The Spatial Ecology of Manta Rays: Movement, Habitat Use and Connectivity

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Abstract

Understanding the movements and habitat use of manta rays is key to improving the conservation status and management of these highly mobile, threatened species. Here I investigate the spatial ecology of reef manta rays *Mobula alfredi* and oceanic manta rays *M. birostris*, from their fine-scale habitat use, to their global scale connectivity. The drivers of individual *M. alfredi* fine-scale movements and habitat use were investigated at the reef off Lady Elliot Island, Great Barrier Reef. An array of acoustic listening stations was deployed around a high-use cleaning station area to monitor 42 acoustically-tagged rays. In-water surveys, conducted on SCUBA, mapped structural features within the array, and determined the number of cleaner wrasse *Labroides dimidiatus* and their location (cleaning stations). Space use by *M. alfredi* was significantly associated with *L. dimidiatus* distribution and hard coral structures. Manta rays visited these sites during daylight hours, with use affected by certain wind and tidal regimes. Cleaning behaviour dominated at this location, taking precedence over other behavioural traits, providing evidence that cleaning station attendance is a driver of reef visitation for this species.

To determine what may drive foraging aggregations of manta rays in surface waters, the food environment for *M. alfredi* in Hanifaru Bay, Maldives was investigated. This location acts to attract the largest known feeding aggregation of this species. Zooplankton dynamics in relation to manta ray presence, abundance and behaviour in the bay were sampled and analysed. It was found that *M. alfredi* was more abundant and fed more regularly when zooplankton biomass in the Bay was high. The prey density threshold for when manta rays were more likely to be feeding was established. This prey density was more than twice that calculated to meet their theoretical energy requirements, and more than four times that observed at other surface feeding sites for large planktivores. Also, the zooplankton community was significantly different between when manta rays were feeding, and when they were not. The dominant zooplankton species present when *M. alfredi* was feeding was of oceanic origin, which infers that intrusions of oceanic water occur at the site.

Citizen science and photo-identification (photo-ID) were used to update the known distance of travel for reef manta rays. Individual *M. alfredi* were confirmed to make point-to-point movements of over 1,150 km along the east coast of Australia – a significant extension of the known distance that this species may travel. Movements of this magnitude demonstrate that *M. alfredi* is clearly capable of making movements across jurisdictional boundaries where they may be subject to differing levels of protection. Non-traditional data collection approaches were used to update the distribution of both *M. alfredi* and *M. birostris* around the Australian coastline through a combination of: photo-ID databases; online sightings via social media channels (youtube, facebook, Instagram, vimeo etc); government records and; aerial surveys. A semi-continuous distribution of *M. alfredi* around the northern extent of the Australian coast, and along both east and west coastlines to approximately 30°S was found. Relatively sparse sighting records of *M. birostris* were used to explore the distribution of this less understood species in Australian waters.

On a global-scale, 52 photo-ID databases that contained images of 17,135 individuals of both *M. alfredi* and *M. birostris* were used to explore whether phenotypic traits could provide evidence of connectivity (or isolation) among geographically separated populations. A quantitative analysis of spot patterns on the ventral surface of each species was performed using machine learning and convolutional neural networks. The analyses indicated that spot patterns do differ significantly among regional populations, in support of known isolation or connectivity among populations based on pre-existing genetic and photo-ID information. However, high within-population variability suggests caution should be applied when using this approach. Further analysis, and development of the approach may provide insight into questions such as the heritability of spot patterns and familial lineages.

Through a variety of methodological approaches, questions about the spatial ecology of these large planktivorous rays, were answered. The findings in this thesis are of value in our overall understanding of the species – from the drivers of local-scale habitat use, to regional and global distributions.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

The following publication has been incorporated as Chapter 2.

Armstrong, A. O., A. J. Armstrong, M. B. Bennett, A. J. Richardson, K. A. Townsend, J. D. Everett, G. C. Hays, H. Pederson, and C. L. Dudgeon. 2021. Mutualism promotes site selection in a large marine planktivore. Ecology and Evolution. https://doi.org/10.1002/ece3.7464

Contributor	Statement of contribution	%
Asia Armstrong	**writing of text** **proof-reading** **theoretical derivations** **numerical calculations** **preparation of figures** **initial concept**	**80** **70** **50** **80** **70** **80**
Amelia Armstrong	proof-reading theoretical derivations initial concept	2 5 5
Mike Bennett	writing of text proof-reading supervision, guidance theoretical derivations initial concept	5 5 20 5 5
Anthony Richardson	writing of text proof-reading supervision, guidance theoretical derivations numerical calculations preparation of figures	5 10 35 10 10 10
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Graeme Hays	writing of text proof-reading	5 2
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Asia Armstrong	**writing of text**	**50**
	proof-reading	**60**
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	preparation of figures	**50**
	initial concept	**40**
Amelia Armstrong	writing of text	25
	proof-reading	10
	theoretical derivations	10
	numerical calculations	50
	preparation of figures	50
	initial concept	40
Mike Bennett	writing of text	5
	proof-reading	10
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Amelia Armstrong	writing of text proof-reading theoretical derivations numerical calculations preparation of figures initial concept	50 60 70 50 70 40
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Frazer McGregor	writing of text proof-reading	2 2
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Adam Barnett	writing of text proof-reading	2 2
Anthony Richardson	writing of text proof-reading	2 2
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Christine Dudgeon	writing of text proof-reading supervision, guidance theoretical derivations preparation of figures initial concept	5 5 40 10 10 10

Amelia and I worked in a highly collaborative way on chapters 4.1 and 4.2. It was decided *a priori* by the advisory team (essentially the same team for both students: Mike Bennett, Anthony Richardson, and Christine Dudgeon) that there would be a shared lead co-authorship, with the physical first authorship position taken by myself for chapter 4.1 and taken by Amelia for chapter 4.2. Both authors were intimately involved in the many aspects of outreach and engagement of citizen scientists, other researchers and groups that enabled the two parts of this chapter to be completed. This included a series of long road trips that the authors undertook to connect with useful collaborators such as dive and tourism companies. Both authors were involved in the collection and collation of the data as part of the *Project Manta* research group that formed a critical role in each of the papers, and both authors contributed equally in terms of intellectual input, planning, analysis, and interpretation of these chapter parts. The chapter parts complement one another and dovetail nicely to achieve the goal of determining the distribution and broad-scale movements of the focal species.

Submitted manuscripts included in this thesis

The following submitted manuscript has been incorporated as Chapter 3 of this thesis:

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Guy Stevens	proof-reading supervision, guidance initial concept	5 30 10
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Annie Murray	proof-reading numerical calculations	2 10
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Other publications during candidature

Peer-reviewed papers

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McEnnulty, F. R., C. H. Davies, **A. O. Armstrong**, N. Atkins, F. Coman, L. Clementson, S. Edgar, R. S. Eriksen, J. D. Everett, and J. A. Koslow. 2020. A database of zooplankton biomass in Australian marine waters. Scientific Data **7**:1-9.

McGregor, F., A. J. Richardson, A. J. Armstrong, **A. O. Armstrong**, and C. L. Dudgeon. 2019. Rapid wound healing in a reef manta ray masks the extent of vessel strike. PLoS ONE **14**:e0225681.

Stewart, J. D., F. R. A. Jaine, A. J. Armstrong, **A. O. Armstrong**, M. B. Bennett, K. B. Burgess, L. I. E. Couturier, D. A. Croll, M. R. Cronin, M. H. Deakos, C. L. Dudgeon, D. Fernando, N. Froman, E. S. Germanov, M. A. Hall, S. Hinojosa-Alvarez, J. E. Hosegood, T. Kashiwagi, B. J. L. Laglbauer, N. Lezama-Ochoa, A. D. Marshall, F. McGregor, G. Notarbartolo di Sciara, M. D. Palacios, L. R. Peel, A. J. Richardson, R. D. Rubin, K. A. Townsend, S. K. Venables, and G. M. W. Stevens. 2018. Research Priorities to Support Effective Manta and Devil Ray Conservation. Frontiers in Marine Science **5**.

Book chapters

Dudgeon, C.L., C. Kilpatrick, **A. O. Armstrong**, A. J. Armstrong, M.B. Bennett, D. Bowden, A.J. Richardson, K.A. Townsend, and E. Hawkins. 2019. Citizen science photographic identification of marine megafauna populations in the Moreton Bay Marine Park. In: Tibbetts IR, Rothlisberg PC, Neil DT, Homburg TA, Brewer DT, Arthington AH, editors. *Moreton Bay Quandamooka & catchment. Past, present and future*.

Conference abstracts

Armstrong, A. O. Reef manta rays foraging at an aggregation site on the Great Barrier Reef. (Poster) *Joint Australian Society for Fish Biology and Oceania Chondrichthyan Society Conference*. Hobart, 2016.

Armstrong, A. O. Reef manta rays foraging at an aggregation site on the Great Barrier Reef. (Poster) *School of Biomedical Sciences HDR Symposium*. Brisbane, 2016.

Armstrong, A. O. Cleaning station visitation and habitat use of *Manta alfredi* on the east coast of Australia. (Oral) *4th International Conference on Fish Telemetry*. Cairns, 2017.

Armstrong, A. O. Cleaning stations as a driver of aggregative behaviour by the reef manta ray, *Mobula alfredi.*(Oral) *Joint New Zealand Marine Sciences Society and Oceania Chondrichthyan Society Conference*. Dunedin, 2019.

Armstrong, A. O. Spot the difference: Can unique markings be used to delineate populations of manta rays.(Oral) *Oceania Chondrichthyan Society Conference*. Virtual, 2020.

Contributions by others to the thesis

Chapter 1

This chapter is my own work. Critical feedback on drafts was provided by Chris Lawson, Mike Bennett, Christine Dudgeon, and Anthony Richardson.

Chapter 2

This chapter was published in Ecology and Evolution in 2021, and was co-authored by Amelia Armstrong, Mike Bennett, Anthony Richardson, Kathy Townsend, Jason Everett, Graeme Hays, Hugh Pederson, and Christine Dudgeon.

Chapter 3

This chapter is under review in PeerJ, and was co-authored by Guy Stevens, Kathy Townsend, Annie Murray, Mike Bennett, Amelia Armstrong, Julian Uribe-Palomino, Phil Hosegood, Christine Dudgeon and Anthony Richardson.

Chapter 4.1

This chapter was published in Marine Biodiversity Records in 2019, and was co-authored by Amelia Armstrong, Mike Bennett, Anthony Richardson, Kathy Townsend, and Christine Dudgeon.

Chapter 4.2

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Chapter 5

This chapter is my own work. Guy Stevens and Andrea Marshall facilitated data collection. Olga Moskvyak, Cedric van den Berg and Anthony Richardson assisted with data processing and statistical analysis. Critical feedback on drafts was provided by Mike Bennett, Christine Dudgeon Anthony Richardson, and Guy Stevens. Data was obtained from Amelia Armstrong, Calvin Beale, Cécile Berthe, Alice Carpentier, Lydie Couturier, Nakia Cullain, Mark Deakos, Mandy Etpison, Daniel Fernando, Anna Flam, Niv Froman, Karen Fuentes, Elitza Germanov, Luke Gordon, Lydia Green, Joanna Harris, Julia Hartup, Simon Hilbourne, Kary Kumli, Hugo Lassauce, Andrea Marshall, Frazer McGregor, Lauren Peel, Jessica Pate, Alessandro Ponzo, Robert Rubin, Tam Sawers, Edy Setyawan, Ana Sobral, Guy Stevens, Carmen Toanchina, Sarah Travers, Ernst van der Poll and Project Manta.

Chapter 6

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Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research involving human or animal subjects

Approval was obtained from The University of Queensland Animal Ethics Committee (SBS/319/14/ARC/EA/LEIER and SBS/342/17).

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My journey in manta ray research began back in 2008 when I first dived with these animals off North Stradbroke Island with James Griffith of Manta Lodge. James's passion for them was contagious, and I immediately wanted to find out more about them, but the literature at that time was relatively scarce. In 2009 I saw the *Queen of the Mantas* documentary about Andrea Marshall's work, and I wanted to get involved. At that stage you needed to become a marine biologist to volunteer for her organization in Mozambique, so back to university I went to become an undergraduate (again!) at the ripe age of 29. I want to acknowledge Andrea as one of the inspirations for my return to university, and James for his never-ending enthusiasm for all things marine, and his constant unwavering support for Project Manta.

Anthony Richardson gave me my first break to get involved with Project Manta. I started out managing the database of sightings after a conversation with Ant where I shared with him my love of stats (over a beer of course!) Ant has always provided me with my benchmark of how to be a good academic, do good science and be a good human at the same time. I owe a real debt of gratitude for his support through my Honours first, and now my PhD. I often look at problems, and think "What would Ant say?"

