new sampling technologies.

7.2 Chapter summaries

The following is an overview of how each thesis chapter contributes to the themes of theoretical and practical approaches to aid the conservation of cetaceans.

Chapter two provides guiding principles for the entire thesis. Clarification of the terms *threat* and *process thresholds* contributes to the conservation literature by providing an approach that avoids misdirecting effort to individual "welfare cases" rather than resourcing mitigation of a stressor or threatening process which would support needed conservation goals. This refines threat categorization and can be used to inform conservation priorities for cetaceans, especially for those which are data deficient. By reviewing threats to cetaceans based on their proximate drivers (what causes the threat) and pressures (the impact) of known threats, this chapter identifies commonalities in threats to cetaceans.

The conceptual framework I developed in Chapter two clarify the analysis and decision-making process, and incorporates assessment of the impact of cumulative threats in decision making. This chapter will be of direct benefit to conservationists and policy/decision makers when prioritizing research and management resources for cetacean conservation, especially for data poor species. Furthermore, conservation managers can use the decision tree and framework to systematically assesses threats and consider the cumulative impacts from multiple threats.

Chapter three explored an alternative approach to identifying impact and designing mitigation of one specific, but pervasive, human threat to cetaceans and other marine giants: shipping. The adoption of a terrestrial road ecology framework within the marine environment provides a mitigation approach to protecting cetaceans from shipping impacts. I demonstrate how road ecology concepts can be used by managers to help reduce the impact of future marine road expansion in a systematic fashion by incorporating knowledge of existing shipping impacts in the marine environment within a conceptual framework based on road ecology concepts such as routing and buffer zones. This chapter makes a timely contribution to the conservation literature regarding shipping consequences not only for the great whales but other giant marine species such as basking and whale sharks.

In chapter four I continued the theme of identifying alternative methods for informing conservation. I explored the role of citizen science as a way of leveraging information about growing populations to support conservation management. I analysed a 20-year dataset (1997-2017) of whale numbers off Cape Solander, Sydney, Australia collected by citizen scientists from the Cape Solander Whale Migration Study. Using data from this study, I estimated an exponential growth rate of 0.099 (95% CI = 0.079-0.119) using a generalised linear model, based on observer effort (number of observation days) and number of whales observed, equating to 10% per annum growth rate since 1997. These findings demonstrate citizen science based studies can provide a robust, cost-effective and citizen empowered approach to monitoring wildlife over the time necessary to detect change in a population. This study also demonstrates the benefits of applying well-established ecological principles and by making processes simple and robust, to use citizen science to derive reliable data on population recovery and other aspects of cetacean monitoring such as documenting different cetacean species.

Findings from Chapter four contribute directly to Australia's international obligations for the monitoring of humpback whale recovery. It is timely as citizen science in the marine literature is growing and is recognised as an underutilised methodology (Cigliano et al. 2015). This chapter demonstrates the benefits of citizen empowered wildlife monitoring and strengthens the case for citizen science being *complimentary* in assisting science (Dickinson et al. 2010). It also provides an opportunity for the results of the Cape Solander Whale Migration Study to be integrated into the scientific literature.

Chapters five and six explored the application of new animal-safe techniques for sampling bacteria and viruses from whale blow. I successfully developed bespoke technology to assess lung health of the recovering east coast humpback whale population. My collaboration with industry partners led to the development of a waterproof, remotely piloted drone for sampling whale blow. The use of the flip lid mechanism set our drones apart from similar studies, which have modified existing off the shelf products but remain vulnerable to sample contamination due to sampling with an open petri dish (no lid). I was able to demonstrate that the flip-lid petri dish addressed several sampling challenges: accessibility; safety; cost, and critically, minimizing the collection of atmospheric and seawater microbiota and other potential sources of sample contamination.

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The application of this novel drone and the collaborations it inspired, also led to advances in whale biological sampling techniques. The collection of whale-blow specific microbial taxa in chapter five provides the first assessment of baseline bacteria complements collected by drone from humpback whales in Southern Hemisphere waters. In addition, the collection of viruses via drones was the first time this method has been used to sample viruses in whale blow and we identified six novel virus species from five viral families. Advances in microbiome characterization techniques and results described in these two chapters contributes to the growing body of evidence that will eventually be useful to assess whale health. This technique would also be useful to monitor changes in population health over time. This is particularly important for the growing east Australian humpback whale population, which has yet to reach its maximum carrying capacity. In addition, knowledge of the types of baseline bacteria found in whale lungs of this population may be used to help inform the on the health status of stranded whales by comparing lung microbiota profiles. Furthermore, increased knowledge of the types of bacteria and viruses which whales may host also has implications for understanding the potential for zoonotic disease, and may contribute to protocols when working in close proximity of stranded or entangled individuals.

7.3 Future directions

This thesis presents a number of different approaches and techniques all intended to inform future conservation action for cetaceans. Each chapter contributes to the overall theme of monitoring as a tool for conservation by exploring components of cetacean conservation and presenting new approaches important for cetacean management action (Grech 2009). For example, the implementation of the decision tree and conservation framework can be adopted in future management decisions when establishing conservation priorities for cetaceans. This may result in the refinement and development of future conservation frameworks, which may be adopted for conserving other wildlife in addition to cetaceans. Additionally, mitigation of future shipping impacts using the road ecology framework will hopefully allow managers to respond to the rapid changes occurring in the marine environment, with implications for cetaceans. The combination of terrestrial and marine themes explored within the marine roads chapter may lead to future collaborations between scientists and wildlife management/decision makers, encouraging the use of cross discipline approaches to conserving wildlife. Furthermore, the adoption of citizen science in future wildlife based

programs may provide an economical and community-empowering way of enhancing long-term population monitoring. The Cape Solander Whale Migration study could serve as an exemplary example of a citizen science program for others wanting to supplement future cetacean research. This could be adopted for monitoring and filling knowledge gaps for other non-cetacean wildlife as well.

Sampling of whale blow over multiple years will help expand our knowledge of lung microbiota and better understand changes in microbes over time. Future sampling of the virome may also lead to the discovery of additional novel viruses. For example, sampling in other areas on humpback whale migratory routes, such as feeding areas in Antarctica, will expand the scope of this research as would sampling other Southern Ocean species such as southern right, dwarf minke, fin, and blue whales. An important implication of this initial work is that migrating whales traversing ocean basins latitudinally have the potential to be a disease vector. Comparing microbial and viral communities from whales sampled in polar and temperate latitudes could help identify whether whales do indeed act as mobile host vectors. Multiple other whale species are sympatric with humpback whales, and indications of co-occurrence or non-transmission of microbial and viral communities between species, will be important to understanding potential disease transfer. Research of this nature would provide baseline data in the face of continued ocean warming and range changes.

7.4 Conclusion

This thesis provides a timely contribution to the conservation of cetaceans by developing a diverse array of theoretical and practical research methods. By clearly defining key conservation terms, I have enabled a more targeted approach to prioritising conservation needs for cetaceans. In addition, I explored alternative methods for mitigating by applying a terrestrial road ecology framework in the marine environment to help mitigate the impacts from shipping on marine giants. This highlighted the importance of protecting marine giants in a changing environment as a result of climate change and the growth of anthropogenic activities. I demonstrated the utility of citizen science based projects being complimentary to traditional scientific monitoring efforts of a recovering humpback whale population in Australian waters. I also documented how citizen science can be a robust, cost-effective and citizen-empowered approach to monitoring wildlife over the time necessary to detect changes in abundance in a population. Finally, I developed and refined new technologies for cetacean

conservation by collaborating with industry to design, develop and build novel drones for collecting microbial information from whales non-invasively. Further comparative sampling using this method can be used to help understand the health of this and other whale populations. For some populations, this may be a crucial step to understanding underlying mechanisms potentially limiting recovery.

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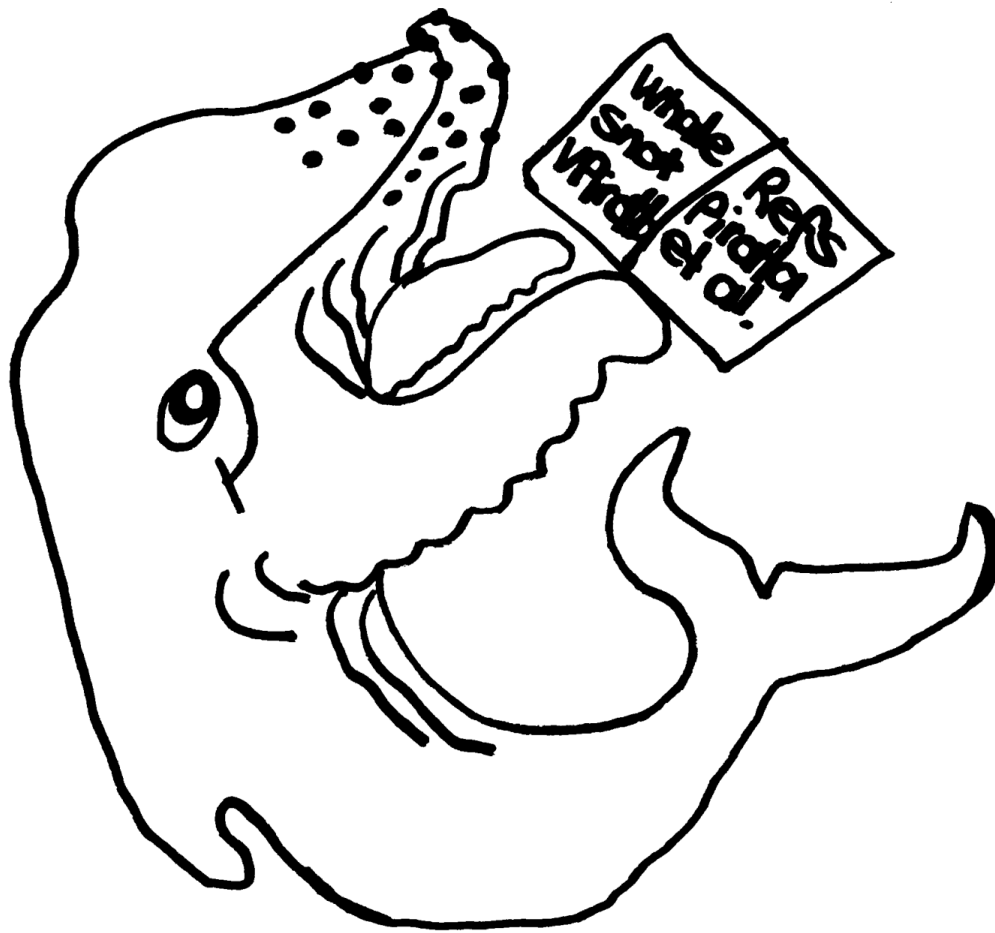
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Appendix

Supplementary material for Chapter five.

Table 1: Whale and air samples collect by drone.