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Keywords

mobulid, elasmobranch, planktivore, zooplanktivore, movement ecology, foraging threshold, animal aggregation, citizen science, mutualism, artificial intelligence

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FoR code: 0602, Ecology, 60%
FoR code: 0608, Zoology, 40%

Dedications

To imagining a healthier planet for Aya,
the axis upon which my earth turns.

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List of Abbreviations used in the thesis

AIC	Akaike Information Criterion
CNN	Convolutional Neural Network
DW	Disc Width
GBR	Great Barrier Reef
GLM	Generalised Linear Model
GLMM	Generalised Linear Mixed Effect Model
HPE	Horizontal Positioning Error
HPEm	measured Horizontal Positioning Error
IUCN	International Union for the Conservation of Nature
LEI	Lady Elliot Island
NBSS	Normalised Biomass Size Spectra
NSI	North Stradbroke Island
NSW	New South Wales
PCA	Principle Components Analysis
Photo-ID	Photographic Identification
SD	Standard Deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism
TL	Total Length
VPS	VEMCO Positioning System
WA	Western Australia

Chapter 1

General Introduction

The study of spatial ecology is concerned with how the arrangement of animals, their populations, and the environment are linked to each other and ecological phenomena (Collinge 2010, de Knegt et al. 2010). This can include core questions such as: how habitat use can structure communities; how productivity influences space use; and how body size effects spatial patterns of diversity (Currie 2007). The spatial structure of a community, i.e., what spaces different species occupy across their varying life history stages, may derive from interspecific and intraspecific interactions such as predation, reproduction, and competition (Perry et al. 2002). This suggests that spatial ecology can be key to the concept of ecological niche theory (Hirzel and Le Lay 2008), and confirms the importance of including community level processes when investigating species ecology (Vellend 2010).

Scale is an important consideration when addressing questions that relate to spatial ecology (Wiens 1989). Investigations need to be conducted at the appropriate spatial scale for the question and are often hampered by sampling effort and / or an inability to scale up the findings from fine-scale studies. For instance, patterns in nature are often observed at a local scale due to sampling constraints, but the mechanisms underlying these processes can operate at broader scales (Levin 1992). The issue is that when patterns and processes are measured at small scales, they do not necessarily prevail at larger scales, meaning problems in ecology cannot be addressed simply by extrapolating locally measured variables to larger areas, and vice versa (Schneider 2001). For researchers to understand the spatial ecology of organisms it is important to integrate the different scales at which ecological processes occur.

In recent times, the study of spatial ecology has become closely related to conservation biology, as the focus in many scenarios has shifted to preventing habitat loss and fragmentation (Collinge 2010). This is particularly true when seeking to understand the drivers of space use in threatened species, whose population viability depends on effective, informed management and habitat protection. By protecting habitat and connectivity on a large scale, it allows for species to select small-scale habitat use that best suits them from a variety of options (Briand et al. 2009). Investigations into the space use of threatened species must extend beyond the species in question, and take into account the ecosystems they utilise, and the communities they interact with. This thesis aims to explore numerous aspects of the spatial ecology of manta rays, from fine-scale behaviour measured in metres, to the global scale that looks at widely dispersed populations across the Indo-Pacific Oceans, as there are many interesting and potentially useful questions that relate to their habitat use, feeding ecology, movements and distribution, and the connectivity of their global populations. Questions such as how important is cleaning station attendance for these species, how far can they travel, and how connected are global populations.

Manta rays

During the past decade there has been a concerted research effort to address questions related to the biology and ecology of manta ray species globally. Manta rays belong to the family Mobulidae within the reasonably diverse group of fishes, the elasmobranchs, which includes sharks, rays and skates (Last and Stevens 2009). Recent taxonomic revision of the family Mobulidae has discarded the genus *Manta* and determined that both of the described manta ray species are nested within the genus *Mobula* alongside the devil rays (White et al. 2017). However, their common names - the reef manta ray (for *M. alfredi*) and the oceanic manta ray (for *Mobula birostris*) - will likely persist and will be used within this thesis. Manta rays are the largest known ray species, reaching disc widths of up to 7 m for the oceanic manta ray, and 5 m for the reef manta ray (Marshall et al. 2009). *Mobula birostris* is more regularly associated with offshore environments, in proximity to continental shelves and deep seamounts, while *M. alfredi* is regularly seen in relatively shallow coastal waters and is particularly associated with reef environments (Kashiwagi et al. 2011). The two species were only formally separated less than a decade ago, leaving a question mark about which species much of the earlier literature relates to (Marshall et al. 2009).

Like many elasmobranch species, manta rays exhibit conservative life history traits, including slow growth rates, late age of maturity and low fecundity (Couturier et al. 2012, Dulvy et al. 2014). These factors make the species particularly vulnerable to exploitation, and targeted fisheries have led to population declines in some regions (Rohner et al. 2013a, Ward-Paige et al. 2013, Acebes and Tull 2016, Croll et al. 2016). Owing to their threatened conservation status, much of the recent research in relation to both species has arisen from the urgent need for baseline knowledge about their movements (Dewar et al. 2008, Clark 2010, Jaine et al. 2014, Stewart et al. 2016a), distribution (Anderson et al. 2011, Couturier et al. 2011) and population dynamics (Marshall et al. 2011b, Kitchen-Wheeler et al. 2012, Couturier et al. 2014a). The ability of manta rays to travel long distances and dive to depths of hundreds of metres (Braun et al. 2014, Stewart et al. 2016b), as well as their large size, has made it difficult to gain even basic biological information. However, similar to some other elasmobranch species, manta rays aggregate at predictable locations in tropical and sub-tropical oceans around the world which can present ideal study sites for exploring different aspects of the species' ecology (Economakis and Lobel 1998, Rowat et al. 2011, Dudgeon et al. 2013).

Habitat selection

Habitat preferences of animals are variously related to resource availability, reproductive activities or predator avoidance. Site preference is observed in both terrestrial and marine species, where individuals aggregate at predictable locations for various reasons including to feed (Rydell 1991, Houghton et al. 2006, Van Doorn et al. 2010), reproduce (Christal and Whitehead 1997, Sinclair et al. 2000, Van Dam et al. 2008) and pup (Lunn and Boyd 1991, Crampe et al. 2007, Chapman et al. 2009, Feldheim et al. 2014). In the marine environment, numerous elasmobranch species aggregate at the same locations with some regularity; be it seasonally, annually or inter-annually depending on the species' life cycle (Wilson et al. 2001, Bansemer and Bennett 2009, Francis et al. 2015). In addition to feeding and courtship, manta rays are also observed attending cleaning stations at aggregation sites (Marshall and Bennett 2010, Deakos 2012, Jaine et al. 2012). However, despite the frequent observation of cleaning station attendance, these sites have scarcely been explored in terms of site fidelity, and consequently the ecological importance of these sites for the spatial ecology of manta rays is little understood.

Foraging ecology

Both species of manta rays are planktivorous filter feeders (Couturier et al. 2013, Armstrong et al. 2016), similar to the whale shark *Rhinocodon typus* and basking shark *Cetorhinus maximus* (Sims and Quayle 1998, Nelson and Eckert 2007, Rohner et al. 2015), and in some ways to baleen whales (Mayo and Marx 1990, Goldbogen et al. 2011). The diet of manta rays primarily comprises temporally and spatially patchy zooplankton, and their movements are likely driven by food availability. However, teasing apart what conditions result in large aggregations of animals at their feeding sites remains a challenge for researchers in the marine environment. Research into aggregation sites for large planktivores has invariably found a link to productivity and thus food availability (Sims et al. 2000, Wilson et al. 2001, Fossette et al. 2010, Rohner et al. 2013a). For manta rays, this may be linked to tidal influences (Dewar et al. 2008, Armstrong et al. 2016), chlorophyll-a concentrations (Sleeman et al. 2007, Weeks et al. 2015) and/or the apparent island mass effect, whereby flow dynamics around islands act to concentrate productivity (Kitchen-Wheeler 2010, Anderson et al. 2011). An understanding of the food environment at these sites is vital for determining drivers of foraging and aggregative behaviour, but currently little *in situ* data are available in this space.

Movement and distribution

The movements and distributions of pelagic fish species are relatively poorly understood when compared to other marine species such as sea turtles, cetaceans and seabirds (Fossette et al. 2010, Cheney et al. 2013, Hennicke and Weimerskirch 2014). Manta rays display aggregative behaviours at particular locations that provide good opportunities to investigate their biology and ecology, and have revealed site fidelity to specific habitats (Deakos et al. 2011, Graham et al. 2012, Peel et al. 2019b). For both species, there appears to be limited evidence of large-scale movements (greater than 1,000 kms). With one exception, of tagged *M. birostris* making straight-line movements of up to 1,500 kms (Couturier et al. 2011, Hearn et al. 2014, Stevens and Peschak 2016). Monitoring the movements and distribution of large marine animals, especially non-airbreathers that may not be suitable for satellite-based location of tagged animals, can be challenging. This is particularly true for species such as manta rays, that inhabit sparsely populated coastlines or pelagic environments, which can make research logistically difficult. Thus, non-traditional research approaches may provide insights into movements and distribution of these large mobile animals where existing methodology is unviable.

Global connectivity

A vital component in understanding spatial ecology is an understanding of the connectivity of populations. Connectivity can influence fundamental processes such as evolution, population dynamics, and responses to climate change (Kool et al. 2013). For example, shortfin mako *Isurus oxyrinchus* show high genetic connectivity at large geographical ranges likely due to the long-distance movements of just a few reproductive migrants (Corrigan et al. 2018). For manta rays, the two recognised species have near global distributions in tropical and warm temperate oceanic waters. *Mobula alfredi*, is distributed throughout much of the tropical and sub-tropical Indo-Pacific region (Marshall *et al.* 2009, Couturier *et al.* 2012; Figure 1.1a); whereas *M. birostris,* has a highly patchy global distribution extending into temperate waters (Couturier et al. 2012, Burgess 2017; Figure 1.1b). For each species, there appears to be numerous sub-populations with no evidence of connectivity between populations (Stewart et al. 2018a). Photo identification has revealed connectivity between aggregations sites for *M. alfredi* within the Andaman Sea (Marshall and Holmberg 2021), along the east Australian coastline (Couturier et al. 2011), and within the Indonesian archipelago (Germanov and Marshall 2014); however this connectivity is at a local-scale, rather than representing broad-scale movements. Genetic evidence has clearly discriminated between the two described species of manta rays (Marshall et al. 2009, Kashiwagi et al. 2012), shown connectivity of rays across international borders in southern Africa (Venables et al. 2020a), and has also confirmed isolation across larger spatial scales (Stewart et al. 2016a, Venables et al. 2020a). Currently no large-scale analysis of the connectivity of global populations has been undertaken, likely due to the difficulty in accessing samples across their global distributions. However, recent advances in the application of artificial intelligence to photo identification databases could provide a novel method for addressing questions of connectivity and isolation.

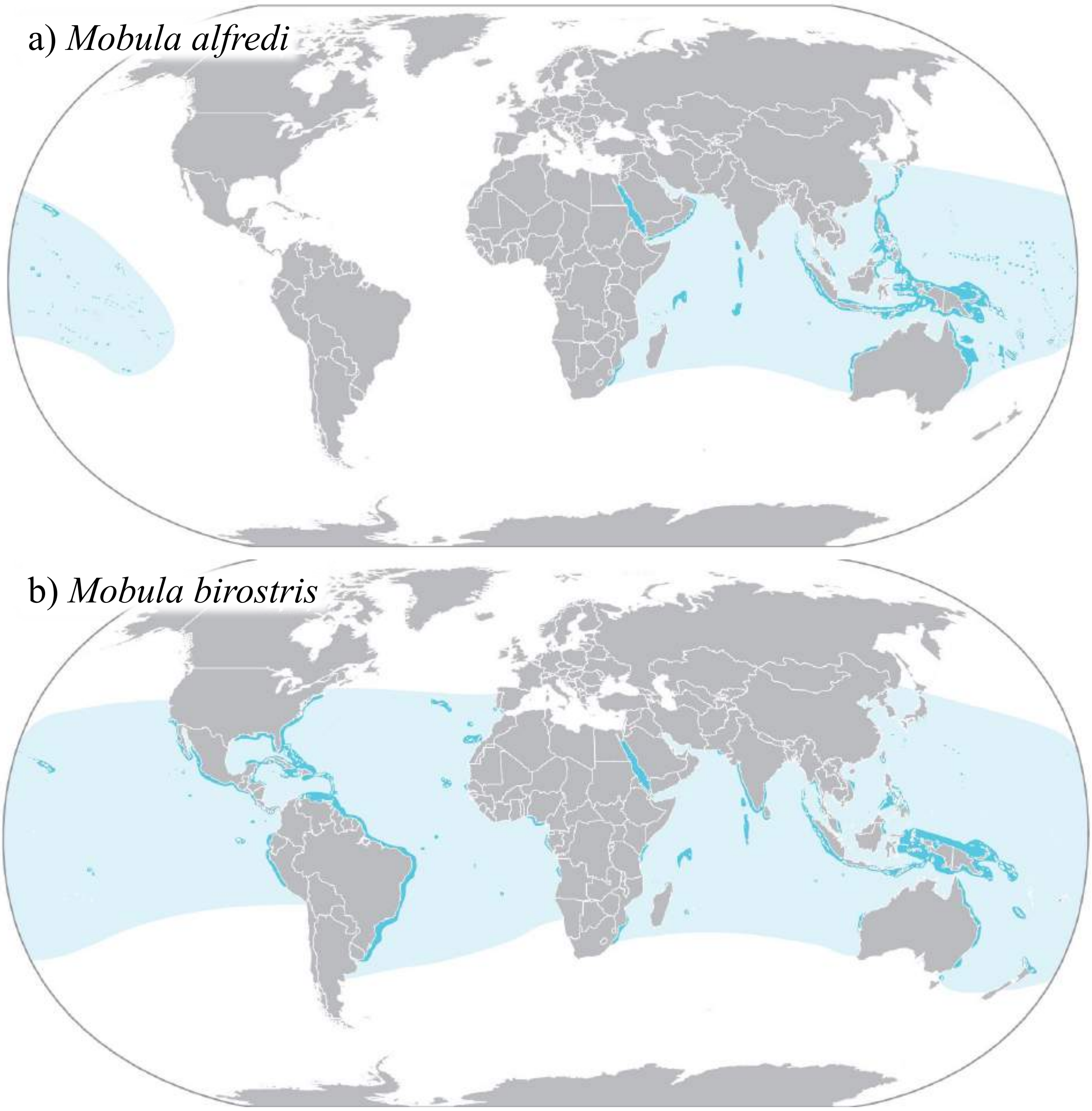

Figure 1.1. Global distribution of manta ray species: a) *Mobula alfredi*; and b) *M. birostris.* Darker areas indicate confirmed range, lighter areas indicate expected range (maps sourced from Stevens et al. 2018).

Significance and Aims

Manta rays are of economic importance to both ecotourism and fisheries industries. Both species form the basis of a multi-million dollar ecotourism industry worldwide (O'Malley et al. 2013), due largely to their interactive nature and predictable aggregations which allow tourism operators to offer reliable wild encounters. Manta rays are also targeted by commercial and artisanal fisheries for their highly valued gill rakers for use in traditional Chinese medicine (O'Malley et al. 2017). These fisheries have resulted in reef manta rays and oceanic manta rays being listed respectively as Vulnerable to Extinction and Endangered on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Marshall et al. 2019, Marshall et al. 2020). Owing to their conservative life history (Dulvy et al. 2013), these species cannot sustain heavy exploitation (Croll et al. 2016), and this targeted trade is believed responsible for driving population declines upwards of 80% in some locations (Rohner et al. 2013a). Thus, it is critical to clarify key aspects of these species' spatial ecology, including their space use, habitat preferences, foraging requirements and connectivity, in order to aid future conservation efforts and to bring clarity to their distributions and population structures, which is needed to inform and improve management strategies.

The overall goal of this thesis is to address outstanding questions pertaining to the spatial ecology of manta rays. Despite a concerted effort to investigate the ecology of these rays over the past two decades, there remain questions about their habitat use, distributions, long distance movements, and connectivity (Stewart et al. 2018a). This thesis will address some of these gaps in knowledge by working across various spatial scales, from fine-scale to global analyses, and employing a range of research approaches to overcome methodological challenges that have resulted in these gaps in understanding (Figure 1.2). The four main aims of the thesis are outlined briefly below:

Chapter 2

The objective of this chapter is to examine the role of cleaning stations in the site selection and habitat use of manta rays in reef environments. This will be achieved using a combination of fine-scale animal tracking and in-water observations of manta ray behaviour, cleaner fish distribution and habitat mapping.

Chapter 3

The objective of this chapter is to investigate the food environment for *M. alfredi* at their largest known aggregation site of Hanifaru Bay in the Maldives. This will be achieved by *in situ* sampling of the zooplankton community, alongside observations of manta ray abundance and behaviour.

Chapter 4

The objective of this chapter is to explore the long-distance movements of manta rays and update their distribution in Australian waters. This will be achieved using non-traditional research methods of citizen science submissions and social media posts to improve otherwise sparse records.

Chapter 5

The objective of this chapter is to explore whether unique spot patterns of individual manta rays are likely to be a heritable trait, and one that can be used to examine global connectivity of populations. This will be achieved by collating photographic identification databases from across the global range for each species, and analysing the pattern expression of the animals using artificial intelligence.

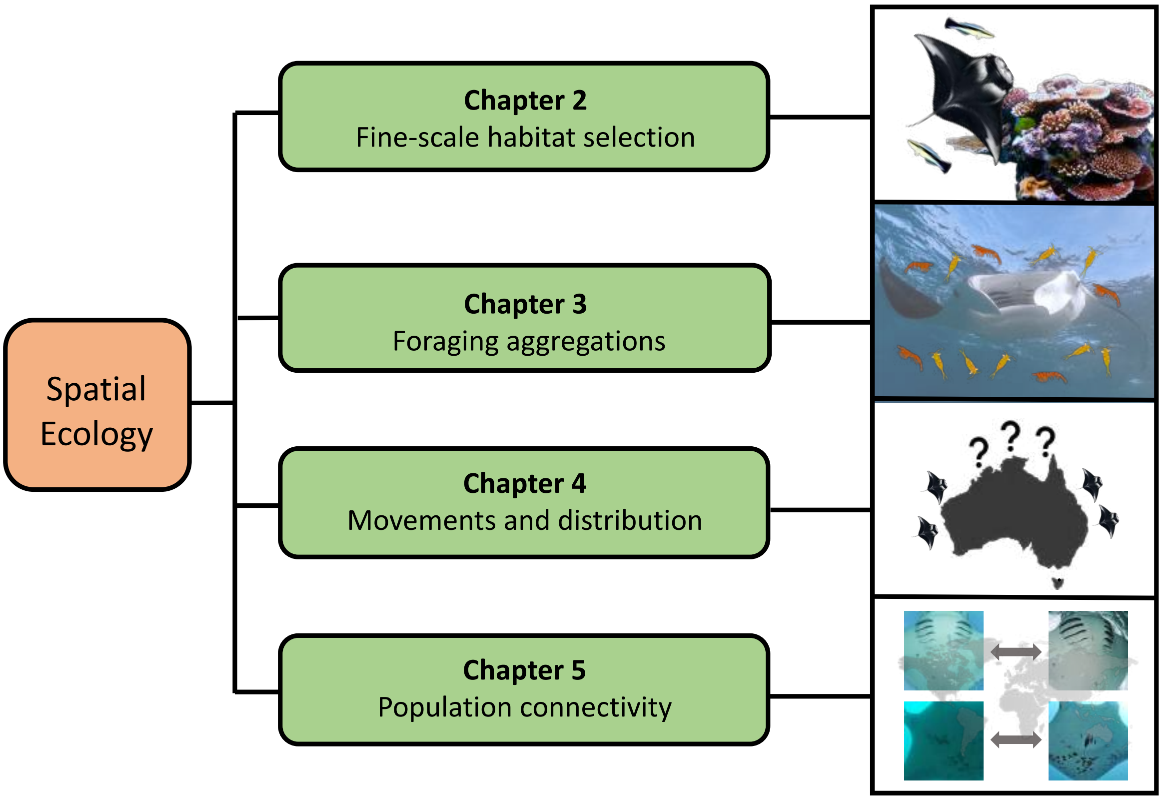

Figure 1.2. Investigating the spatial ecology of manta rays. Schematic of the structure of the thesis. The focus of each data chapter is to address an outstanding question concerning the spatial ecology of manta rays: Chapter 2. Habitat use at cleaning stations; Chapter 3. The food environment at feeding aggregation sites; Chapter 4. Movements and distribution; and Chapter 5. Connectivity of global populations inferred from pattern expression analysis.

Chapter 2

Mutualism promotes site selection in a large marine planktivore

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Abstract

1. Mutualism is a form of symbiosis whereby both parties benefit from the relationship. An example is cleaning symbiosis, which has been observed in terrestrial and marine environments. The most recognised form of marine cleaning symbiosis is that of cleaner fishes and their clients.

2. Cleaner species set up cleaning stations on the reef, and other species seek out their services. However, it is not well understood how the presence of cleaning stations influence movements of large highly mobile species. We examined the role of cleaning stations as a driver of movement and habitat use in a mobile client species.

3. Here we used a combination of passive acoustic telemetry and in-water surveys to investigate cleaning station attendance by the reef manta ray *Mobula alfredi*. We employed a novel approach in the form of a fine-scale acoustic receiver array set up around a known cleaning area, and tagged 42 rays. Within the array, we mapped structural features, surveyed the distribution of cleaner wrasse, and observed the habitat use of the rays.

4. We found manta ray space use was significantly associated with blue-streak cleaner wrasse *Labroides dimidiatus* distribution and hard coral substrate. Cleaning interactions dominated their habitat use at this site, taking precedence over other life history traits such as feeding and courtship.

5. This study has demonstrated that cleaning symbiosis is a driver for highly mobile, and otherwise pelagic, species to visit inshore reef environments. We suggest that targeted and long-term use of specific cleaning stations reflects manta rays having a long-term memory and cognitive map of some shallow reef environments where quality cleaning is provided. We hypothesise that animals prefer cleaning sites in proximity to productive foraging regions.

Keywords

Elasmobranch, megafauna, movement ecology, acoustic tracking, animal navigation, coral reef, location accuracy, VEMCO Positioning System

Introduction

Mutualism is the exchange of goods and services between organisms that provides a net benefit to those involved. A classic example is pollination, in which an animal vector receives food in the form of pollen or nectar, in exchange for fertilising a plant’s ovules (Cushman and Beattie 1991). Another is cleaning symbiosis, whereby a client species has ectoparasites removed by a host cleaner species (Limbaugh 1961). On land, this symbiosis is commonly observed in bird species such as oxpeckers *Buphagus* spp. removing ticks and blood-sucking flies from ungulates (Sazima 2011). Cleaning symbiosis is also found, though less commonly, in other taxa including small mammals: an example is the banded mongoose *Mungos mungo* removing ticks from common warthogs

Phacochoerus africanus (Sazima 2010). In the ocean, over 100 marine fishes and numerous invertebrate species act as cleaners to a wide range of taxa including cephalopod molluscs, fishes, mammals and reptiles (Côté 2000). Host cleaner species in marine systems are generally more site attached than their terrestrial counterparts, setting up ‘cleaning stations’ that clients seek out and visit. However, to date there is little understanding of how this mutualism promotes site selection in large-bodied, vagile marine species.

Many species of marine megafauna have extensive home ranges, moving 100s of kilometres in search of food or undergoing reproductive migrations (e.g. leatherback turtles *Dermochelys coriacea*, sperm whales *Physeter macrocephalus*, and lemon sharks *Negaprion brevirostris*; (Christal and Whitehead 1997, Houghton et al. 2006, Chapman et al. 2009). Yet large migratory species also seek out the services of site-attached cleaners (e.g. oceanic sunfish *Mola mola*, pelagic thresher sharks *Alopias pelagicus*, whale sharks *Rhincodon typus*, and manta rays *Mobula birostris* and *M. alfredi*; (Konow et al. 2006, O’Shea et al. 2010, Oliver et al. 2011, Araujo et al. , Murie et al. 2020). Cleaning interactions typically involve the cleaner removing ectoparasites from visiting or resident clients, but cleaners may also feed on host mucus and skin, particularly at wound sites (Grutter 1999). The ecological importance of this symbiosis in terms of body maintenance, especially for mobile client species, has been demonstrated on tropical coral reefs via experimental exclusion of the blue-streak cleaner wrasse, *Labroides dimidiatus*. For client species, this has resulted in decreased diversity and abundance, higher rates of fungal infection, smaller body size, and poorer health (Bshary 2003, Grutter et al. 2003, Bshary et al. 2007, Waldie et al. 2011). Mobile client species may therefore benefit from their choice to visit reefs based on the presence of cleaner fish. In turn, reef-associated cleaners benefit from large, mobile species bringing a food source that originates from off the reef, suggesting that this symbiosis is indeed mutualistic.