Sample ID	Sample name	Sequence name	Date sampled	Sample quality (0-3, 0: no sample, 1: minimal sample, 2: medium sample and 3: large sample)*	Dish open duration (sec)**	Clustsig cluster	Sample group	Number of merged sequences
W1	W1	W1_S1	27/6/17	1	4	3	whale blow	88073
W2	W2	W2_S2	27/6/17	3	3	3	whale blow	67136
W3	W3	W3_S3	27/6/17	2	4	3	whale blow	64046
W4	W4	W4_S4	27/6/17	2	3	3	whale blow	230568
W5	W5	W5_S5	27/6/17	2	6	3	whale blow	154359
W6	W6	W6_S6	27/6/17	1	3	3	whale blow	133506
W7	W7	W7_S7	27/6/17	2	4	3	whale blow	203269
W8	W8	W8_S8	27/6/17	3	4	3	whale blow	67927
W9	W9	W9_S9	27/6/17	1	5	3	whale blow	214608
W10	W10	W10_S10	27/6/17	1	4	3	whale blow	94726
W11	W11	W11_S11	27/6/17	1	4	3	whale blow	203629
W12	W12	W12_S12	27/6/17	2	4	3	whale blow	90196
W13	W13	W13_S13	27/6/17	1	5	3	whale blow	183920
W14	W14	W14_S14	27/6/17	2	5	3	whale blow	213654
W15	W15	W15_S15	27/6/17	2	4	3	whale blow	238046
W16	W16	W16_S16	27/6/17	2	4	#N/A	whale blow	1172
W17	W17	W17_S17	27/6/17	2	5	3	whale blow	220172

W18	W18	W18_S18	27/6/17	2	5	3	whale blow	173461
W19	W19	W19_S19	27/6/17	1	5	3	whale blow	257359
W20	W20	W20_S20	27/6/17	0	4	2	whale blow	216649
W21	W21	W21_S21	27/6/17	0	6	2	whale blow	182137
W22	W22	W22_S22	27/6/17	1	6	2	whale blow	179128
W23	W23	W23_S23	27/6/17	0	4	2	whale blow	171078
W24	W24	W24_S24	27/6/17	1	4	2	whale blow	95896
W25	W25	W25_S25	27/6/17	0	5	2	whale blow	76221
W26	W26	W26_S26	27/6/17	0	nd	3	whale blow	220782
W27	W27	W27_S27	27/6/17	1	3	3	whale blow	141432
W28	W28	W28_S28	27/6/17	0	nd	2	whale blow	139869
W29	W29	W29_S29	27/6/17	0	nd	3	whale blow	257561
W30	W30	W30_S30	27/6/17	0	nd	3	whale blow	219153
W31	W31	W31_S31	27/6/17	0	nd	3	whale blow	59919
W32	W32	W32_S32	27/6/17	0	nd	3	whale blow	262133
W33	W33	W33_S33	27/6/17	0	nd	3	whale blow	87835
W34	W34	W34_S34	27/6/17	0	nd	2	whale blow	93029
W35	W35	W35_S35	27/6/17	0	nd	#N/A	whale blow	3113
W36	W36.1	W36.1_S36	27/6/17	0	nd	3	whale blow	71090
W37	W36.2	W36.2_S37	27/6/17	0	nd	3	whale blow	114026
W38	W37	W37_S38	27/6/17	0	nd	2	whale blow	11004
Air1	Control.1	Control.1_S39	27/6/17	0	5	2	Air	76270
W39	W1.2	W1.2_S40	**26/6/17**	2	6	3	whale blow	113398
W40	W2.2	W2.2_S41	**26/6/17**	**3**	**4**	3	whale blow	137592
W41	W3.2	W3.2_S42	**26/6/17**	3	5	3	whale blow	77404

W42	W4.2	W4.2_S43	**26/6/17**	2	4	3	whale blow	253867
W43	W5.2	W5.2_S44	**26/6/17**	2	4	3	whale blow	97402
W44	W7.2	W7.2_S45	**26/6/17**	1	6	3	whale blow	240987
W45	W8.2	W8.2_S46	**26/6/17**	2	4	3	whale blow	18408
W46	W10.2	W10.2	**26/6/17**	2	5	3	whale blow	124852
W47	W11.2	W11.2_S48	**26/6/17**	2	4	3	whale blow	112550
W48	W12.2	W12.2_S49	**26/6/17**	1	4	2	whale blow	49185
W49	W13.2	W13.2_S50	**26/6/17**	2	4	3	whale blow	68356
W50	W14.2	W14.2_S51	**26/6/17**	3	4	3	whale blow	64946
Air2	Control 1.2	Control1.2_S52	**26/6/17**	0	5	2	Air	170637
Air3	Control 1.3	Control1.3_S53	**26/6/17**	0	5	2	Air	151727
W51	W15.2	W15.2_S54	26/6/17	3	4	3	whale blow	101307
Air4	Control 1.3	Ctrl1.3b_S55	26/6/17	0	5	2	Air	93128
Air5	Control 1.4	Control.1.4_S56	26/6/17	0	5	2	Air	17671
W52	W4.3	W4.3_S57	4/6/17	3	4	3	whale blow	199724
W53	W5.3	W5.3_S58	4/6/17	1	5	2	whale blow	78998
W54	W6.3	W6.3_S59	4/6/17	3	4	3	whale blow	249962
W55	W7.3	W7.3_S60	4/6/17	**3**	**4**	3	whale blow	231862
W56	W8.3	W8.3_S61	4/6/17	3	5	3	whale blow	73834
W57	W10.3	W10.3_S62	4/6/17	2	5	3	whale blow	89204
W58	W11.3	W11.3_S63	4/6/17	2	4	3	whale blow	116771
W59	W12.3	W12.3_S64	4/6/17	1	2	3	whale blow	178695
Air6	Control.5	Control.5_S65	4/6/17	0	5	2	Air	42944

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Table 2: Seawater samples.

Sample Code	BPA.Id	full BPA.Id	Date sampled	Depth (m)	Clustsig cluster	Sample group	Number merged sequences
PHB20140212d0	21814	102.100.100/21814	12/2/14	0	1	seawater	87729
PHB20140212d10	21815	102.100.100/21815	12/2/14	10	1	seawater	121393
PHB20150331d0	21862	102.100.100/21862	31/3/15	0	1	seawater	25116
PHB20150331d10	21863	102.100.100/21863	31/3/15	10	1	seawater	32515
PHB20150519d0	21820	102.100.100/21820	19/5/15	0	1	seawater	60918
PHB20150519d10	21821	102.100.100/21821	19/5/15	10	1	seawater	75658
PHB20150630d0	21856	102.100.100/21856	**30/6/15**	0	1	seawater	103795
PHB20150630d10	21857	102.100.100/21857	**30/6/15**	10	1	seawater	117029
PHB20150723d0	21874	102.100.100/21874	**23/7/15**	0	1	seawater	23034
PHB20150723d10	21875	102.100.100/21875	**23/7/15**	10	1	seawater	25596
PHB20150819d0	21886	102.100.100/21886	19/8/15	0	1	seawater	21512
PHB20150819d10	21887	102.100.100/21887	19/8/15	10	1	seawater	25180
PHB20150113d0	21898	102.100.100/21898	13/10/15	0	1	seawater	28536
PHB20150113d10	21899	102.100.100/21899	13/10/15	10	1	seawater	20689
PHB20151115d0	21910	102.100.100/21910	15/11/15	0	1	seawater	25471
PHB20151115d10	21911	102.100.100/21911	15/11/15	10	1	seawater	32532
PHB20151216d10	34194	102.100.100/34194	16/12/15	0	1	seawater	44521
PHB20151216d0	34198	102.100.100/34198	16/12/15	10	1	seawater	27078
PHB20160202d0	34127	102.100.100/34127	2/2/16	0	1	seawater	52154
PHB20160202d10	34128	102.100.100/34128	2/2/16	10	1	seawater	35760
PHB20160311d0	34133	102.100.100/34133	11/3/16	0	1	seawater	39810

PHB20160311d10	34134	102.100.100/34134	11/3/16	10	1	seawater	30409
PHB20160408d0	34121	102.100.100/34121	8/4/16	0	1	seawater	85147
PHB20160408d10	34122	102.100.100/34122	8/4/16	10	1	seawater	65176
PHB20160503d0	34115	102.100.100/34115	3/5/16	0	1	seawater	47560
PHB20160503d10	34116	102.100.100/34116	3/5/16	10	1	seawater	46265

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Supplementary Table 3. List of bacteria identified in humpback whale blow from next generation and sanger sequencing. Evidence of similar bacteria isolated in other cetaceans obtained from blow samples (b), blowhole swabs (s), skin microflora (sm) or lung tissue (t). Bolded wording indicates sanger sequencing.

Phylum	Class	Highest level of OTUs: genus (g)/species(s)/family (f)	Bacteria associated with other cetaceans. Blow (b), swab blowhole (s), skin microflora (sm) or lung tissue (l)	Reference/s
Actinobacteria	Actinobacteria	Corynebacterium_1, Corynebacteriales, Leucobacter, Pseudoclavibacter **Micrococcus, Zimmermannella, Dietzia, Microbacteriaceae**	Bottlenose dolphin (*Tursiops truncatus* and *T. aduncus*) (b) (s) Bowhead whale (*Balaena mysticetus*) (s)	(Shotts Jr et al., 1990; Jensen et al., 2009; Lima et al., 2012)
Bacteroidetes	Bacteroidetes_ BD2-2, Bacteroidia, Cytophagia, Flavobacteriia,	Bacteroidetes_BD2-2, Bacteroides_sp._KhalH BD91, Marinifilum_sp._TWO-1, Marinilabiaceae, Microbacter, Porphyromonas, uncultured_Prevotella_sp., uncultured_eubacterium_E1-K10, Belliella_sp._No.164, pt46, Fluviicola, Gangjinia, Maritimimonas, Empedobacter_sp._C2-7, Pseudofulvibacter, Flexibacter_sp._S4475, **Tenacibaculum** Bergeyella_sp._405	Humpback whale (*Megaptera novaeangliae*) (sm) Bottlenose dolphin (*Tursiops truncatus* and *T. aduncus*) (b) (s)	(Johnson et al., 2009; Apprill et al., 2011; Lima et al., 2012; Apprill et al., 2014)
Candidate_division_SR1	uncultured_bacterium	Candidate_division_SR1		
Firmicutes	Clostridia	Clostridiales_bacterium_canine_oral_taxon_157	Blue (*Balaenoptera musculus*) (b)	(Johnson et al., 2009; Acevedo-Whitehouse et al.,

	Bacilli	Helcococcus, Peptoniphilus_sp._oral_taxon_386_str._F0131 Peptostreptococcaceae_bacterium_feline_oral_taxon_054, Peptostreptococcaceae_bacterium_feline_oral_taxon_060 Peptostreptococcaceae_bacterium_feline_oral_taxon_061, Peptostreptococcaceae_bacterium_feline_oral_taxon_069, e.g., Spp. strain Guggenheimella_bovis, Natranaerovirga_hydrolytica, Clostridiales_bacterium_canine_oral_taxon_100, Clostridiales_bacterium_feline_oral_taxon_019 **Exiguobacterium, Paenibacillus, Streptococcus, Bacillus, Planococcaceae, Staphylococcus, Brevibacillus**	Grey (*Eschrichtius robustus*) (b) Killer whales (*Orcinus orca*) (b) Humpback whale (*Megaptera novaeangliae*) (sm) Bottlenose dolphin (*Tursiops truncatus*) (s) Striped dolphin (Stenella coeruleoalba) (s) (t)	2010; Apprill et al., 2011; Denisenko et al., 2012; Apprill et al., 2014; Stewart et al., 2014; Jaing et al., 2015; Godoy-Vitorino et al., 2017; Raverty et al., 2017)
	Erysipelotrichia	Dielma_fastidiosa, bacterium_enrichment_culture_clone_DPF18, Faecalitalea_cylindroides_T2-87, Erysipelotrichaceae_bacterium_canine_oral_taxon_255		
Fusobacteria	Fusobacteriia	ASCC02, uncultured_Fusobacterium_sp., Leptotrichia_sp._ES2714_GLU		

Gracilibacteria	uncultured_epsilon_proteobacterium	uncultured_epsilon_proteobacterium		

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2016/010-2   **Date of Expiry:** 01 June 2017

Full Approval Duration: 01 June 2016 to 01 June 2019 (36 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

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Vanessa Pirotta   0439 480 600

> **In case of emergency, please contact:**
> *the Principal Investigator / Associate Investigator named above*
> **or Animal Welfare Officer**: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project :   Use of unmanned aerial systems (UAS) to assess southern right whale (Eubalaena australis) body condition as part of a global assessment of right whale health.

Purpose: 4 - Research: Human or Animal Biology

Aims: To assess body condition of Critically Endangered Southern Right Whales via 1) photogrammetry and 2) microbiome collection.

Surgical Procedures category: 1 - Observation Involving Minor Interference

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex/Age/Weight	Total	Supplier/Source
44F Whale and Dolphins	Southern Right Whale (Eubalaena Australis)	Any/Any/Any	50	Wild
44F Whale and Dolphins	Humpback Whale (Megaptera novaeangliae)	Any/Any/Any	150	Wild
44F Whale and Dolphins	Dwarf/Minke Whale (Balaenoptera acutorostrata)	Any/Any/Any	50	Wild
44F Whale and Dolphins	Brydes Whales (Balaenoptera edeni)	Any/Any/Any	20	Wild
44F Whale and Dolphins	Sperm Whales (Physeter microcephalus)	Any/Any/Any	20	Wild
		TOTAL	**290**	

Location of research:

Location	Full street address
In - Situ / Wild	Australian coastal waters, primarily Victorian waters (e.g. Logans Beach, Warrnambool) and NSW waters.

Amendments approved by the AEC since initial approval:
1. *Amendment #1 - Add David Slip as Associate Investigator. (Executive Approved. To be ratified by AEC 16 June 2016).*

Conditions of Approval:
1.   **Approval subject to relevant permits/licences being submitted.**

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

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Associate Professor Jennifer Cornish (Chair, Animal Ethics Committee)   **Approval Date: 26 May 2016**

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

ANIMAL RESEARCH AUTHORITY (ARA)

MACQUARIE University

AEC Reference No.: 2016/010-4
Full Approval Duration: *01 June 2016 to 01 June 2019 (36 months)*

Date of Expiry: 01 June 2018

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:
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or Animal Welfare Officer: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title: **Use of unmanned aerial systems (UAS) to assess southern right whale (*Eubalaena australis*) body condition as part of a global assessment of right whale health.**

Purpose: **4 - Research: Human or Animal Biology**

Aims: **To assess the body condition of critically endangered southern right whales via 1) photogrammetry 2) microbiome collection, and 3) tissue biopsy.**

Procedures category: 3 - Minor Conscious Intervention

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex/Age/Weight	Total	Supplier/Source
44F - Whales and Dolphins	Southern Right Whale (*Eubalaena australis*)	Any	50	Wild
	Humpback Whale (*Megaptera novaeangliae*)		150	
	Dwarf/Minke Whale (*Balaenoptera acutorostrata*)		50	
	Bryde's Whale (*Balaenoptera edeni*)		20	
	Sperm Whale (*Physeter microcephalus*)		20	
		TOTAL	**290**	

Location of research:

Location	Full street address
In - Situ / Wild	Australian coastal waters, primarily Victorian waters (e.g. Logans Beach, Warrnambool) and NSW waters.

Amendments approved by the AEC since initial approval:
1. *Amendment #1 - Add David Slip as Associate Investigator. (Executive Approval ratified by AEC 16 June 2016).*
2. *Amendment #2 - Amend aims and add an additional sampling procedure to include trialling the use of UAVs (unmanned aerial vehicles) to collect biopsy samples (Approved by AEC 18 May 2017).*
3. *Amendment #3 - Add Dr Double as Associate Investigator. (Executive Approval ratified by the AEC 18 May 2017).*

Conditions of Approval:
1.   Approval subject to relevant permits/licences being submitted.

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal supplier's licence.

_____
Associate Professor Jennifer Cornish (Chair, Animal Ethics Committee)

Approval Date: 18 May 2017

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

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An Economical Custom-Built Drone for Assessing Whale Health

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Drones or Unmanned Aerial Vehicles (UAVs) have huge potential to improve the safety and efficiency of sample collection from wild animals under logistically challenging circumstances. Here we present a method for surveying population health that uses UAVs to sample respiratory vapor, 'whale blow,' exhaled by free-swimming humpback whales (*Megaptera novaeangliae*), and coupled this with amplification and sequencing of respiratory tract microbiota. We developed a low-cost multirotor UAV incorporating a sterile petri dish with a remotely operated 'blow' to sample whale blow with minimal disturbance to the whales. This design addressed several sampling challenges: accessibility; safety; cost, and critically, minimized the collection of atmospheric and seawater microbiota and other potential sources of sample contamination. We collected 59 samples of blow from northward migrating humpback whales off Sydney, Australia and used high throughput sequencing of bacterial ribosomal gene markers to identify putative respiratory tract microbiota. Model-based comparisons with seawater and drone-captured air demonstrated that our system minimized external sources of contamination and successfully captured sufficient material to identify whale blow-specific microbial taxa. Whale-specific taxa included species and genera previously associated with the respiratory tracts or oral cavities of mammals (e.g., *Pseudomonas*, *Clostridia*, *Cardiobacterium*), as well as species previously isolated from dolphin or killer whale blowholes (*Corynebacteria*, others). Many examples of exogenous marine species were identified, including *Tenacibaculum* and *Psychrobacter* spp. that have been associated with the skin microbiota of marine mammals and fish and may include pathogens. This information provides a baseline of respiratory tract microbiota profiles of contemporary whale health. Customized UAVs are a promising new tool for marine megafauna research and may have broad application in cost-effective monitoring and management of whale populations worldwide.

Keywords: UAV, UAS, drone, blow, humpback whale, microbiota, technology, conservation

INTRODUCTION

Conservation biology is entering a new era of innovation, with unprecedented growth across a range of techniques, from genetics and genomics to telemetry and remote sensing (Allendorf et al., 2010; Hussey et al., 2015). Rapid advances in the technology underpinning Unmanned Aerial Vehicles (UAVs also known as Unmanned Aircraft Systems or drones), are driving new and

innovative environmental applications (Koh and Wich, 2012; Anderson and Gaston, 2013; Christie et al., 2016; Smith et al., 2016; Duffy et al., 2017). The application of UAVs in conservation science makes it possible to collect information from dangerous and inaccessible environments and answer research questions that were previously limited to the hypothetical (Harvey et al., 2016). UAVs also provide an alternative, safer, quieter and often cost-effective option for monitoring fauna and flora, from individuals and populations to entire ecosystems, and in so doing are replacing expensive manned systems such as helicopters and fixed-wing aircraft (Christiansen et al., 2016; Christie et al., 2016). UAV applications in wildlife research now encompass almost all environments, from arid deserts, through rainforests, oceans to polar regions (Linchant et al., 2013, 2015; Durban et al., 2015; Goebel et al., 2015; Duffy et al., 2017).

UAVs are transforming the way scientists monitor and conserve wildlife (Gonzalez et al., 2016). In the terrestrial world, UAVs have been used for a wide variety of conservation applications (van Gemert et al., 2014; Gonzalez et al., 2016). Some examples include, counting elephants (*Loxodonta africana*) (Linchant et al., 2013; Vermeulen et al., 2013), UAV surveillance (anti-poaching tools) for elephants and rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) (Marks, 2014; Mulero-Pázmány et al., 2014; Hahn et al., 2017), locating chimpanzee nests (*Pan troglodytes*) (van Andel et al., 2015) and mapping Sumatran orangutan (*Pongo abelii*) habitat, distribution and density (Wich et al., 2015; Szantoi et al., 2017). UAV applications now extend to the polar regions where they have been used to monitor and estimate abundance of penguin populations (gentoo, *Pygoscelis papua,* and chinstrap, *Pygoscelis antarctica*) and estimate size and condition of leopard seals (*Hydrurga leptonyx*) (Goebel et al., 2015; Ratcliffe et al., 2015). In the marine environment, UAVs are revolutionizing the way marine species can be studied due to their small size, apparent minimal disturbance of wildlife and improved safety for both operators and animals (Nowacek et al., 2016; Fiori et al., 2017). UAVs have been utilized for a wide variety of applications including aerial surveys, monitoring, habitat use, abundance estimates, photogrammetry and biological sampling e.g., whale "blow" (Hogg et al., 2009; Acevedo-Whitehouse et al., 2010; Hodgson et al., 2013; Durban et al., 2015; Pomeroy et al., 2016; Schofield et al., 2017).

There are widespread concerns about the health of marine mammal populations in the face of global anthropogenic stressors (Gulland and Hall, 2007). Yet health assessments typically involves collecting samples from stranded animals, which are often biased as these animals are most likely to be health-compromised (Geraci and Lounsbury, 2005). Sampling exhaled breath or 'blow' from wild whales may therefore provide a more representative assessment of the health status of individuals because samples can be randomly taken from the population. From a single sample of whale blow, scientists may be able to collect respiratory bacteria, lipids, proteins, DNA and hormones (Hogg et al., 2005, 2009; Schroeder et al., 2009; Acevedo-Whitehouse et al., 2010; Hunt et al., 2013, 2014; Thompson et al., 2014; Burgess et al., 2016; De Mello and De Oliveira, 2016; Raverty et al., 2017). This information is

important for whale conservation, as it can be collected over time to help monitor the recovery of whale populations post-whaling. Early approaches to sampling whale blow involved passing a cotton gauze or nylon stocking on the end of a carbon fiber pole through the blow when the animal surfaced (Hogg et al., 2009; Hunt et al., 2014). Recent advancements on this method have seen the use of a pole with a number of petri dishes with lids to sample wild killer whales (Raverty et al., 2017). However, this method requires extremely close vessel approaches to whales (Hogg et al., 2009). Given the large size, mass and power of whales, this approach involves high risk to both researchers and to the whale itself. Even under ideal circumstances this method is likely to disturb the animal, potentially compromising the validity of some of the measures such as stress hormones which elevate rapidly (Harcourt et al., 2010). Accordingly, alternative approaches have long been sought. Acevedo-Whitehouse et al. (2010) deployed a single-rotor UAV (a remote-controlled helicopter) to sample whale blow. Their study demonstrated the feasibility of the approach but loss of samples from the UAV as it careers through the sea air proved a potential issue as did contamination from airborne particulate not expired by the whale.