The preference of mobile species for particular sites indicates they have a spatial memory of the sites and the service they have received. The species of cleaner fish at a site may influence the quality of the cleaning station, and large mobile marine species have multiple options when it comes to habitat choice. Mobile clients would likely opt for cleaning stations where they receive quality service (i.e. parasite removal with few or no adverse events, such as biting from the cleaners; (Bshary and Schäffer 2002), and would be less likely to return to a site where they were previously not attended to promptly (Bshary and Grutter 2002). Perhaps then it is not surprising that clients with multiple choices of cleaning stations are given better service than clients with reduced options, suggesting that cleaners can distinguish between resident and visiting clients (Adam 2010). A key determinant of a mobile client’s attendance at a particular cleaning station site may be the memory of quality cleaning service and the animal's spatial cognition. How animals move in relation to learning and memory remains a key question in megafauna studies (Hays et al. 2016a), and the role of spatial cognition in relation to fine-scale habitat use is not well understood.

Many large, mobile elasmobranch species have been observed attending cleaning stations, including spotted eagle rays *Aetobatus narinari*, pelagic thresher sharks, silky sharks *Carcharhinus falciformis,* Galapagos sharks *C. galapagensis,* bull sharks *C. leucas,* demersal lemon sharks *Negaprion acutidens*, whale sharks, and scalloped hammerheads *Sphyrna lewini* (Keyes 1982, Oliver et al. 2011, Quimbayo et al. 2016). Ectoparasite loads may drive these visits, as individuals with high parasite loads frequent cleaning stations more regularly than individuals with low parasite loads (Grutter 1999). Ectoparasite loads are lower after cleaning interactions (Keyes 1982), and cleaner fish spend longer foraging on body regions with more ectoparasites (Oliver et al. 2011). Larger elasmobranchs may also receive preferential treatment from the cleaner community (Keyes 1982, Oliver et al. 2011). Larger fish with more parasites are inspected more often and for longer than smaller fish with fewer parasites (Grutter 1995). Further, facultative cleaners favour interactions with planktivore clients over piscivores, which is likely to be related to a lower risk of being eaten by the client fish (Francini-Filho and Sazima 2008). However, drivers of site selection by large mobile clients at cleaning station habitats has not previously been investigated.

The reef manta ray is a large, mobile planktivorous species that demonstrates site affinity to reef environments, including cleaning stations (Fig 2.1). This affinity has been documented at aggregation sites in the Maldives (Stevens 2016), eastern Australia (Couturier et al. 2018), Indonesia (Germanov et al. 2019), the Seychelles (Peel et al. 2019b), and Mozambique (Venables et al. 2020c). These studies have documented that manta rays visit cleaning stations, but the methods used in most studies have limitations for determining behaviour and fine-scale spatial use. For example, while observations by SCUBA or free divers or via remote underwater cameras can provide fine-scale information, they are limited to short observational windows. By contrast, aquatic telemetry approaches such as acoustic or satellite tagging can facilitate continuous, long-term detections, but typically lack spatial resolution. However, combining these approaches, and enhancing the fine-scale tracking of animal movements, could elucidate the site preferences and drivers of mobile client's visitation to particular sites.

Figure 2.1 Reef manta ray *Mobula alfredi* attending a cleaning station at Lady Elliot Island, Australia.

Here we investigated the role of mutualism in determining site selection in vagile marine megafauna. Our aim was to determine the role of cleaning stations as a driver of movement and habitat use in a mobile client species. Through a combination of in-water observations and a novel application of fine-scale passive acoustic tracking, we show how high-accuracy tracking can elucidate the memory of particular shallow reef habitats and space use in a largely pelagic species, and provide a mechanism for nutrient exchange between reef and open ocean environments.

Materials and methods

Study site

Mobula alfredi is found in tropical and subtropical waters of the Indo-Pacific and Indian Ocean. In Australia, it is found in coastal waters north of ~30°S (Fig. 2.2a; (Armstrong et al. 2020a), with the largest known aggregation on the east coast around Lady Elliot Island (LEI, Fig. 2.2b; (Couturier et al. 2014b). The peak of the *M. alfredi* aggregation at LEI is in winter, and during summer many individuals migrate south, with North Stradbroke Island (Fig. 2.2a) a seasonal aggregation site (Couturier et al. 2011). At LEI, previous research identified a high-use area on the western side of the island where *M. alfredi* individuals cruise, feed, court, and are cleaned (Jaine et al. 2012, Couturier et al. 2018). This area has a series of coral reef features 8–15 m deep (Fig. 2.2c).

Figure 2.2. Key *Mobula alfredi* sites in eastern Australia. a) Two aggregation sites of *M. alfredi* in southern Queensland, LEI = Lady Elliot Island and NSI = North Stradbroke Island; b) Aerial view of LEI, white boxed area highlighting the study region; and c) Zoomed in view of the study region on the western side of LEI. Black circles indicate positions of the acoustic receivers, and black stars indicate the location of temperature loggers and sentinel transmitters (Image credit: Jeremy Somerville).

Acoustic tracking

To monitor the presence and location of acoustically-tagged *M. alfredi* on the western side of LEI, an array of eight acoustic receivers (VR2W; Vemco, Nova Scotia) were deployed as a VEMCO Positioning System (VPS) between February 2017 and October 2018 (Fig. 2.2c). Receivers were ~76 m apart in a grid formation placed at GPS-verified locations. Each receiver was paired with an acoustic transmitter or sync tag, attached 1 m above the VR2W, which emitted a unique coded pulse-train every 500-700 s. These transmissions allowed for the relative position of each receiver within the array to be determined throughout the study, to provide an estimate of the location error associated with the calculated positions of tagged animals. To assess the magnitude of the positioning error, two sentinel transmitters and temperature loggers were placed at GPS-verified locations within the array for the duration of the study. The VPS facilitates precise locational tracking of tagged animals in and around the acoustic array (Espinoza et al. 2011, Roy et al. 2014), and receivers in the LEI array were set up conservatively (close together) to ensure stable performance over a 24-hr cycle.

A total of 42 individual *M. alfredi* (27 female, 15 male) were tagged with V16 acoustic transmitters (Vemco, Nova Scotia) with a random delay of 60-90 s (see appendix Table A1). Each transmitter was attached to a Domeier umbrella-dart tag head with a 10 cm shrink-wrapped braided wire tether, and was inserted into the dorsal musculature of a pectoral fin of a free-swimming ray using a modified Hawaiian sling spear. Antifoul was not applied due to the relatively short retention times of external Domeier umbrella-dart head tags on manta rays (median detection time of 121 days; Couturier et al 2018). Prior to tagging, individual *M. alfredi* were photographed for subsequent identification by comparing the image with those in an existing photo-ID database (Armstrong et al. 2019). Sex was assessed based on the presence (male) or absence (female) of claspers (Marshall et al. 2011b). Maturity status was determined by the clasper size (in males), and pregnancy and/or presence of mating scars (in females; (Marshall and Bennett 2010). Animal size was estimated visually to the nearest 0.5 m using stationary objects for scale, and all females were assigned as adult if their disc width was ≥ 3.5 m (Couturier et al. 2014b). Tagging commenced in February 2017 at North Stradbroke Island (n = 10), and the remaining tags were deployed at LEI between February 2017 and June 2018 (n = 32).

Processing acoustic data

The position of a tagged *M. alfredi* within the array was calculated from the time-difference-of-arrival of its transmitter pulse-train at each receiver (minimum three receivers required to calculate a position estimate). The process relies on knowing the precise relative positions of the receivers and their sync tags. For all position estimates (animal transmitters and sync tags), a relative Horizontal Positioning Error was calculated based on ranges of water temperature, depth, and salinity of the particular VPS, as well as the geometry of the transmitter and detecting receivers for specific transmissions (VEMCO 2018). For each sync tag position estimate, a measured Horizontal Positioning Error value (in metres) is also calculated, as their true location is known. Using the relationship between the Horizontal Positioning Error values and the measured Horizontal Positioning Error values from the sync tags we extrapolated measured Horizontal Positioning Error values for animal transmitters (Coates et al. 2013). We could then provide an estimate (in metres) of the error associated with each animal's transmitter position. To improve precision of position estimates and provide confidence in our conclusions regarding manta ray space use at the site, we removed 10% of the positions with the greatest measured Horizontal Positioning Error (Roy et al. 2014). Sentinel transmitter positions were used as a control to reduce the potential for making erroneous inferences regarding animal behaviour (Payne et al. 2010) and to rule out the possibility that animal position estimates were heavily influenced by background reef noise.

In-water observations

Stationary point surveys of *M. alfredi* cleaning interactions with host cleaner fish were made between June 2017 and June 2019 (n = 67). Surveys were conducted for 10 minutes by two SCUBA divers at four locations within the acoustic array cleaning area, during which the abundance and diversity of cleaner fish species that interacted with *M. alfredi* was recorded. Each dive (~60 minutes) could have up to four surveys, allowing for transit time between locations. There were regularly two dives in one day (one in the morning, one in the afternoon). The maximum number of cleaner fish counted per survey (MaxN) was used in subsequent analyses, as this alleviates concerns that an individual may be counted more than once if it leaves the observers field of view (Bosch et al. 2017). Locations were chosen based on previous observations of manta rays cleaning at the site. A manta ray was deemed to be getting cleaned when two criteria were met: (1) it approached the cleaning station (to within ~2 m) and maintained position or started circling the station for more than one minute; and (2) it had cleaner fish attending it. If *M. alfredi* was encountered within the array, but not at a cleaning station, an image was taken for photo-ID and behaviour recorded as feeding, cruising (as defined by Jaine et al. 2012), or courtship (as defined by Stevens 2016). Photo-ID was used to ensure individuals were only documented once during each SCUBA dive (~60 mins) and that their dominant behaviour was recorded.

To investigate associations among substrate type, cleaner fish and *M. alfredi*, we mapped the habitat and the obligate cleaner *L. dimidiatus* within the boundary of the acoustic array (Fig. 2.3). For the habitat mapping, two divers on SCUBA conducted gridded surveys using transect tapes and photographs to create a map of reef features (see appendix Fig. A1). Transect tapes were laid out to form 10 m by 10 m grid squares using a compass to maintain direction, and all features >2 m in diameter were measured and plotted on the gridded map. Substrate types were categorised as sand (bare sand and <2 m diameter sparsely-distributed corals), hard coral, soft coral, or dense coral ridge. To map the distribution of cleaner fish within the acoustic array we conducted three surveys on SCUBA using the aforementioned habitat map to provide accurate positions for spatial analysis. Counts *of L. dimidiatus* were obtained and cross checked by two divers on SCUBA. Surveys were conducted in June to coincide with the observed peak of *M. alfredi* visits to the study site (Couturier et al 2011). The focus of the distribution surveys was the obligate cleaner fish, *L. dimidiatus*, as this species is found across all habitat zones (Green 1996). *Labroides dimidiatus* establishes cleaning stations at fixed locations (Potts 1973), whereas the other cleaner species observed in the current study are facultative cleaners, and less site attached.

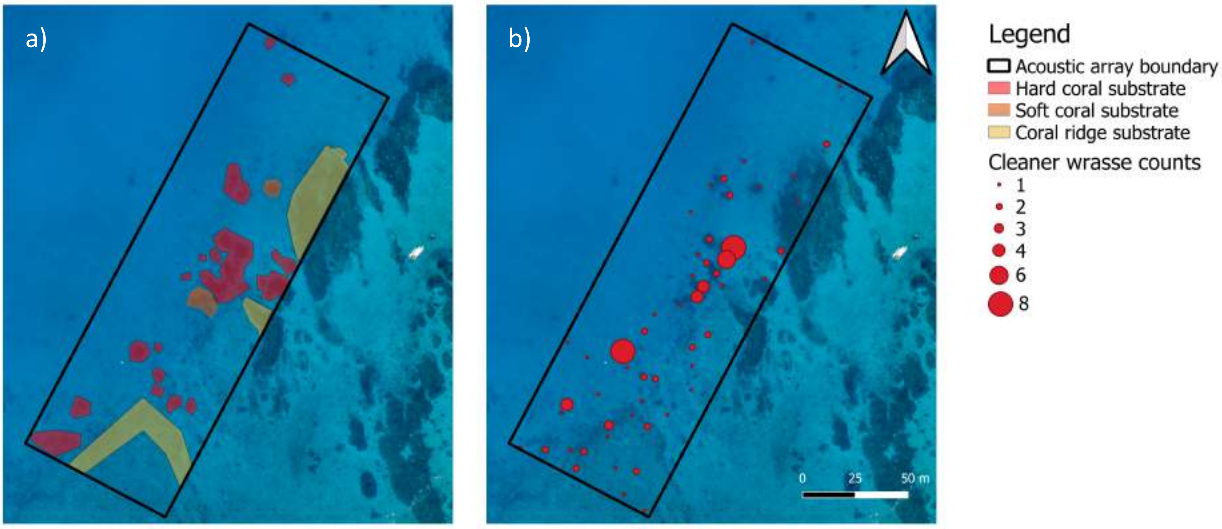

Figure 2.3. Distribution of structural features and cleaner wrasse within an acoustic array off Lady Elliot Island. a) Coloured polygons represent different substrate types, with the remaining regions being sand or structural features <2 m in diameter; and b) Distribution and abundance of blue-streak cleaner wrasse, *Labroides dimidiatus* (Image credit: Jeremy Somerville).

Statistical analyses

To quantify the fine-scale habitat use of *M. alfredi*, we excluded position estimates calculated outside the acoustic array boundary and focused on the location of tagged animals within the array. The region within the array boundary is where positional accuracy of tagged *M. alfredi* was highest (see appendix Fig. A2), and where we mapped structural features and *L. dimidiatus* locations. Positions of individual *M. alfredi*, *L. dimidiatus*, and substrate polygons were transformed into spatial data using a 5 x 5 m grid in the R packages “raster” (Hijmans 2020) and “sf” (Pebesma 2018). To explore associations between locations of *M. alfredi* and substrate types, and between locations of *M. alfredi* and *L. dimidiatus,* we used a modified t-test in the R package “SpatialPack” (Osorio and Vallejos 2019).

Cleaning area visitation events for tagged *M. alfredi* were calculated using the R package GLATOS (Holbrook et al. 2017). A visitation event was based on a minimum of two position estimates in and around the acoustic array to allow for animals in transit. Visitation events were deemed separate if the time between position estimates exceeded 300 s.

To investigate potential drivers of *M. alfredi* visits around the VPS array, we constructed models in the R package “lme4”, (Bates et al. 2014). To test for significant predictors we used the R package “lmerTest” (Kuznetsova et al. 2017). We conducted analyses using two different response variables: (i) Count (the number of *M. alfredi* position estimates per hour); and (ii) Duration (the length of each *M. alfredi* visitation event in s). For the Count model we used a generalised linear model (GLM) with a negative binomial error structure (as a Poisson error structure was overdispersed), and for the Duration models we used a generalised linear mixed effect model (GLMM) incorporating individual tagged *M. alfredi* as a random effect with a Gamma error structure (as the variance generally increased with the square of the mean and there were zeros present). Both models had a log-link function and were constructed using the glm.nb and glmer function for the Count and Duration models respectively in the R package “lme4” (Bates et al. 2014). During model development, we visually inspected diagnostic plots to assess assumptions of homogeneity of variance and normality.

Predictors in the Count model were Time of Day (continuous), Tide (continuous), Wind Direction (continuous), and Wind Speed (continuous). Fixed effect predictors in the Duration model were Time of Day (continuous), Sex of the Ray (factor), Maturity (factor), Size Estimate (continuous), Tide (continuous), Wind Direction (continuous), and Wind Speed (continuous). Individual ray attributes were not appropriate for the Count model as this dataset was summarised by hour and there were zeros present. Environmental information was obtained from the Australian Bureau of Meteorology. Tide was calculated as the hours from low tide. To account for the circular nature of Tide (~12-hr cycle), Wind Direction (360° cycle) and Time of Day (24-hr cycle), variables were transformed using a truncated Fourier series (a harmonic function of sine and cosines). This ensures that the cyclical nature of these predictors is captured, while guaranteeing that the response values predicted at the extremes of the predictor range are the same (e.g. the same prediction for Count at times of 0 and 24 hours). Wind Speed was smoothed using a natural spline in the R package “splines” (R Core Team 2019). Explained deviance was calculated in R using delta values in the package “MuMIn” (Barton 2009). Final models were selected based on AIC values and significance of variables was taken at $p < 0.05$. To visualise the generalised linear mixed effects models, we present contrast plots using the response scale in the R package “visreg” (Breheny and Burchett 2017). Model output with confidence limits on the response scale is provided in the Results, and output on the log-link scale with residuals is available in the supporting information (see appendix Fig. A3).

Results

Acoustic tracking data

Between February 2017 and September 2018, 34 of the 42 tagged *M. alfredi* were detected multiple times by the VPS on the western side of LEI, with 114,575 detections in total. From these detections, 13,507 unique *M. alfredi* position estimates were calculated. It is the position estimates that subsequent analyses are based on. We removed 10% of the positions with the greatest measured Horizontal Positioning Error (> 10.82 m), leaving 12,157 positions for subsequent analysis (Females n = 7465, Males n = 4692). Error estimates for the remaining animal positions were relatively small (<1.1 m for 50% of animal position estimates and <2.9 m for 75% of animal position estimates; Appendix Fig. 2). There was a mean of 357.5 positions per tagged *M. alfredi* (SD = 344.8, Range = 29-1603) and tagged animals were detected over a mean duration of 92.3 days (SD = 68.4 days, Range = 5-241 days). The greatest number of consecutive days an individual was detected by the acoustic array was 11 days (see appendix Fig. A4).

There were 741 *M. alfredi* visitation events during the study, with a mean duration of 19.9 minutes (SD = 24.1 mins, Range = 1.5-230.0 mins). The mean duration for female visitation events was 21.0 mins (n = 432), and 18.3 mins for male visitation events (n = 309). For all animals the median visitation duration was 12.8 mins, as the data were heavily right-skewed. There was variation in the space use within the array during visitation events, both for the same individual and among individuals, here illustrated by eight visitation events (Fig. 2.4). Here we observe some individuals moved less decisively between different reef features in the array (e.g. Tag #32438; Fig. 2.4), whereas other individuals moved more directly, with their time in the array focused at particular sites (e.g. Tag #32455; Fig. 2.4).

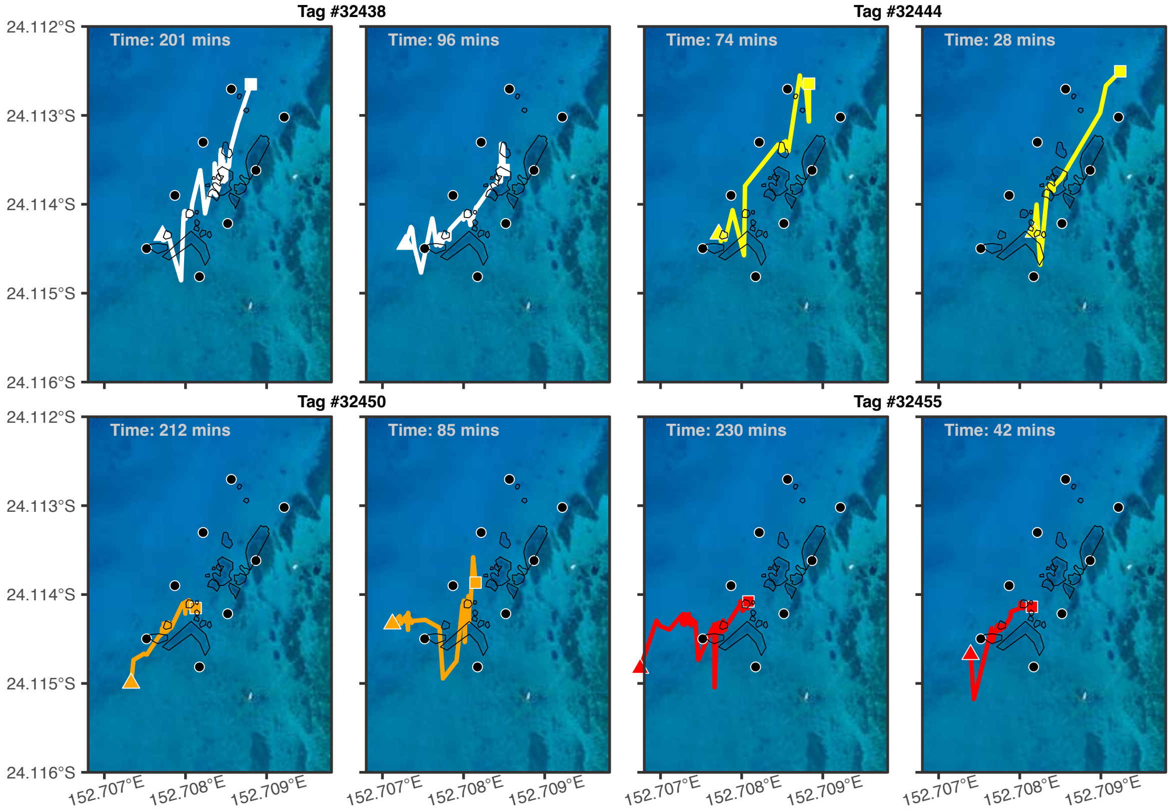

Figure 2.4. Eight examples of fine-scale tracking of *Mobula alfredi* in the acoustic array off Lady Elliot Island. Each colour represents an individual manta ray, and each plot a separate visitation event. The coloured triangle denotes the start of the track and the square is where the track ends. Black circles represent the locations of the acoustic receivers, and the black outlines are the mapped reef structures.

A total of 6,973 positions fell within the boundary of the VPS array (57.4%). The relative density of these tagged *M. alfredi* positions was highest around structural features (Fig. 2.5a), and where *L. dimidiatus* numbers were highest (Fig. 2.5b). *Mobula alfredi* position was significantly associated with structural features within the array (modified t-test, F = 6.03, df = 1, 247, p = 0.015, r = 0.15, Fig. 2.5a). Post-hoc analysis of substrate types found *M. alfredi* position was significantly associated with hard coral structure >2 m in diameter (modified t-test, F = 27.47, df = 1, 263, p < 0.001, r = 0.31), but not with soft coral structure or the coral ridge structures. In terms of the association with cleaner fish, *M. alfredi* position was significantly related to the position of *L. dimidiatus* (modified t-test, F = 49.15, df = 1, 510, p < 0.001, r = 0.30, Figure 2.5b). Overall, *L. dimidiatus* position was significantly associated with hard coral features >2 m (modified t-test, F = 48.97, df = 1, 488, p < 0.001, r = 0.30), but not with soft coral features or coral ridge features. There was some variation in the space use between male and female *M. alfredi* (see appendix Fig. A5), however there was no difference in their association with structural features or *L. dimidiatus*.

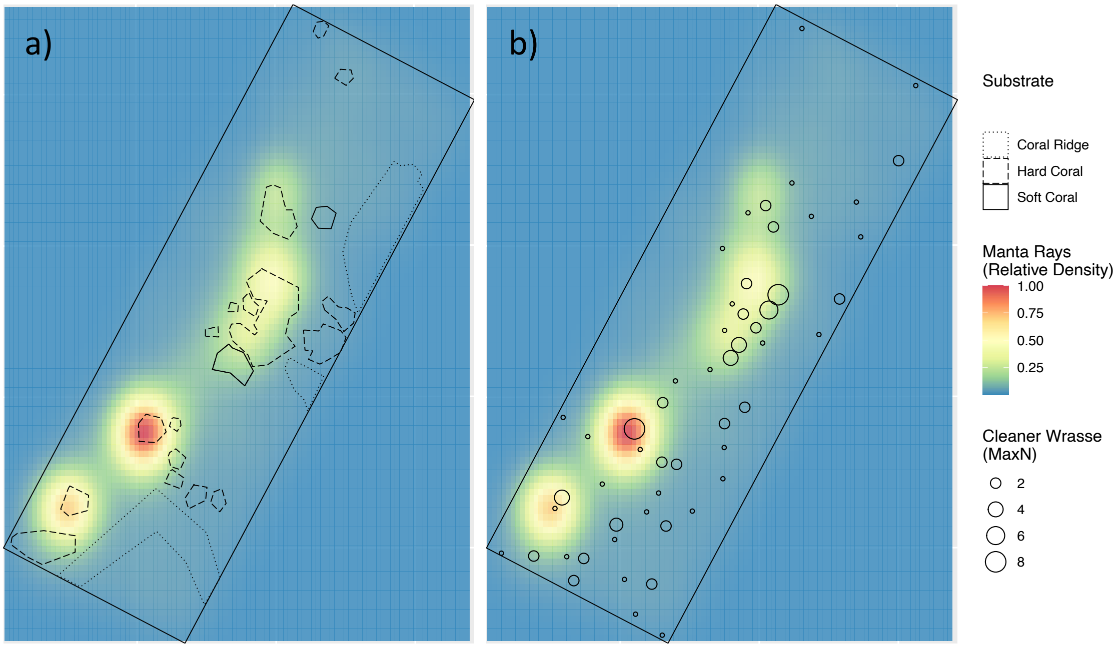

Figure 2.5. Habitat use of tagged *Mobula alfredi* in the Lady Elliot Island acoustic array between February 2017 and September 2018. a) Relative density of manta ray positions overlaid with structural features of the substrate; and b) Relative density of manta ray positions overlaid with density of cleaner wrasse denoted by size of black circles. The black rectangle in the figures represents the bounds of the acoustic array.