Here we describe a purpose-built UAV designed to sample whale blow in the field with minimal contamination. Our goal was to provide a snapshot of whale health. We specifically targeted northward migrating humpback whales (*Megaptera novaeangliae*) off the East coast of Sydney, Australia for the collection of baseline microbiota information. The UAV used in our study has a unique combination of features that represent a significant advance over existing UAVs. It is fast, highly maneuverable, durable, waterproof, low-cost (< \$USD 1000) and provides flexible payload mounting options. The UAV is scaled to the sampling gear (in this case a 100 mm petri dish), which is held in a mechanism that allows the dish to be opened/closed during flight–minimizing sample contamination or loss.
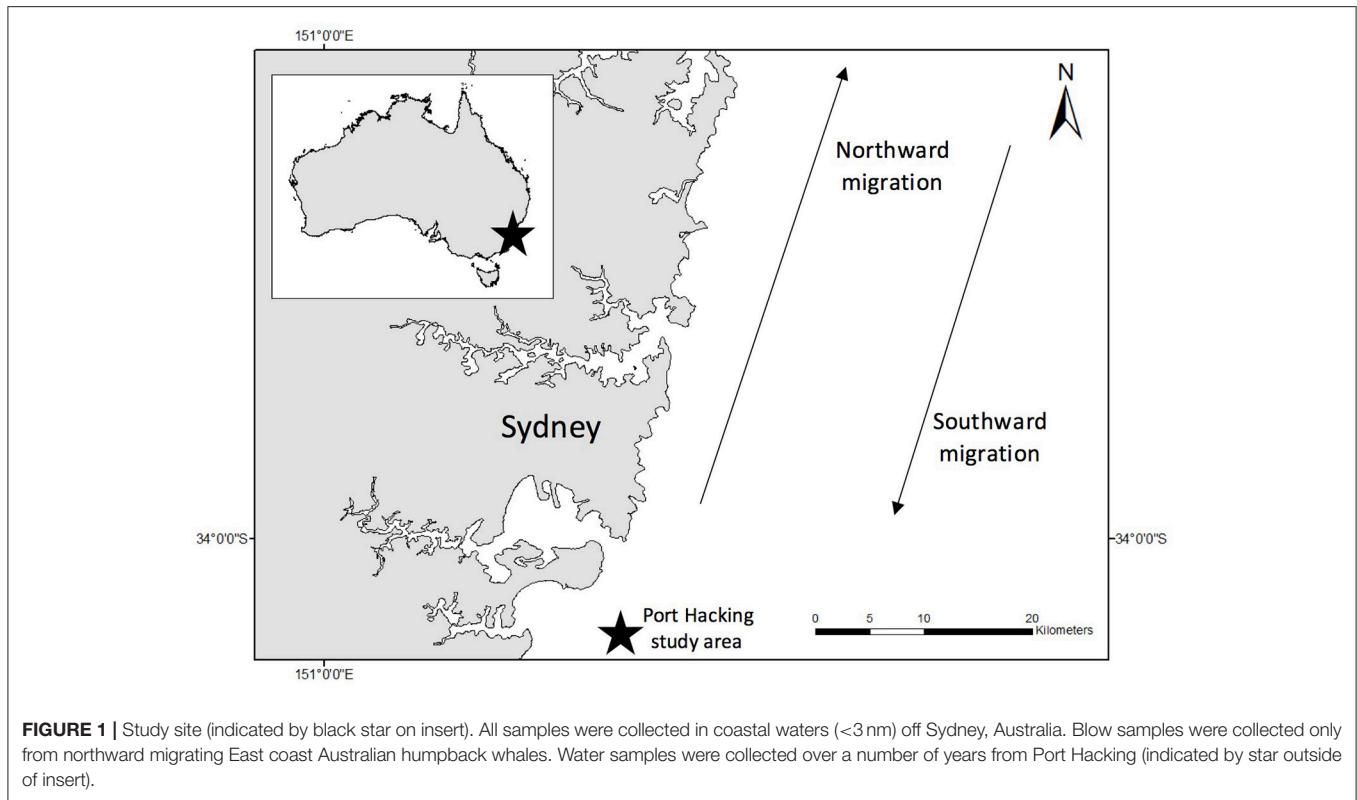
MATERIALS AND METHODS
Study Site and Species
All flights were conducted offshore Sydney, Australia (**Figure 1**). Each year from May to November, migratory Group V (Stock E1) humpback whales migrate past Sydney, as they swim from high latitude feeding areas in Antarctica to low latitude breeding waters off Queensland (Chittleborough, 1965). All sampling took place in coastal waters <3 nautical miles from Sydney between 30 May 2017 and 27 June 2017.

UAV Design
The UAV is a 4-motor electric multirotor (quadcopter) 500 mm across (motor to motor, diagonally) (**Figure 2A**). It has a relatively high power to weight ratio making it fast, maneuverable, resistant to strong wind gusts and relatively quiet while hovering. It carries the bare minimum of hardware and is operated in 'manual mode' (no GPS or autolevelling assistance) with a heavy reliance of the onboard video feed for control, navigation and sampling operations. The airframe structure of the UAV is a 'sandwich' style construction cut from

FIGURE 1 | Study site (indicated by black star on insert). All samples were collected in coastal waters (<3 nm) off Sydney, Australia. Blow samples were collected only from northward migrating East coast Australian humpback whales. Water samples were collected over a number of years from Port Hacking (indicated by star outside of insert).

carbon fiber plate, with a top shell molded from impact-resistant polycarbonate. This seals against the airframe to create a waterproof compartment which houses the power distribution, flight control, motor control, radio control transceiver, and video transmitter components. The float booms/legs were cut from expanded polypropylene (EPP)—a closed-cell foam, chosen for high strength, resistance to bending loads and excellent water resistance. A clear acrylic tube at the front of the aircraft houses a forward facing, tilting camera that provides a real-time position reference to the pilot (First Person View). The resulting composite structure is light, stiff, strong and waterproof. Buoyancy is provided by the two watertight compartments and EPP foam floats under the arms. In the event of a crash or forced landing over water, the UAV floats in an upright position so it can be recovered or take off again. Two reinforced mounting areas on the top shell accept payloads of around 100 g. For this configuration, the blow-sampling apparatus was mounted at the front. This is a hinged frame which opens to 180 degrees and holds a 100 mm diameter petri dish with suction cups. A servo motor opens and closes the dish remotely, during flight. Airflow testing using smoke indicated the best position for the sampling dish relative to the propellers. A forward-looking waterproof video camera (GoPro® Hero Session™) is positioned at the rear and logs video to an internal memory card. The dish is in the frame of the recorded video, so the footage can be used to confirm the source of the sampled material.

Sampling Method

This study was approved by the Macquarie University Animal Ethics Committee, and carried out in accordance with the

Animal Research Authority (2016/010). This research was permitted by New South Wales National Parks and Wildlife Services (NSWNPWS) to fly UAVs over whales in New South Wales coastal waters (permit number SL101743). To adhere to Australian legislative requirements, the UAVs (including backup UAV) were registered with the Civil Aviation Safety Authority (CASA) and operated by a CASA certified operator (Heliguy Pty. Ltd.). All flights were conducted in good weather (no rain, Beaufort < 3), from small research vessels, where the UAV was launched and landed on a launch pad at the bow or stern of the boat. A closed, sterile petri dish with nutrient agar covering the base of the petri dish was secured using eight suction cups affixed on the UAV before each flight.

Members of the team scanned the area for humpback whales. Once an individual was selected, the vessel was driven maintaining a constant speed and distance from the whale (>200 m). Once the respiratory rhythm of an individual was determined (downtime length in minutes), the UAV was launched to coincide with the individual surfacing. The UAV pilot was directed by spotters on the vessel and positioned the UAV with the aid of the live feed from the forward-facing camera. To minimize sample contamination, the petri dish remained closed until just before the whale surfaced, when the dish remotely opened as the UAV accelerated toward and through the densest part of the whale blow, collecting the maximum amount of sample in the dish and lid (**Figures 2B,C** and Supplementary Video 1). The petri dish was immediately closed and the UAV was returned to the vessel. The petri dish containing the sample was removed from the UAV and Parafilm® was wrapped around the closed petri dish to secure the sample. All samples were
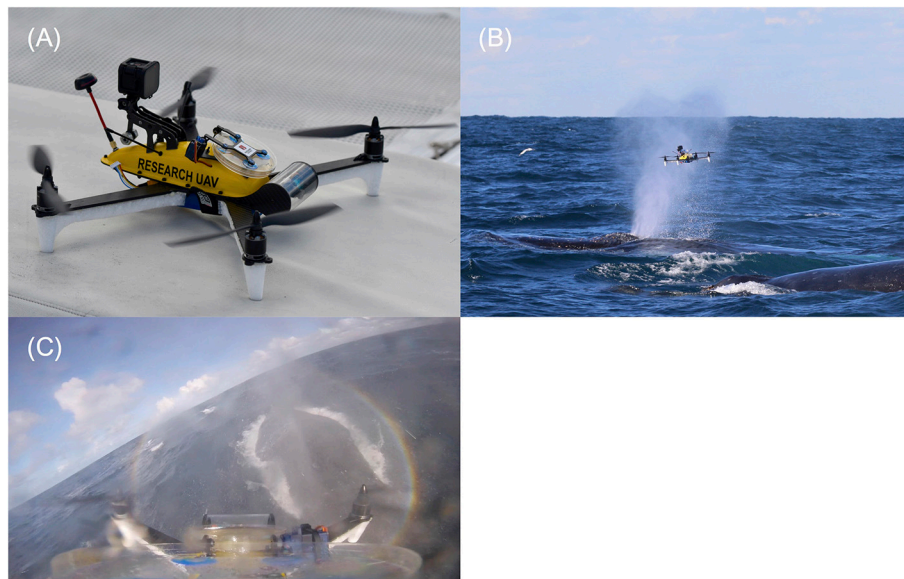
FIGURE 2 | (A) Purpose-built UAV designed to sample whale blow. The UAV consists of a sandwich style carbon fiber body. White foam floats support the UAV during take-off and landings and provide floatation in water. The yellow shell houses all electrical equipment. A GoPro® hero session is mounted at the back of the yellow shell to record flights. A hinge mechanism with disposable petri dish is located in the center of the yellow shell. This can be remotely operated to minimize sample contamination in the field. The clear round tube at the front of the UAV houses the first-person camera to assist with sampling. **(B)** UAV sampling whale blow. This photo was taken just as the UAV had passed through the visible blow (plume of spray). The petri dish is still in the open position. Sample was collected on both the lid and bottom (nutrient agar filled) side. The petri dish was shut immediately after collection to minimize sample contamination and the drone was flown back to the research vessel >200 meters away. **(C)** Screenshot from the UAVs on-board GoPro® camera mid whale sample collection. This footage shows the petri dish at the bottom of the picture. The whale is located on the right-hand side. The petri dish is completely extended (open) with blow droplets visible on both sides of the dish and GoPro® lens.

temporarily stored in a cooler box on ice until further processing in the laboratory at the end of each day.

Attempts were made to sample a different whale each flight. Individuals within a pod were chosen based upon unique markings (e.g., white flanks/patterns/scarring/barnacle arrangements). To ensure the same individual was not sampled twice, a live video feed was used to target individuals. Cross contamination among whales was avoided by not triggering the opening of the flip lid until only the targeted whale respired. Footage collected from the GoPro® throughout each flight was used to validate sample collection and eliminate repeated sampling of the same individuals by post-hoc identification. The behavioral response of whales was recorded for each pass using by scoring system of one to three (one: 'no response,' two: 'minor response' minor surface activity such as logging, spy hopping and three: 'severe/elevated Response' e.g., breaching, peduncle throw or chin slap).

Air and Seawater Samples

To enable direct comparison of UAV-captured air and whale blow samples with bacteria inhabiting the adjacent seawater, the data were combined with 16S sequence libraries prepared from 26 surface seawater samples. This represents a complete annual cycle, collected from the National Time Series Station known as Port Hacking 100 (PH100). All UAV-captured samples were collected within 20 km of PH100.

Laboratory Processing of Samples

Initial processing of samples occurred in two stages. First, in an Ultra Violet-sanitized class II biosafety hood, the top of the petri dish lid (non-agar) side was swabbed using a dry sterile cotton tip and then placed in a sterile 1.5 ml tube and stored in the freezer at $-30°C$. Secondly, the petri dish (both the lid and nutrient base) was placed in an incubator at $37°C$ after the lid was swabbed, simulating average mammalian body temperature $36-37°C$ (Whittow, 1987; Cuyler et al., 1992). Plates were observed daily for colony growth. If growth occurred, colonies were counted and a representative number of colonies were picked from each plate, resuspended in 100 μl of sterile water, vortexed for 10 s and immediately frozen at $-30°C$ until further processing. Plates were then stored in the fridge for future reference if needed.

Bacterial DNA Extraction

DNA extractions were conducted using the *Quick*-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, California, USA) with minor modifications to the manufacturer's instructions. Each swab was transferred to a tube containing 1.2 g of ZR BashingBeads™ (equivalent to ∼half of the portion supplied for each extraction). The original storage tube was rinsed with lysis solution (750 μl) to ensure the complete transfer of material into the extraction tube. The swab was then bead-beaten on a Vortex-Genie® 2 (Mo Bio Laboratories/QIAGEN, California, USA) for 20 min at room temperature. All other steps

were followed according to the manufacturer's instructions, with the exception that two successive final elutions were carried out, each with 20 μl of sterile DNA elution buffer.

Amplification and Sequencing

Amplicons targeting the bacterial 16S rRNA gene (27F—519R; Lane et al., 1985; Lane, 1991) were generated and sequenced for each sample at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) using 250 bp paired end illumina sequencing according to established protocols (http://www.bioplatforms.com/wp-content/uploads/base_illumina_16s_amplicon_methods.pdf).