In-water observations

Surveys (n = 67) confirmed that the cleaner fish community for *M. alfredi* at the four identified cleaning stations was dominated by the obligate and site-attached cleaner *L. dimidiatus* (mean = 3.6, SD± = 3.1), and the facultative, less site-attached cleaner, the moon wrasse *Thalassoma lunare* (mean = 11.9, SD± = 7.2), with occasional inspections by the bicolor wrasse *L. bicolor* (mean = 0.2, SD± = 0.6), brown butterflyfish *Chaetodon kleinii* (mean = 0.4, SD± = 1.4), and birdnose wrasse *Gomphosus varius* (mean = 0.1, SD± = 0.3). The four identified cleaning stations comprised hard coral substrate and were the larger, more prominent reef features (5+ m wide and 2+ m high) in the array. There were 99 *M. alfredi* encounters during in-water photo-ID surveys, comprising 79 identified individuals. The majority were cleaning (81%, n = 80), with relatively few feeding (6%, n = 6), cruising (3%, n = 3) or courting (10%, n = 10).

Drivers of Mobula alfredi visitation

The Count of *M. alfredi* positions from the VPS array was significantly related to Time of Day ($p < 0.001$), Wind Direction ($p = 0.01$), and Tide ($p < 0.001$; Fig. 2.6a), while Wind Speed was not significant ($R^2 = 0.26$; Table 2.1). The Count of *M. alfredi* positions was highest during daylight hours, when winds were northerly, and around high and low tides.

Table 2.1. Model selection table for Count of *Mobula alfredi* positions (C1-C4) and Duration of *M. alfredi* visitation events (D1-D6). The df is the degrees of freedom of the Fixed effects in the model. Deviance (%) is the Total Explained Deviance from the model, with the bracketed value the Explained Deviance from the Fixed effects in the model. AIC = Akaike information criterion. Variables were removed in a stepwise approach based on AIC. The original model and the top 5 models based on AIC are displayed. The final model of each set is highlighted in bold.

Model	*Variables*	*df*	*R^2*	*Deviance (%)*	*AIC*
C1	TimeofDay(k=1)+WindDirection(k=1)+Tide(k=2)+WindSpeed(df=2)	10	0.26	-	13233
C2	WindDirection(k=1)+Tide(k=2)+WindSpeed(df=2)	8	0.26	-	13852
C3	TimeofDay(k=1)+WindDirection(k=1)+WindSpeed(df=2)	6	0.25	-	13249
C4	**TimeofDay(k=1)+WindDirection(k=1)+Tide(k=2)**	**8**	**0.26**	**-**	**13233**
D1	Sex+Maturity+SizeEstimate+TimeofDay(k=2)+WindDirection(k=2)+Tide(k=2)+WindSpeed(df=2)	17	-	15.6 (7.1)	5641.5
D2	Sex+Maturity+TimeofDay(k=2)+WindDirection(k=2)+Tide(k=2)+WindSpeed(df=2)	16	-	15.6 (7.1)	5639.5
D3	Sex+Maturity+TimeofDay(k=2)+WindDirection(k=2)+WindSpeed(df=2)	12	-	14.5 (6.2)	5639.8
D4	Sex+TimeofDay(k=2)+WindDirection(k=2)+WindSpeed(df=2)	11	-	14.4 (5.9)	5638.5
D5	Sex+TimeofDay(k=1)+WindDirection(k=2)+WindSpeed(df=2)	9	-	14.3 (5.8)	5636.2
D6	**TimeofDay(k=1)+WindDirection(k=2)+WindSpeed(df=2)**	**8**	**-**	**14.2 (5.1)**	**5635.4**

The Duration of *M. alfredi* visitation to the VPS array was significantly related to Time of Day ($p < 0.001$), Wind Direction ($p < 0.001$), and Wind Speed ($p = 0.01$; Fig. 2.6b). The Duration of *M. alfredi* visitation was not related to Tide, Size Estimate, Maturity, or Sex of the Ray (Table 2.1). Manta rays visited cleaning stations for longer periods during daylight hours, when winds were from a SSW direction and the wind speed was low (Total explained deviance = 14.2%, Fixed effects = 5.1%, and Random effects = 8.9%).

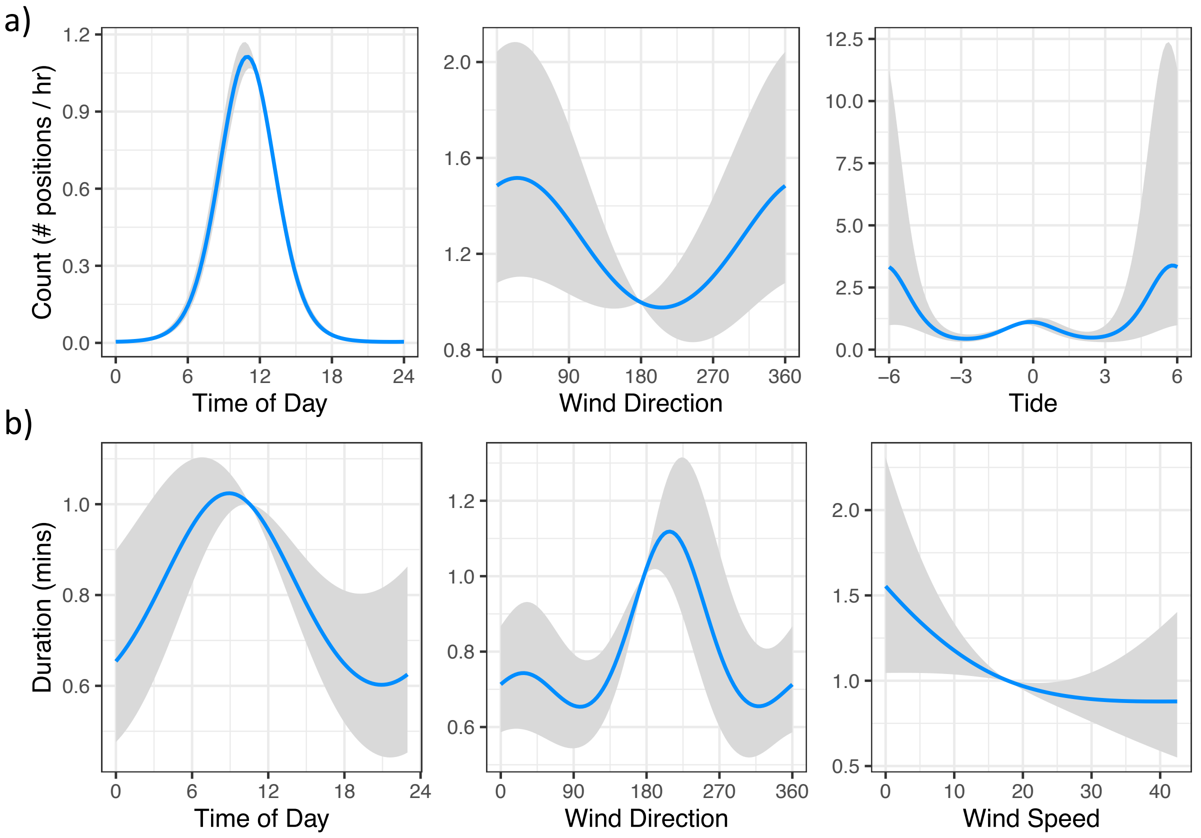

Figure 2.6. Models of tagged *Mobula alfredi* at Lady Elliot Island cleaning stations: a) Count per hour of positions by Time of Day (0 – 24 hrs), Wind Direction (South = 180°, North = 360°), and Tide (Hours from low tide at 0); and b) Duration in minutes of visitation by Wind Speed (km/hr), Wind Direction (South = 180°, North = 360°), and Tide (Hours from low tide at 0). Output on the y-axis is the response scale.

Analysis of sentinel transmitter positions revealed that the minimal performance of the array decreased slightly at night. The correction to the total hourly counts of animal positions using the standardised positioning frequency of the sentinel transmitters did not alter the overall pattern of attendance to the site from tagged manta rays (see appendix Fig. A6). We can thus be confident that the patterns observed are driven by the physics and biology, rather than an artefact of under-performance in the acoustic array due to reef noise.

Discussion

We found that mutualism – in the form of cleaning symbiosis – can promote site selection in a large-bodied, mobile marine species. Fine-scale site selection by a client species was associated with the distribution of the obligate cleaner *Labroides dimidiatus,* and the hard coral structures where this site-attached species has established cleaning stations. We found that interactions with cleaner species were the most commonly observed behaviour in the client, taking priority over other traits such as foraging and reproductive behaviour at these sites.

Generally movement studies focus on how a few key issues, including foraging, predator avoidance and breeding, influence animal movements (Hays et al. 2016a). Set against this backdrop, our results show how an important function of daily movements may also be body maintenance, with manta rays regularly visiting cleaning stations. This regular use of cleaning stations is common in many vagile marine species, that includes bony fishes, sea turtles and elasmobranchs (Konow et al. 2006, Oliver et al. 2011, Schofield et al. 2017, Araujo et al. 2020b, Murie et al. 2020). The relationship between pelagic species and their cleaners could be a common form of mutualism that controls the fine-scale habitat use of many species and deserves more consideration. Similar to cleaning excursions, other marine taxa may take a break from foraging for other forms of body maintenance. For example, some fish that forage below the thermocline come to the surface to rewarm (Pope et al. 2010, Evans et al. 2014), whereas pinnipeds haul-out ashore to rest (Andrews-Goff et al. 2010, Hamilton et al. 2018). While the amount of time spent in the vicinity of cleaning stations may be small in absolute terms (tens of minutes over a 24-hour period), it appears to be a key component of the daily time-budget for manta rays. Similarly, many terrestrial species spend time each day grooming to remove parasites (e.g chimpanzees (Lehmann and Boesch 2008, Foster et al. 2009), with an important distinction being that in these cases animals do not need to travel to specific locations to undertake such behaviour.

Quality cleaning habitat is an important driver of pelagic species visitation to inshore reefs. Here we established that the number of individual *L. dimidiatus* present at a particular cleaning station may influence manta ray site preference, demonstrating the disproportionate effect that a small and sparsely distributed species can have on coral reef communities (Waldie et al. 2011). *Labroides dimidiatus* is ubiquitous in tropical reef systems globally (Green 1996), however our findings suggest that its importance in attracting pelagic visitors to the reef may have been previously underrepresented. A remaining question in the movement ecology of marine megafauna is; can movement data provide information on the ecosystem role of marine megafauna (Hays et al. 2016a)? Information provided by movement studies on the spatiotemporal patterns of abundance and behaviours (e.g. foraging and cruising) of animals is key to understanding their ecological roles. For the reef environment, it may be that visits to cleaning stations from animals that spend considerable time in the open ocean (e.g. pelagic thresher sharks (Oliver et al. 2011) and manta rays (Murie et al. 2020)), provides a mechanism for nutrient exchange between these environments. This is similar to the broader-scale example of large whales (baleen and sperm whales) migrating from high latitudes translocating nutrients to oligotrophic tropical systems (Roman et al. 2014). Thus, understanding the movement ecology and site selection of threatened species is not only crucial for informing effective management strategies, but also for gaining insights into their role in ecosystem function.

Many individuals repeatedly visited the same localised sites across many weeks, and we propose that manta rays likely locate these cleaning stations using conspicuous landmarks that they remember. Relatively little is known about effects of learning and memory on the movement patterns of marine megafauna (Hays et al. 2016a). An unresolved question in marine megafauna movement ecology is how learning and memory or innate behaviours drive animal movements. It is likely that manta rays have a cognitive map of particular reef areas, akin to how animals with distinct home-ranges know their environment intimately (Harten et al. 2020). In common with other taxa such as sea turtles, that alternate between oceanic and coastal areas, manta rays likely use coarse-scale navigational cues in the open ocean, and precisely orientated movement in coastal areas (Hays et al. 2020). Manta rays visit the same reef systems across many years, as demonstrated by photo-ID records (Couturier et al. 2014b, Harris et al. 2020), and such repeated site use is similar to that seen in sea turtles and many bird species that maintain strong site fidelity to particular areas interspersed with long-distance migration (Alerstam et al. 2006, Armstrong et al. 2019, Shimada et al. 2020). These observations imply that many migratory taxa, including manta rays, have a long-term memory of particular focal sites.

We found that *M. alfredi* prefer hard coral structure, rather than soft coral cover or continuous coral ridge substrate, and that cleaner wrasse density was also more associated with this type of substrate. Cleaner species often use prominent coral heads or outcrops to set up their cleaning stations (Côté et al. 1998), and choosing a conspicuous location is likely to be beneficial to them for attracting clients. But for large, mobile species such as *M. alfredi*, these structures also provide suitable habitat to allow manoeuvrability and facilitate cleaning interactions. As for many other pelagic elasmobranch species, *M. alfredi* is a ram ventilator and has to swim continuously to irrigate its gills for uptake of oxygen. They are unable to rest on the bottom to facilitate cleaning interactions, as do some demersal elasmobranch species (Keyes 1982, Sazima and Moura 2000). Hard corals are particularly susceptible to the impacts of climate change, and the potential for coral reefs to recover from multiple stressors is declining (Hughes et al. 2018). Given the preference for hard coral structures, climate change could present a threat to the habitat of numerous cleaner species. Loss of habitat for cleaner species could have downstream consequences on the movements and site selection of large mobile clients like manta rays.

Wind conditions and tidal cycles are known to influence the movements of large animals, and this study also confirmed the importance of these environmental variables. There were contrasting patterns in how Wind Direction influenced reef manta ray Counts and the Duration of their visits, and this may be explained by the location of the study site on the western side of the island. Previous work has suggested manta rays favour this side of the island due to the shelter provided from prevailing winds (Couturier et al. 2018), and it may be that south to south-westerly winds are more favourable for longer cleaning station attendance, but that the protection afforded on this side of the island means manta rays may be detected during other wind regimes as well. We found that Wind Speed influenced the Duration of visits here, but did not impact their detection rate, supporting that manta rays are still present during less favourable conditions but for shorter periods of time. Foraging opportunities may present a reason for the Tidal influence of manta ray detections at the site. Prior research has shown zooplankton concentrations in the vicinity of the current study site are found to peak prior to low tide, and manta rays are more commonly observed feeding during this tidal phase (Armstrong et al. 2016). It may be that manta rays attend the cleaning station for longer periods either side of this foraging opportunity. Tide and current movements have been implicated for cleaning behaviour at other locations (O'Shea et al. 2010, Rohner et al. 2013a, Murie et al. 2020), as moderate currents are favourable to a manta ray's ability to hold station and facilitate cleaning. This suggests that the preference of *M. alfredi* for prominent hard coral structures in the current study may be related both to suitable habitat for the cleaner wrasse and the hydrodynamics of the location that facilitates cleaning interactions.

We found a clear diurnal signal for *M. alfredi* attendance within the study region. First arrival occurred in the morning, after sunrise, and individual visits gradually declined throughout the day. Early in the day was also when individuals were more likely to spend longer periods of time in the cleaning station area. Similar findings have been reported for manta rays in other regions (Venables et al. 2020c) and for other elasmobranchs (Oliver et al. 2011). Diurnal visitation likely reflects the behaviour of the cleaner fish, since *L. dimidiatus* individuals are inactive at night, and do not return to their cleaning station habitats until after dawn (Potts 1973). However, it is also likely a product of the behaviour of the manta rays themselves, as they move offshore to forage at night. Satellite tracking has revealed night-time diving behaviour in manta rays (Braun et al. 2014, Stewart et al. 2016b), and investigations using stable isotope analysis have suggested manta rays source their food from deep, benthic or epipelagic environments (Couturier et al. 2013, Burgess et al. 2016, Peel et al. 2019a). Planktivores from a range of taxa exhibit diurnal patterns in foraging behaviour (Hays 2003, Brierley 2014), to take advantage of diel vertical migrating zooplankton that come into shallower waters at night.

We showed the utility of automated high-accuracy acoustic tracking, which contrasts with historic active acoustic tracking that often has low accuracy, is limited in time, and is labour intensive (Nelson et al. 1997). Levels of location accuracy we achieved in a reef environment (within a few metres), is similar to that recorded by others using the VPS approach in freshwater (Espinoza et al. 2011, Roy et al. 2014). By comparison, modern satellite tracking approaches such as Fastloc-GPS, where locations are typically within a few 10s of metres of the true location (Dujon et al. 2014, Thomson et al. 2017), can provide continuous broad-scale tracking at the cost of such precision. These modern approaches are transforming our understanding of the patterns of small-scale space use for a range of marine species. For manta rays, a blended use of acoustic arrays and satellite tracking would provide a comprehensive understanding of space use over a range of spatial scales, and further clarify links between cleaning stations and adjacent feeding grounds (Jaine et al. 2014).

Combining tagging methods could also offer redundancy for tag failure or loss. Almost 20% of the tags that we deployed were never detected after their initial deployment, likely because of animal migration, tag shedding or tag failure. Understanding why transmitters stop relaying data is important to help drive improvements to tag design and deployment (Hays et al. 2007). Three of the eight tagged manta rays that were not detected by the VPS array were identified at the site by photo-ID within the study period, showing that for these individuals at least, the issue was tag shedding or failure (tag attachment was not confirmed via photo-ID). Nevertheless, for 34 individuals (81% of those tagged), tracking revealed repeated visits to cleaning areas. This large sample size, when compared to many tracking studies from a recent review (Sequeira et al. 2019), suggests that fidelity to cleaning areas is a general feature of manta ray ecology.

We hypothesise from the findings of the current study, together with other recent work (Stevens 2016, Murie et al. 2020), that preferred cleaning station sites are likely paired with rich feeding grounds nearby. The current study location is about seven kilometres from the shelf edge, and the mesoscale oceanographic feature of the Capricorn Eddy (Weeks et al. 2010). The productivity of the Capricorn Eddy is a result of increased frontal activity and upwelling, providing foraging opportunities to seabirds such as wedge-tailed shearwaters *Puffinus pacificus* (McDuie et al. 2018), and has been associated with foraging of manta rays (Jaine et al. 2014). Cleaning station environments, where manta rays sometimes stay in close proximity for long periods (i.e. weeks to months), would need to be in the vicinity of places that fulfill the multiple biological and ecological functions of these animals. Therefore, the feeding-cleaning hypothesis – where mobile species select cleaning sites close to productive foraging opportunities – may also explain the habitat preferences of other large, mobile client species.

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Authors' Contributions

AOA, AJA, CD, HP and KT conceived the ideas and designed methodology; AOA, AJA, CD and KT collected the data; AOA, JE, HP and AR analysed the data; AOA, MB, CD, GH and AR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of Interest

The authors declare no conflict of interest.

Data Accessability Statement

Detection logs from the acoustic array have been archived on the UQ eSpace repository, accessible via the following link: https://doi.org/10.14264/2e79b25

Chapter 3

Reef manta rays forage on tidally driven, high density zooplankton patches in Hanifaru Bay, Maldives

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Abstract

Manta rays forage for zooplankton in tropical and subtropical marine environments, which are generally nutrient-poor. Feeding often occurs at predictable locations where these large, mobile cartilaginous fishes congregate to exploit ephemeral productivity hotspots. Investigating the zooplankton dynamics that lead to such feeding aggregations remains a key question for understanding their movement ecology. The aim of this study is to investigate the feeding environment at the largest known aggregation for reef manta rays *Mobula alfredi* in the world. We sampled zooplankton throughout the tidal cycle, and recorded *M. alfredi* activity and behaviour, alongside environmental variables at Hanifaru Bay, Maldives. We constructed generalised linear models to investigate possible relationships between zooplankton dynamics, environmental parameters, and how they influenced *M. alfredi* abundance, behaviour, and foraging strategies. Zooplankton biomass changed rapidly throughout the tidal cycle, and *M. alfredi* feeding events were significantly related to high zooplankton biomass. *Mobula alfredi* switched from non-feeding to feeding behaviour at a prey density threshold of 53.7 mg m^{-3}; more than double the calculated density estimates needed to theoretically meet their metabolic requirements. The highest numbers of *M. alfredi* observed in Hanifaru Bay corresponded to when they were engaged in feeding behaviour. The community composition of zooplankton was different when *M. alfredi* were feeding (dominated by copepods and crustaceans) compared to when they were present but not feeding (more gelatinous species present than in feeding samples). The dominant zooplankton species recorded was *Undinula vulgaris*, a large-bodied calanoid copepod species which blooms in oceanic waters, suggesting offshore influences at the site. Here, we have characterised aspects of the feeding environment for *M. alfredi* in Hanifaru Bay and identified some of the conditions that may result in large aggregations of these threatened planktivores, and this information can help inform management of this economically important marine protected area.

Keywords

foraging ecology, foraging threshold, megafauna, zooplanktivore, mobulid ray, *Undinula*, ZooScan

Introduction

Manta rays are large planktivores that inhabit tropical and subtropical waters globally, which are generally oligotrophic (Marshall et al. 2009). Therefore, to meet their metabolic needs, manta rays need to locate pulses of zooplankton productivity. Similar to other tropical planktivores, such as leatherback turtles *Dermochelys coriacea* (Hays et al. 2006) and whale sharks *Rhincodon typus* (Rohner et al. 2015), manta rays aggregate where and when conditions result in elevated local productivity (Dewar et al. 2008, Anderson et al. 2011, Jaine et al. 2012). However, these productivity 'hotspots' are ephemeral in nature and often difficult to locate and characterise (Harris et al. 2020, Harris et al. 2021), which makes the direct study of planktivore feeding ecology challenging.

There are a variety of approaches used to study a species' diet, including stomach contents analysis, biochemical analyses, and direct observation. Two studies have recently explored the diet of manta ray species based on stomach contents: one on oceanic manta rays *Mobula birostris* taken in a fishery in the Philippines (Rohner et al. 2017), and one on a historic stomach sample from a reef manta ray *M. alfredi* collected from eastern Australia (Bennett et al. 2017). Traditionally, lethal approaches for dietary analysis, such as stomach contents analysis, are inappropriate for vulnerable marine fishes (Cortés 1997), and only offer a 'snapshot' of a species' diet (Rohner et al. 2013b). Instead, biochemical approaches, including stable isotope and fatty acid analysis, are non-lethal methods that provide an integrated signal that represents the long-term diet and trophic position of species. Biochemical analysis has inferred that *M. birostris* off Ecuador derive much of their calorific intake by feeding at depth (Burgess et al. 2016), as do *M. alfredi* off eastern Australia (Couturier et al. 2013), and *M. alfredi* in the Seychelles target pelagic zooplankton sources (Peel et al. 2019a). Nevertheless, biochemical analyses lack resolution, such as identifying and quantifying preferred prey species, whereas direct observation of animal feeding allows simultaneous sampling of the feeding environment.

Currently, the only detailed direct observation of the diet in manta rays is from an aggregation site off eastern Australia, where *M. alfredi* was observed feeding near the surface (Armstrong et al. 2016). The study found *M. alfredi* feeding events were significantly associated with greater zooplankton biomass, but were not influenced by zooplankton size or species composition. Further, feeding activity and zooplankton density was tidally driven at this site. Similarly, the occurrence of *M. alfredi* in Komodo National Park in Indonesia was heavily influenced by tide, and was considered likely to be related to feeding activity (Dewar et al. 2008). An *in situ* prey density threshold of 11.2 mg m^{-3} was determined for *M. alfredi* foraging in eastern Australia (Armstrong et al. 2016). However, a theoretical estimate of the density threshold to meet their metabolic requirements (25.2 mg m^{-3}) suggests they require additional energy from alternate food sources, such as foraging at depth (Armstrong et al. 2016).