Amplicons generated from drone-captured air and whale blow were combined with 27F—519R sequences generated from 26 surface (2 m and 10 m depth) seawater samples collected over a complete annual cycle from the nearby National Reference Station (PH100) time series (Dec 2014–Mar 2016). Monthly microbial sampling has been conducted at the Port Hacking100 reference station since 2009 (Seymour et al., 2012). All UAV-captured whale and air samples were collected within 20 km upstream of this reference station, within 1–3 km from shore. We reasoned that this dataset, which was sampled and sequenced using standardized protocols at the same sequencing center, would provide a comprehensive and unbiased assessment of bacterial species characteristic of seawater in this region, which could be excluded as potential contaminants from the whale blow samples. Whale, air and seawater samples analyzed in this study are detailed in Supplementary Tables 1, 2.

Sequence Operational Taxonomic Units (OTUs) tables were prepared after (Bissett et al., 2016). Briefly, paired-end reads were filtered using Trimmomatic (ILLUMINACLIP: NexteraPE-PE.fa:2:30:10 SLIDINGWINDOW:4:15 MINLEN:76) (Bolger et al., 2014) then merged using PEAR (Zhang et al., 2014). The combined amplicon data were clustered into OTUs at 97% sequence similarity using an open reference OTU picking pipeline in USEARCH 64 bit v8.1.1756 (Edgar, 2010), which included *de novo* chimera detection. Clusters with < 4 sequences were removed, and reads were mapped to representative OTU sequences using USEARCH (97% ID) to calculate read abundances. From an initial pool of 10.5 million paired-end reads, a total of 7.62 million filtered, merged sequences, with chimeras removed, were added to the OTU table. OTU tables were sub-sampled to a constant sampling depth of 10,000 sequences using rarefy in vegan (Oksanen, 2017). All subsequent analyses were conducted on sub-sampled OTU tables. Sequences generated over the course of this project are deposited in the European Nucleotide Archive under project PRJEB23634. All seawater sequence data are deposited in the NCBI Sequence Read Archive PRJNA385736.

Data Analyses

Hierarchal clusters of OTU abundance profiles generated from seawater, drone-captured air and whale blow were compared using the simprof test following square-root transformation and conversion to a Bray-Curtis dissimilatory matrix in the r package clustsig (Whitaker and Christman, 2014). Data from samples that were near misses, which would reflect a mixture of air and whale blow microbiota, were set aside from the subsequent statistical analyses. The community structure dissimilarity between samples was observed with non-metric multidimensional scaling. Significant differences in communities sampled in seawater, UAV-captured air or whale blow samples were defined using generalized linear models within mvabund (Wang et al., 2012). Briefly, a negative binomial model was fit to the OTU abundance data and the sample grouping was analyzed using Analysis of Variance (ANOVA). OTUs that were significantly overrepresented in seawater, drone-captured air or specific for whale blow samples were defined using ANOVA with the 'p.uni="adjusted"' option. OTUs were classified against the Silva 123 release database (Quast et al., 2013) using mothur "classify.seqs" with default parameters (v1.36.1, Schloss et al., 2009).

Identifying Bacteria Isolated from Agar Plates

Bacterial 16S rRNA genes were directly amplified from cell suspensions obtained from colony picks using conserved primers 27F and 519R (Lane et al., 1985; Lane, 1991). PCR amplifications consisted of 1.0 μl of template and cycle specific for 16S consisted of 95°C for 10 min, 94°C for 30 s, 55°C for 10 s, 72°C for 45 s and 72°C for 10 min, and Taq DNA Polymerase (Qiagen). Amplified DNA was prepared for Sanger sequencing using Agencourt®AMPure® XP beads (Beckman Coulter). Sequences were trimmed to q20, and classified against the Silva Database (version 123).

RESULTS

A total of 74 flights were conducted over 4 days of sampling. Each pod was considered independent as all whales were on their annual northern migration (Pirotta et al., 2016). Overall, 59 successful samples were collected from at least 48 different whales (11 whales were sampled but not identified via video due to occasional failure of the GoPro® camera e.g., low battery or maximum storage capacity reached). Sample volume varied between 50 and 150 μl of exhaled breath. The average opening time of the flip lid was 4 s (min 2 s, max 6 s). The UAV had a maximum flight time (battery time) of 15 min and sampling attempts on average were 4 min 28 s long (range: 27 s to 7 min). The majority of flight time was used to search for the whale's next surfacing position. The time that the UAV was in close proximity to a whale (UAV approximately within 5 m horizontal distance) varied but was on average 53 s (range: 2 s to 2.36 min or 141 s). The most number of samples collected in 1 day was 38. In all cases, there was no behavioral response to the drone (level 1, *n* = 48). Twice there were strong social interactions that occurred prior to the drone approaching the whales (one tail slap, one breach) but sampling was continued on the group in each case and samples successfully collected.

Next Generation Sequencing Results

A total of 7.62 million filtered bacterial 16S ribosomal gene sequences were produced from 59 UAV-captured whale blow and six air samples. These were combined with 0.91 m sequences generated from 26 seawater samples to generate bacterial OTU

abundance profiles. Distance-based clustering of blow, air or seawater bacterial community profiles defined at least three significant clusters (simprof, $P < 0.05$), encompassing one group exclusively composed of seawater, one group exclusively composed of whale blow samples and a third group which clustered the six air samples along with 11 whale-blow samples (**Figure 3A**). Whale blow samples in this group may correspond to UAV sorties that missed, or narrowly missed, capturing whale blow material and were highly correlated with low capture scores based on a visual score of the amount of whale material recovered (Supplementary Table 1).

Bacterial OTUs correlated with seawater, whale blow or air samples were identified using Analysis of Variance (ANOVA) based on generalized linear models fit to the data (Wang et al., 2012). OTU diversity and abundance profiles for air and whale blow were significantly different ($p < 0.05$) from each other and bear little similarity with communities characteristic of the adjacent seawater. At the Class level whale blow bacteria were dominated by *Gammaprotobacteria, Flavobacteriia, Clostridia* and *Fusobacteria*, in contrast to seawater communities, where species composition reflected values typical for sub-tropical waters of the Tasman Sea, i.e., ∼60% Alphaproteobacteria, 15% *Cyanobacteria* and smaller proportions of Gammaproteobacteria and Flavobacteriia (**Figure 3B**; Seymour et al., 2012).

Overall, whale blow samples displayed the greatest OTU diversity, followed by seawater and air (Supplementary Figure 1). Model-based multivariate analyses identified 198 OTUs that were seawater-specific and 35 OTUs that were significantly correlated with air samples (ANOVA, $P < 0.1$; Supplementary Tables 3, 4). Successfully collected whale blow samples contained a small proportion seawater and air-specific OTUs, contributing on average $15.7(\pm 10.8)\%$ and $11.5(\pm 4.4)\%$, respectively, of total sequences. The proportion of air-specific and seawater OTUs in near-miss samples was significantly higher (41.0% and 24.1%, respectively). Subtraction of seawater and air specific OTUs from the total enabled us to define 129 OTUs that were highly specific to whale samples (ANOVA, $P < 0.05$, **Figure 4**, Supplementary Table 5). Abundant bacterial species identified as whale-blow-specific include multiple OTUs belonging to the genera Cardiobacteriaceae and species *Tenacibaculum*, followed by OTUs related to *Pseudomonas* sp. Strain wp33, *Leptotrichia* sp. and *Corynebacteria* spp. While these analyses identified which OTUs were highly specific for whale, air and seawater, an addition set of whale-related OTUs could be identified in the remaining non-significant OTUs. We used the following criteria: present in greater than five whales and >100 sequences, to add an additional 145 OTUs that were highly specific to whales but found only in a small proportion of the sampled whale population (5–17 individuals, out of a total of 57) (Supplementary Table 6). Many of the OTUs in this group are closely related to whale-specific OTUs at the genus and species levels, e.g., *Cardiobacteriaceae, Tenacibaculum,* and *Fusibacter* strains. However, potential respiratory pathogens were also detected, such as Balneatrix (Gammaproteobacteria), and a range of Gram positive Clostridia and Bacilli, such as *Staphylococcus* and *Streptococcus*. In the context of monitoring whale respiratory health, potential pathogens may be present in a subset of the

population only. OTUs in this whale-associated group were present in low abundance, and on average constituted $13(\pm 5.7)\%$ of the total sequences detected in each whale sample.
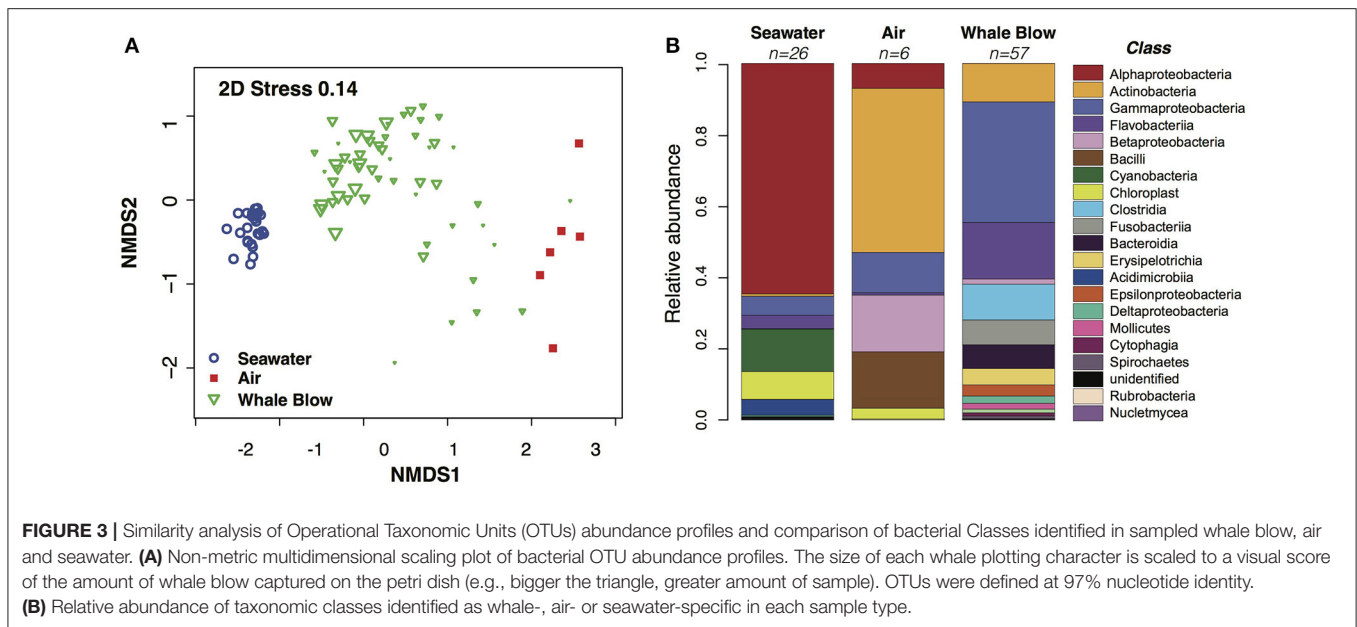
Comparison with Culture-Dependent Identification of Whale Blow Microbiota

Bacterial growth was observed on 48 UAV-mounted agar plates exposed to whale blow. Unexposed control plates displayed no bacterial growth. Sequencing of rRNA genes amplified from single colonies identified 18 different bacteria taxa isolated from 19 different whales (Supplementary Table 7). Overall, the most common bacteria identified at the phylum level included Proteobacteria ($n = 7$), Firmicutes ($n = 7$) and Actinobacteria ($n = 4$). Two samples were identified to the family level, Brucellaceae ($n = 1$) and Microbacteriaceae ($n = 1$). At the genus level, *Micrococcus* ($n = 3$), *Acidovorax* ($n = 3$), *Bacillus* ($n = 3$), *Enterobacteriaceae* ($n = 2$), *Paenibacillus* ($n = 2$), *Streptococcus* ($n = 2$), and *Staphylococcus* ($n = 2$) were most common. Seven whales had more than one bacterium identified. *Staphylococcus* was identified in both an individual sampled via our UAV.

DISCUSSION

UAVs are rapidly transforming the way scientists collect information on their study species (Christie et al., 2016; Lowman and Voirin, 2016; Nowacek et al., 2016; Duffy et al., 2017). In whale research, UAVs have enabled sampling methods to be refined and have eliminated the need for close vessel approaches. To our knowledge, this study is the first to successfully demonstrate the use of a purpose-built UAV designed to sample humpback whale blow in Southern Hemisphere waters. The minimal behavioral disturbance observed suggests this method is an excellent, low-impact alternative to pole sampling methods for large, migrating whales. Humpback whales may have been aware of the UAV and did not react or, mostly likely, were not even aware of the UAV's presence. Underwater noise generated from the UAV was likely to be very low level at the heights flown (<10 m), as it is smaller, lighter and has a lower disc loading than comparable off-the-shelf UAVs shown to transmit minimal noise transmission underwater (e.g., SwellPro Splashdrone and the DJI Inspire 1 Pro) (Christiansen et al., 2016). The combination of the waterproof design and the remotely operated flip lid petri dish designed to minimize airborne contamination, is a significant improvement over existing UAV types.

Our results demonstrate that whale blow can be effectively sampled while minimizing species associated with likely sources of contamination, i.e., air and seawater, to define microbes specifically associated with whales. Amplification of DNA extracted from UAV-captured air highlights the sensitivity of PCR-based approaches for detecting microbiota, even from low amounts of extracted DNA, while also demonstrating the sensitivity of this approach to contamination from external sources. The development of a flip-lid sampling system using sterile petri-dishes enabled us to effectively reduce contamination

FIGURE 3 | Similarity analysis of Operational Taxonomic Units (OTUs) abundance profiles and comparison of bacterial Classes identified in sampled whale blow, air and seawater. **(A)** Non-metric multidimensional scaling plot of bacterial OTU abundance profiles. The size of each whale plotting character is scaled to a visual score of the amount of whale blow captured on the petri dish (e.g., bigger the triangle, greater amount of sample). OTUs were defined at 97% nucleotide identity. **(B)** Relative abundance of taxonomic classes identified as whale-, air- or seawater-specific in each sample type.

from typical seawater bacteria, which may exist in aerosols above the sea surface. While the presence of abundant seawater species (Alphaproteobacteria SAR11 and cyanobacteria) in air and whale blow samples is not surprising, the source of some major species detected in air samples is less clear. Some of the most abundant species detected in air samples, *Propionobacteria, Arthrobacter,* and *Staphylococcus,* are common commensal organisms of mammalian (human) skin and nasal cavities (Human Microbiome Project Consortium, 2012; Prussin and Marr, 2015). A potential source of some non-marine material may have been contamination during the DNA extraction or amplification procedure, especially when the amount of captured material was low (i.e., for air or near-miss samples). In the context of developing indicators of whale health the presence or absence of species that are common in humans should be interpreted cautiously. Nevertheless, in the UAV-sampled blow where a sufficient amount of material was collected, our analyses indicate that ~70% of the total sequences were specific to whales, a group of whale associated sequences accounted for a further ~12% and the remainder could be confidently identified as seawater- or air-specific.

To our knowledge this is the first study to utilize a long-term seawater dataset to identify and subtract seawater bacteria from community profiles of field-captured mammalian samples. The seawater data provided a comprehensive, temporal assessment of the composition of microbial communities present in sea water off Sydney. Critically, a much larger quantity of seawater was collected (2 L) and analyzed in comparison to the whale samples. This method minimized the impact of external sources of contamination and allowed for the greater coverage of the seawater community diversity. We used this resource to filter out all sequences characteristic of seawater to produce a whale blow dataset that could be used as a diagnostic for whale health. The distinct differences observed between statistically-defined

bacteria in whale, sea water and air samples indicates that this method was effective for collecting whale microbiota with minimal contamination.

The successful collection of bacterial DNA in this study provides baseline information of microbiota found in migrating humpback whale blow. Due to the infancy of sampling whale breath as an assessment of whale health (Acevedo-Whitehouse et al., 2010; Hunt et al., 2013), it is not clear as to the type of microflora/bacteria species that are considered 'normal' for northward migrating humpback whales off Sydney. Despite this, there are similarities in our collection of bacterial genera from the few studies that have collected blow for the assessment of microbiota (Acevedo-Whitehouse et al., 2010; Denisenko et al., 2012; Hunt et al., 2013). For example, Streptococcus and Staphylococcus genera were detected in our samples and have been detected in the blow of blue whales (*Balaenoptera musculus*), gray whales (*Eschrichtius robustus*) and Southern resident killer whales (Acevedo-Whitehouse et al., 2010; Denisenko et al., 2012; Hunt et al., 2013; Raverty et al., 2017). Bacteria from the Streptococcus genus is common in mucous membranes of animals (and humans) and is known to be found in the upper respiratory tract (Krzyściak et al., 2013). Streptococcus bacteria has previously been responsible for pneumonia causing death in cetaceans (Acevedo-Whitehouse et al., 2010). *Bacillus sp.* was also identified via blow collection from western North Pacific gray whales and Southern resident killer whales (Denisenko et al., 2012; Hunt et al., 2013; Raverty et al., 2017).

Next generation sequencing identified *Cardiobacteriaceae* (family) and *Tenacibaculum* (genus) to be the most abundant bacterial rRNA genes in whale blow. *Cardiobacteriaceae* has previously been isolated as a dominant taxa in the respiratory system of "healthy" captive bottlenose dolphins (*Tursiops aduncus* and, *T. truncates*) and free-ranging species (*T. truncates*)
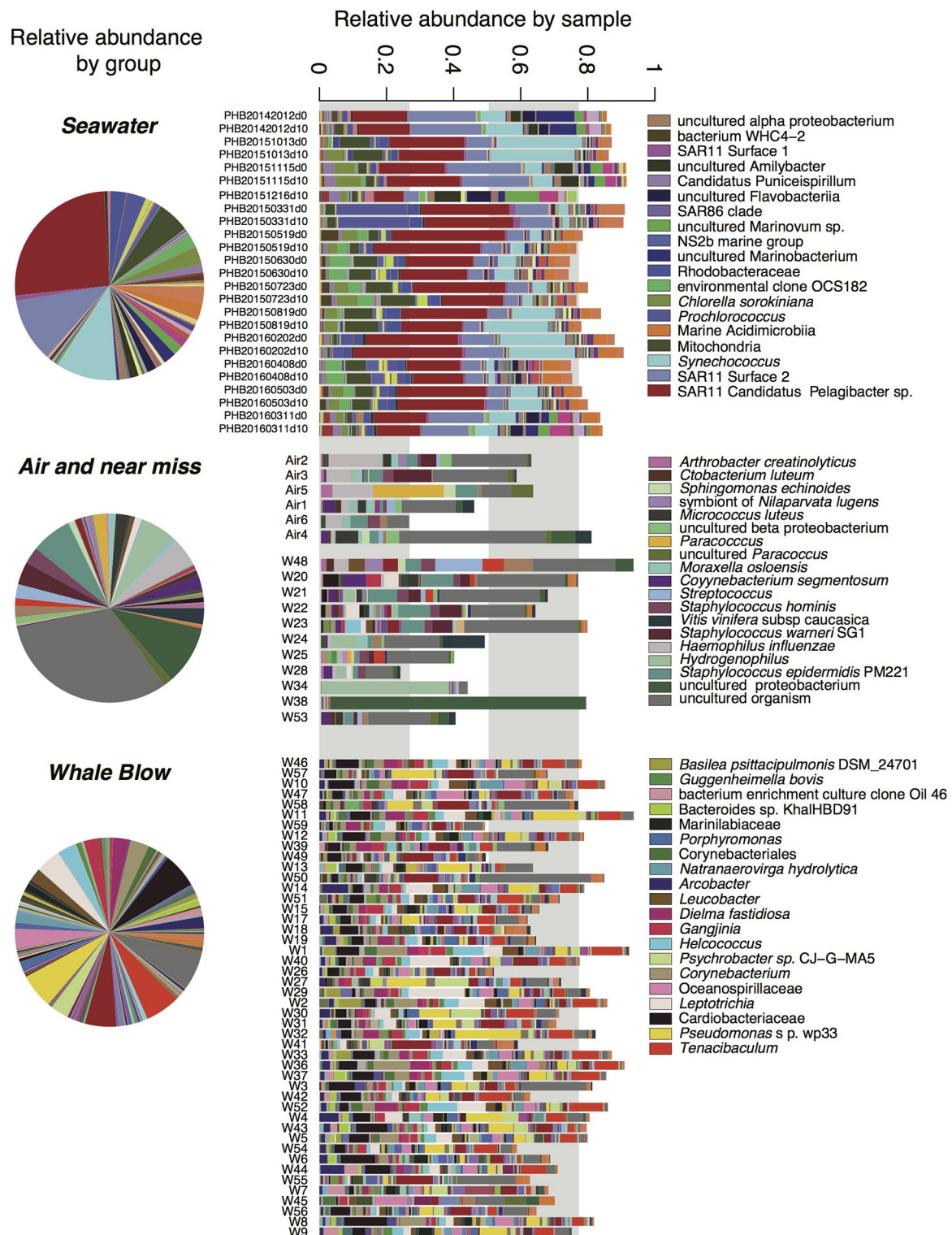
FIGURE 4 | Relative abundance of bacterial taxa identified in seawater, UAV captured air and whale blow. OTUs with abundance <9 across the entire dataset were omitted for clarity. Relative abundances are presented for each group (i.e., seawater, air plus "near-miss" samples and whales, as well as for each sample. Taxa names correspond to the highest taxonomic level identification, full taxonomies are present in Supplementary Tables 3–6) only the top taxa by abundance are shown in the legend.

(Johnson et al., 2009; Lima et al., 2012). These findings may indicate that these genes are part of the normal microflora of dolphins, whilst presence in whales until now was unknown. *Cardiobacteriaceae* are abundant on humpback whale skin (*Gammaproteobacteria* genus), as is *Tenacibaculum* (Apprill et al., 2011, 2014). It may be possible that bacteria found on whale skin also occur within the respiratory tract or epithelial cells. *Tenacibaculum* has been associated with the microbiome of other marine species such as southern bluefin tuna (*Thunnus maccoyii castelnau*) (Valdenegro-Vega et al., 2013), while *Psychrobacter* is part of the thresher shark and rainbow trout skin microbiome (Lowrey et al., 2015; Doane et al., 2017).

The collection of bacterial microbiota is as an indicator of cetacean health is growing (Hogg et al., 2009; Schroeder et al., 2009; Acevedo-Whitehouse et al., 2010; Lima et al., 2012; Hunt et al., 2013; Nelson et al., 2015; Raverty et al., 2017). We were able to sample a number of individuals from a single population over a very short time frame. The use of the waterproof GoPro® camera made identification of different individuals reliable and therefore reduced repeated sampling. Our remotely operated "flip dish" design proved effective at reducing possible contamination from the pilot/research team (e.g., breath, touch, clothing) and vessel vapor/fumes. The placement of Parafilm® around the dish after sampling ensured that the sample remained unexposed until back in the laboratory for processing. Recently published work by Burgess et al. (2016) found polystyrene dishes (petri dish) to be the most effective surface for sampling whale blow in comparison to other sampling materials like veil nylon and nitex nylon mesh. In addition, the use of ice chilling of our samples for temporary storage was also consistent with Burgess et al. (2016), which found storage in cooler box with ice packs was appropriate for preserving samples (at least for hormones) for daylong fieldwork at sea (<6 h). Our samples only contained a fine mist [we estimated between 50 and 150 μL per sample, similar to amounts collected by Hogg et al. (2009)], and so we were unable to directly pipette samples but we found that swabbing the non-agar lid of the petri dishes to be effective. Variability in blow sample volumes appear to be a common issue (Hogg et al., 2009; Acevedo-Whitehouse et al., 2010) and therefore the need for repeated sampling is recommended. Sample success increased with effort/experience and we recommend effort be made early in any study to improve pilot skill, sample collection, quality and quantity.

While overall highly successful, UAVs still require a high level of skill and effort. Predicting when the whale is about to surface, positioning the UAV and opening the petri dish in time remains challenging. This may be complicated when a whale comes to the surface to breath but does not respire forcefully. When this happens, the plate is exposed to the air and so the UAV must return to the boat so the petri dish can be exchanged, our miss/near-miss rate was 11/59 = 20%. Second, not using an off-the-shelf product requires a high level of UAV competence both to fly and to fix problems as they arise. Third, the flight time for this UAV is 15 min, restricting the number of opportunities for sampling before the UAV must return to the vessel in order to replace the battery. Flight time

will increase as battery technology progresses (Nowacek et al., 2016).

Our dataset details the diversity and abundance of the microbiota found in a migrating whale population which provides the baseline to identify pathogenic species. Ultimately, the isolation of pathogens from healthy or diseased animals will be an important step toward understanding the causes of disease and the factors that contribute to virulence. Culture-dependent techniques remain a viable option for the surveillance of pathogens in populations. In this study, nutrient agar was an effective way of culturing a subset of whale blow microbiota, including species commonly associated with respiratory disease in mammals. The use of both sides of the petri dish effectively doubled the chance of obtaining bacterial samples. While next generation sequencing has the capacity to probe the diversity of whale blow microbiota, at present, the isolation and identification bacteria from agar plates can be achieved within 3–5 days, compared to a practical timeframe of weeks for illumina sequencing. Selective media could be used to target potential pathogens in conjunction with opportunistic sampling of diseased or distressed animals.

CONCLUSIONS

Our purpose-built UAV proved highly successful in sampling whale blow for microbial community analysis. It is cost-effective, has low risk of contamination and greatly reduces disturbance of whales. Future applications include other free-ranging whale species (e.g., southern right whales, *Eubalaena australis*), as well as sampling smaller cetaceans (e.g., dolphins). Our UAV is useful addition to the conservation scientist's tool box, enabling collection of health information and therefore the ability to monitor changes in individual health as populations recover and to provide an early warning system for potential future changes.

AUTHOR CONTRIBUTIONS

Paper conception: VP, AS, RH, MO, IJ, and AG. Experiment design: VP, AS, RH, and IJ. Field work: VP, AS, and RH. Laboratory work: VP, DR, and MO. Analysis and interpretation of data: VP, MO, RH, IJ, AG, and DR. Wrote paper: VP, MO, RH, IJ, AG, DR, and AS.

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SUPPLEMENTARY MATERIAL

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Virological Sampling of Inaccessible Wildlife with Drones

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Abstract: There is growing interest in characterizing the viromes of diverse mammalian species, particularly in the context of disease emergence. However, little is known about virome diversity in aquatic mammals, in part due to difficulties in sampling. We characterized the virome of the exhaled breath (or blow) of the Eastern Australian humpback whale (*Megaptera novaeangliae*). To achieve an unbiased survey of virome diversity, a meta-transcriptomic analysis was performed on 19 pooled whale blow samples collected via a purpose-built Unmanned Aerial Vehicle (UAV, or drone) approximately 3 km off the coast of Sydney, Australia during the 2017 winter annual northward migration from Antarctica to northern Australia. To our knowledge, this is the first time that UAVs have been used to sample viruses. Despite the relatively small number of animals surveyed in this initial study, we identified six novel virus species from five viral families. This work demonstrates the potential of UAVs in studies of virus disease, diversity, and evolution.

Keywords: whale; virome; drone; mammalian host; virosphere

There is a growing interest in understanding the diversity, evolution, and disease associations of viruses in natural populations [1]. Although sampling of many terrestrial species is relatively straightforward, there may be serious logistical challenges for animals that live in inaccessible habitats. Marine environments are one such habitat [2–4]. It has recently been shown that wild populations can be sampled using Unmanned Aerial Vehicles (UAVs) [5,6]. UAVs are rapidly transforming wildlife science, allowing sampling from dangerous and inaccessible environments to address questions previously only approached by theory. Here, we show how UAVs can be used to sample viruses. This approach may ultimately enable a better understanding of the patterns and drivers of disease emergence in wild populations.

There is evidence that marine mammal health is deteriorating as anthropogenic stressors on the world's oceans increase [7]. However, contemporary assessments of marine mammal health are strongly biased towards animals whose health is already compromised, such as stranded animals,

which in part reflects the difficulties in sampling aquatic environments. Sampling from free-ranging marine mammals is therefore critical to assess whether healthy animal populations are potential reservoirs of viruses and other transmittable agents.

Following the use of UAV technology for sampling, we employed a meta-transcriptomic approach [8,9] to help characterize the virome of an important marine mammal, the Eastern Australian humpback whale (*Megaptera novaeangliae*), which serves as a model for work in this area. Recent analyses of whale breath, or "blow", have revealed an extraordinary diversity and abundance of microbiota. Importantly, the microbial communities observed were divergent from those present in the surrounding seawater such that they could be considered as distinctly whale blow associated [5,6]. To date, however, these studies have not included virus sampling, and little is known about the diversity of the whale virome and whether this differs fundamentally from that seen in terrestrial mammals.

We collected whale blow samples from 19 humpbacks during the 2017 annual northward migration from Antarctica to northern Australia (Figure 1a). To adhere to all Australian legislative requirements, our UAVs were registered with the Civil Aviation Safety Authority (CASA) and operated by a CASA-certified remote pilot. All flights were conducted in good weather (no rain, Beaufort < 3), from a small research vessel, where the UAV was launched and landed on a launch pad at the stern of the boat. A closed, sterile petri dish was placed on eight suction cups on the UAV before each flight.

Members of the team visually scanned the area for humpback whales. Once an individual or pod was chosen, the vessel was driven at a constant speed and distance from the whale. Once the respiratory rhythm was determined (i.e., downtime length), the UAV was launched to coincide with surfacing. The UAV pilot was directed by spotters on the vessel and positioned the UAV with the aid of the live feed from a forward-facing camera. To minimize sample contamination, the petri dish remained closed until immediately before the whale surfaced. The dish was remotely opened as the UAV accelerated towards and through the densest part of the whale blow, collecting the maximum amount of sample in the dish and lid (see Video S1). The petri dish was immediately closed and the UAV was returned to the vessel. The petri dish containing the sample was removed from the UAV and secured with Parafilm®. All samples were stored immediately in a portable −80 °C freezer. A different whale was sampled each flight. Different individuals within a pod were chosen based upon unique distinctive markings (e.g., white flanks and barnacle arrangements).

RNA was extracted using an RNeasy Plus Universal mini kit (Qiagen, Australia). Due to low RNA concentration, all 19 samples were pooled and concentrated using a NucleoSpin RNA Clean-up XS kit (Macherey-Nagel, Australia). A single library was produced for RNA sequencing using the Low-Input SMARTer Stranded Total RNA Sample Prep Kit with Mammalian rRNA depletion (Clontech, Australia), with 1 ng of the pooled whale blow RNA as input. Paired-end (100 bp) sequencing of the RNA library was performed on the HiSeq 2500 platform (Illumina, Australia) at the Australian Genome Research Facility.

RNA sequencing of the rRNA-depleted library resulted in 19,389,378 paired reads (100 nt in length) that were assembled de novo into 107,681 contigs. Sequencing reads were first quality trimmed then assembled using Trinity [10]. The assembled transcriptome was annotated based on similarity searches against the NCBI nucleotide (nt) and non-redundant protein (nr) databases using BLASTn [11] and Diamond (BLASTX) [12], respectively, and an e-value threshold of 1×10^{-5}. Transcript abundance was estimated using RSEM [13] implemented within Trinity.

Our transcriptome data revealed that the humpback whale blow contains a wide diversity of DNA and RNA viruses (that we refer to "whale-blow-associated" viruses). BLAST analysis revealed the relative abundance of taxonomic classes present in the non-rRNA transcriptome data, of which bacteria occupied ~45%, while ciliates were the second-most abundant source at ~29%. Importantly, Baleen whale species contributed 0.9% of the transcriptome data and were the most abundant source of mammalian RNA, indicating our sample is indeed whale associated. Viruses occupied ~0.01% of the non-rRNA transcriptome, which falls within the range of other meta-transcriptome studies of

vertebrates [9]. Despite this relatively low abundance, the viral contigs observed fell into 42 classified viral families, including 29 families of bacteriophage (Figure 1b). The most relatively abundant bacteriophages included the *Siphoviridae* (18.4% of all viruses) and the *Myoviridae* (15.2% of all viruses). Among the most abundant viral families that are known to infect eukaryotes were small single-stranded (ss) DNA viruses, specifically the *Circoviridae* (and *Circoviridae*-like viruses) (6.5% of all viruses), as well as members of the *Parvoviridae* (2.4%) and an RNA virus family, the *Tombusviridae* (0.9%).
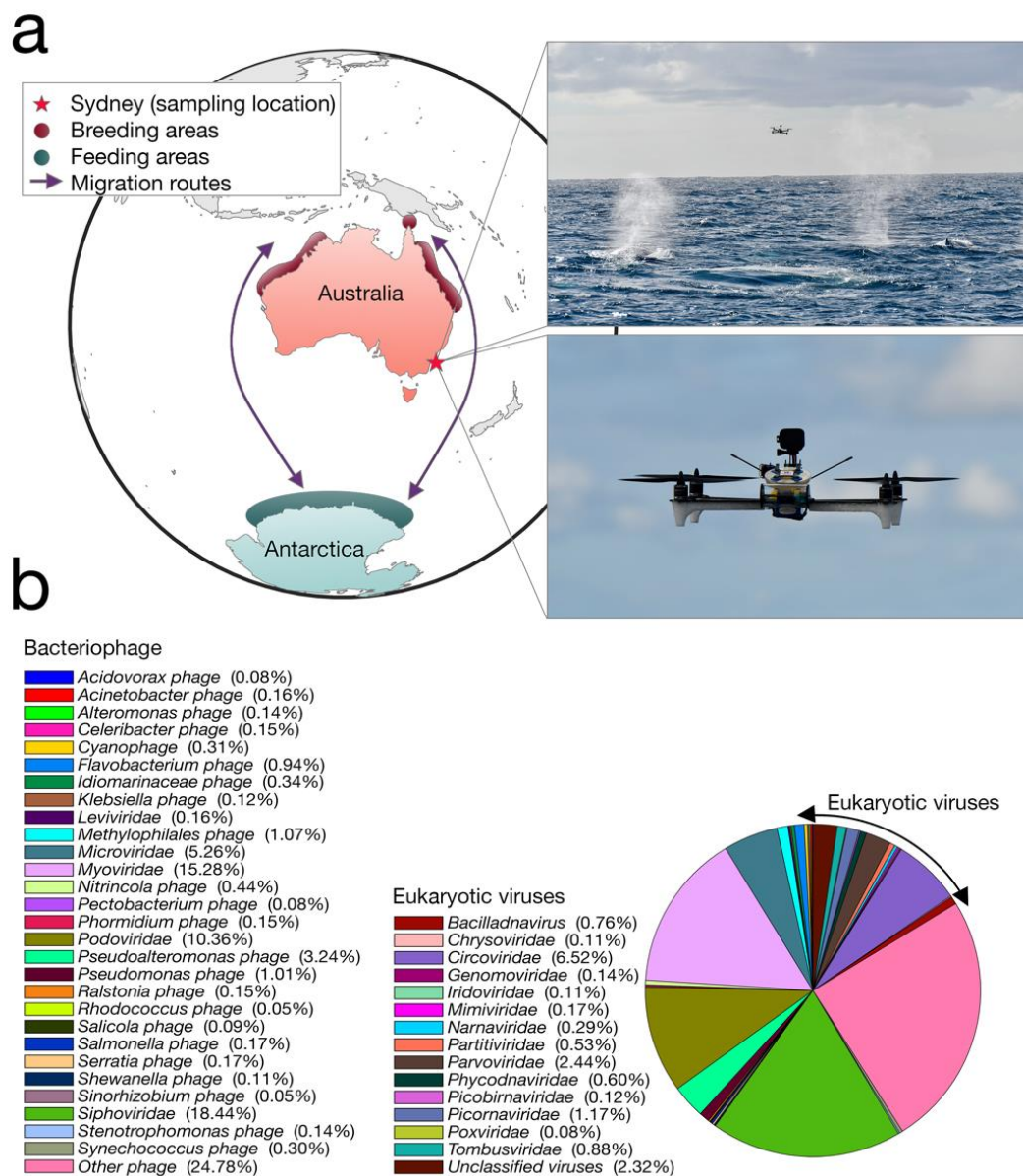
Figure 1. (**a**) Map showing the humpback whale sampling location (red star), approximately 3 km off the coast of Sydney, New South Wales, Australia. Purple arrows indicate the typical seasonal migratory routes of the humpback whale from their likely feeding ground in Antarctica (dark green) to their breeding areas around northern Australia (dark red). Photographs demonstrate the Unmanned Aerial Vehicle (UAV) in action. (**b**) Relative abundance of viruses and their taxonomic families. Taxonomy was based on both protein and nucleotide BLAST search results, taking the best e-value for each (for those with identical e-values, we used the taxa with the closest percentage identity). This included 42 viral families, including 29 families of bacteriophage. Percentages indicate relative abundance of all viruses in the sequence library.

We next inferred the evolutionary relationships of the viruses contained in whale blow with their closest phylogenetic relatives. Translated open reading frame segments were combined with protein sequences obtained from GenBank, using the top search results from BLAST (see Table 1 for more details of the sequences analyzed). Sequences were aligned using MAFFT v.3.4 [14], employing the E-INS-I algorithm with poorly aligned regions removed using trimAl v.1.2 [15]. To estimate phylogenetic trees for the virus data sets, we selected the optimal amino acid substitution model identified using the Bayesian Information Criterion as implemented in Modelgenerator v.0.85 [16] and analyzed the data using the maximum likelihood approach available in PhyML v3.1 [17] with 1000 bootstrap replicates. Phylogenetic trees were annotated with FigTree v.1.4.2.

Table 1. Amino acid identity, contig length, and relative frequency of the viruses identified in this study. All sequence reads generated in this project are available under the NCBI Short Read Archive (SRA) under accession number SRP149185 and virus sequences have been deposited in GenBank.

Virus Family	Virus Species	Contig Length (nt)	% Relative Abundance in Library	% Amino Acid Identify	Closest Match (GenBank Accession Number)
Circoviridae	Humpback whale blow-associated circo-like virus 1	702	0.000115%	51%	Sewage-associated circular DNA virus-29 (YP_009117067)
Circoviridae	Humpback whale blow-associated circo-like virus 2	909	0.000164%	46%	McMurdo Ice Shelf pond-associated circular DNA virus-5 (YP_009047137)
Parvoviridae	Humpback whale blow-associated denso-like virus	315	0.000143%	47%	*Periplaneta fuliginosa* densovirus (NP_051022.1)
Tombusviridae	Humpback whale blow-associated tombus-like virus	279	0.000164%	41%	Changjiang tombus-like virus-9 (YP_009337417.1)
Picornaviridae	Humpback whale blow-associated picornavirus	255	(N/A–assembled contigs from raw reads)	61%	Quail picornavirus (NC_016403)
Astroviridae	Humpback whale blow-associated astrovirus	130	(N/A–assembled contigs from raw reads)	76%	Porcine astrovirus 5 (YP_009010969)

Of the most abundant eukaryotic viruses, two novel (as determined by phylogenetic analysis) circular Rep-encoding ssDNA viruses (CRESS-DNA viruses) *Circoviridae*-like viruses were identified, denoted here as humpback whale blow-associated circo-like virus 1 and 2 (Table 1; Figure 2). Related viruses have previously been identified in many aquatic systems, for which marine invertebrates, particularly crustaceans, are thought to be a primary host [18]. Humpback whale blow-associated circo-like virus-1 exhibited 51% amino acid identity to the replication-associated protein (Rep) of its closest genetic relative, sewage-associated circular DNA virus-29, and 46% amino acid identity to the Rep of Lake Sarah associated circular virus-32. Humpback whale blow-associated circo-like virus-2 shared 46% amino acid identity to the Rep of McMurdo Ice Shelf virus-5, isolated from a freshwater pond in Antarctica [19]. As these ssDNA viruses appear to be major virome components in many aquatic environments [18], they are likely associated with aquatic ecosystems in general.

Another relatively abundant viral contig was a partial genome of a novel densovirus (family *Parvoviridae*). The most similar amino acid sequence to this new virus, denoted here as humpback whale blow-associated denso-like virus, was a densovirus isolated from a *Periplaneta fuliginosa* (i.e., a cockroach), sharing only 47% sequence similarity to the nonstructural protein (Table 1; Figure 2). Similarly, a novel tombus-like viral partial genome, falling into the *Tombusviridae*, was identified and was closely related to Changjiang tombus-like virus-9 isolated from crayfish, with 41% sequence

similarity to the RNA-dependent RNA polymerase (RdRp). We denote this virus humpback whale blow-associated tombus-like virus (Table 1; Figure 2).
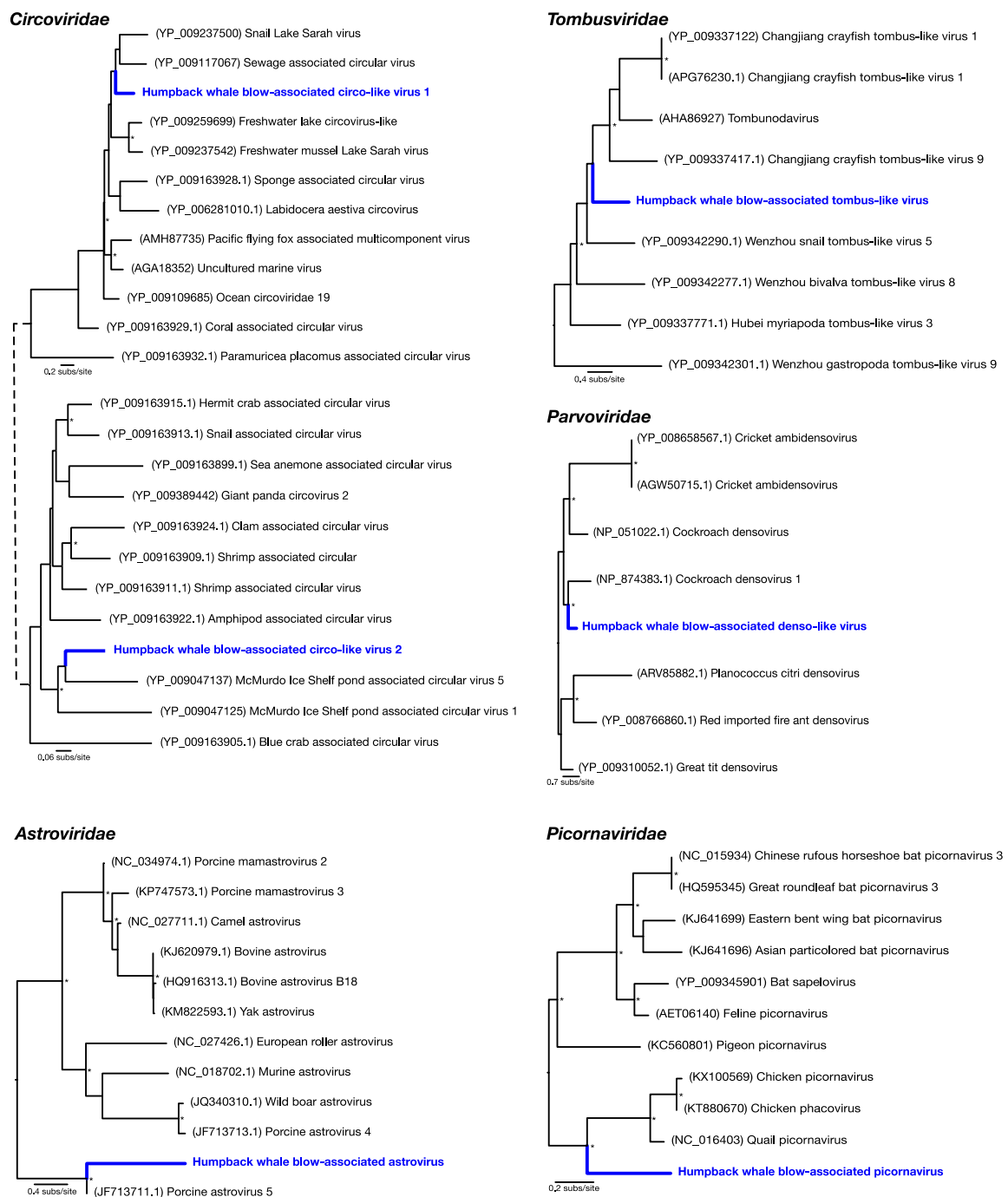

Figure 2. Phylogenetic relationships of the viruses discovered from assembled contigs along with their closest genetic relatives obtained from GenBank (accession numbers in parentheses). The families described here are *Circoviridae*-like, *Parvoviridae*, *Tombusviridae*, *Picornaviridae*, and *Astroviridae*. The maximum likelihood phylogenetic trees show the topological position of the newly discovered viruses (blue). Asterisks indicate branch support >70%, based on 1000 bootstrap replicates. All branches are scaled per the number of amino acid substitutions per site. Trees were midpoint rooted for clarity only.

To reveal viruses at very low relative abundance, a Diamond BLAST [12] analysis was performed against the raw 100 bp sequencing reads. This process identified several sequencing reads that matched viruses, later assembled into short contigs, that comprised two potentially new RNA viruses from the *Picornaviridae* and the *Astroviridae*. Humpback whale blow-associated picornavirus shared 61% amino acid similarity to the RdRp of the most closely related *Coturnix coturnix* (quail) picornavirus (Table 1; Figure 2). Similarly, humpback whale blow-associated astrovirus shared 76% amino acid identity with the nonstructural protein 1a of porcine astrovirus-5 (Figure 2). Both picornaviruses and astroviruses are single-stranded, positive-sense RNA viruses with small icosahedral capsids and no external envelope which may aid their preservation in harsh marine environments, and viruses from these families are commonly found in aquatic vertebrates [9]. As only short fragments of these viruses' genomes were identified in our data set, their phylogenetic position requires confirmation. This is likely due to the low quantity of RNA isolated from the whale blow samples and the pooling of individual samples. However, that both these viruses were most closely related to other vertebrate viruses suggested that they are likely whale associated rather than sampled from the surrounding seawater. Further sampling of the sea water virome is required to understand the potential enormous diversity that comprises the aquatic virosphere.

Little is known about the transmission of whale viruses. Analyses of whale influenza viruses suggest that they likely originated from gulls and that feeding activities of gulls and whales often place them in close contact, such that oral–fecal transmission through seawater is a likely route [20] and which might explain our observation of viruses associated with aquatic ecosystems. In addition, given the vast aerosol produced by whales, and their close contact within migrating pods as well as at feeding and breeding grounds, respiratory transmission may also play an important role in the movement of viruses in whales.

In sum, we show that drone-based virological surveys of previously inaccessible wildlife populations has the potential to help reveal the diversity of the virosphere, facilitating the detection of viruses infecting wildlife and aiding evaluation of their pathogenic and zoonotic potential.

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