Manta rays exhibit behavioural plasticity in relation to their feeding environment. In eastern Australia (Jaine et al. 2012), Indonesia (Dewar et al. 2008), and the Chagos Archipelago (Harris et al. 2021), surface feeding by manta rays is frequently observed during daylight hours, and other large planktivores, such as basking sharks *Cetorhinus maximus* (Sims and Merrett 1997) and *R. typus* (Prebble et al. 2016) also employ this strategy. In the Red Sea, *M. alfredi* swim in various circular patterns when feeding on zooplankton in shallow water (Gadig and Neto 2014). At Ningaloo Reef in Western Australia, *M. alfredi* is frequently observed to use a combination of feeding modes, including surface feeding, somersaulting, and bottom feeding (AOA *pers. obs.*). A unique strategy of “cyclone” feeding has been described at Hanifaru Bay in the Maldives, where multiple individuals manipulate the water column to create a vortex that concentrates zooplankton on which they then feed (Stevens 2016). Eight different feeding strategies have been described at this site, and have been related to prey density using a subjective visual assessment of the water column (Stevens et al. 2018). However, zooplankton density or composition has yet to be quantified in relation to these strategies. Upwards of 250 individual manta rays aggregate in Hanifaru Bay during peak feeding events, making it the largest known *M. alfredi* aggregation site in the world (Harris et al. 2020). This makes Hanifaru Bay a unique location to test hypotheses regarding habitat use, aggregative behaviour, feeding strategies, and zooplankton dynamics for this species.

Here, we investigate the food environment for *M. alfredi* at Hanifaru Bay. Manta rays are of economic importance to both ecotourism and fisheries industries and have a conservative life history, and so identifying the foraging requirements and habitat preferences of these threatened rays should aid future conservation efforts (Stewart et al. 2018a). We analyse the zooplankton dynamics (biomass, size structure, and community composition) in relation to *M. alfredi* presence, behaviour and feeding strategies, to improve our understanding of the feeding dynamics of this large planktivorous species. This study aims to relate changes in zooplankton biomass to *M. alfredi* behaviour; to establish a critical prey density threshold for feeding at this site, and to determine whether prey density influences the type of feeding strategy *M. alfredi* employ to exploit their prey. Further, we aim to investigate whether *M. alfredi* foraging behaviour is influenced by changes in the zooplankton community composition, or size structure.

Materials & Methods

Study Site

The Maldives has a large resident population of *M. alfredi* which undertake biannual migrations linked to the changing monsoons (seasons) within the archipelago (Anderson et al. 2011; Figure 3.1a). During the Southwest Monsoon, or *Hulhangu* (April – November), *M. alfredi* frequent foraging aggregation sites on the eastern side of the nation's atolls (Harris et al. 2020). One site, Hanifaru Bay, is situated on the eastern edge of Baa Atoll, and attracts large feeding aggregations of this species annually (Stevens 2016, Harris et al. 2020). Hanifaru Bay is a small reef inlet (700 m long by 200 m wide) which forms part of a core marine protected area within the Baa Atoll UNESCO Biosphere Reserve (5°17'N, 73°15'E; Figure 3.1b). The shallow (maximum depth 22 m) inlet is periodically inundated with zooplankton-rich water. Motorised boat activity and SCUBA diving are prohibited in Hanifaru Bay due to the high numbers of manta rays and other megafauna that access the inlet (Murray et al. 2020).

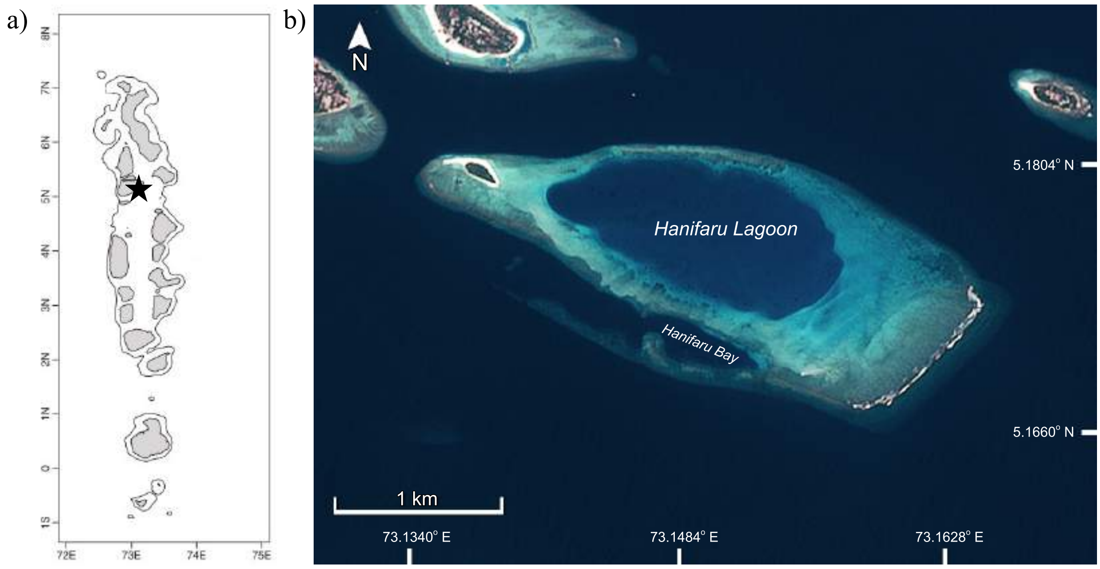


Figure 3.1. Study site in Hanifaru Bay in Baa Atoll, The Maldives. a) Map of The Maldives, black star indicates location of Hanifaru Bay in Baa Atoll; and b) Satellite image of Hanifaru Island and Lagoon, with key study site of Hanifaru Bay (Credit: Copernicus Sentinel data 2020, accessed via USGS EarthExplorer and processed by AJA).

Data collection

Fieldwork was conducted in the lead up to the new moon in August 2017, under Ministry of Fisheries Permit No. (OTHR)30-D/PRIV/2017/280, and Ministry of Environment Permit No's. EPA/2017/RP-01 & EPA/2016/PSR-M02. This time of year was chosen because strong lunar tides appear to overcome the force of the prevailing monsoonal current, drawing plankton-rich water from outside the atoll edge into Hanifaru Bay (Stevens 2016). The currents form a back eddy, trapping and concentrating plankton in this shallow reef inlet, resulting in *M. alfredi* foraging opportunities which peak during spring and high tides (Stevens 2016). Sampling was conducted during daylight hours and across the tidal cycle from 13 – 21 August 2017. Zooplankton was collected by two people using a 200 µm-mesh net towed by hand at the surface for a ~50 m transect between two coral features at the eastern end of Hanifaru Bay (Figure 3.2a). A flowmeter was fitted to the plankton net to allow calculation of the volume of water sampled. Flowmeter calibration was performed prior to the field trip in a swimming pool of known length to establish an accurate measurement of distance per flowmeter revolution. Samples were kept on ice and fixed with 10% buffered formalin solution at the end of each day.

Each net tow was accompanied by an in-water observer recording manta ray activity in vicinity of the tow. This included: (1) manta ray abundance; (2) behaviour (Feeding, Non-feeding – when manta rays were present but not feeding, and Absent – when manta rays were not present); and (3) most common feeding strategy employed (as described in Stevens 2016; Figure 3.2b).

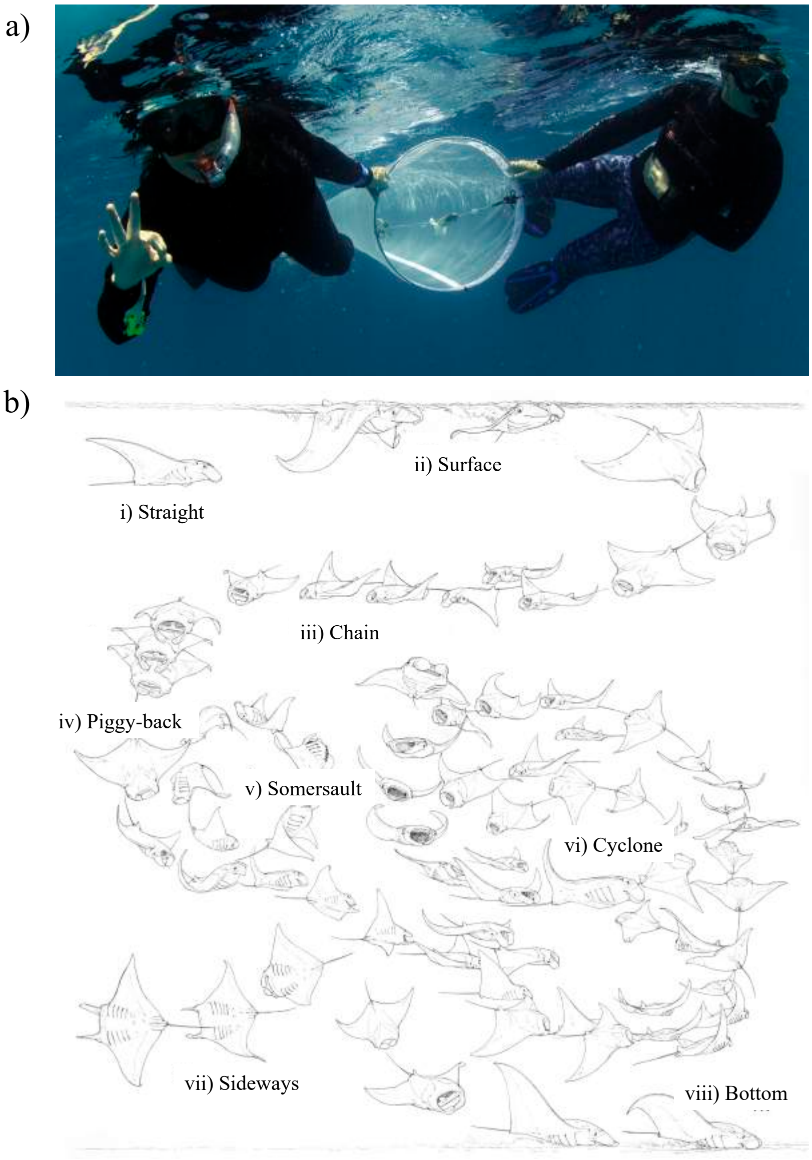


Figure 3.2. Zooplankton sampling and reef manta ray *Mobula alfredi* feeding strategies. a) Zooplankton samples were collected by two snorkellers surface swimming a 200 µm-mesh net with flowmeter for ~50 m; and b) Ethogram of feeding strategies: i) Straight, ii) Surface, iii) Chain, iv) Piggy-back, v) Somersault, vi) Cyclone, vii) Sideways, and viii) Bottom (Illustration credit: Marc

Dando).

Temperature and salinity data were collected at 1 s intervals from a CTD unit deployed at the site for the study duration (except for ~24 hrs from 17 – 18 August for battery exchange). Temperature and salinity ranges were relatively small throughout the study (28.6 – 29.2°C and 34.3 – 35.0 ppt respectively). These variables were excluded from the models as their inclusion resulted in missing values. Tide data were obtained from a local government representative.

Sample processing

Zooplankton samples were processed in the CSIRO Plankton Ecology Laboratory in Brisbane, Australia. Flowmeter readings and the area of the net mouth were used to estimate the volume of water filtered.

Zooplankton biomass

Zooplankton samples were split into two halves using a Folsom splitter (Harris et al. 2000). The first half was used to determine dry mass, with each sample oven-dried at 70°C for 24 hrs prior to weighing. Zooplankton dry mass (hereafter referred to as biomass) per unit volume of filter-seawater for each tow was calculated by dividing the dry mass of the sample (mg) by the volume of filtered water (m^3):

$$\textit{Biomass (mg } m^{-3}\textit{) = Dry mass (mg) / Volume of water filtered (}m^3\textit{)}$$

Zooplankton identification

The second half of the sample was used to examine size structure and community composition via a 2400 dpi ZooScan system and microscopy. The Hydroptic v3 ZooScan (EPSON Perfection V700 Flatbed) is a high resolution, waterproof scanner that digitises particles for size and biovolume measurements (Gorsky et al. 2010). An aliquot of each sample was prepared using a Stemple pipette of known volume and placed on the scanning tray. To avoid overlap, particles were manually separated using a cactus spine (Eriksen et al. 2019). Once separated, the sample was scanned and particles were extracted into vignettes for categorisation into broad taxonomic groups (24 groups) using Plankton ID software (Version 1.2.6) and manual validation (Gorsky et al. 2010).

Objects classified as sand, fibre, detritus, bubbles and shadows were excluded from further analysis (as per Rohner et al. 2015). For visualisation, taxa that comprised <5% of the total abundance were grouped as "other", and these included cnidaria, polychaetes, echinoderm larvae, bryozoan larvae, fish larvae, salps, and various classes of arthropods.

To investigate which species were responsible for the majority of the biomass at the site when overall biomass values in the water were high, samples were analysed taxonomically via microscopy. A subsample was prepared using a Stempel pipette, and organisms were identified and counted in a Bogorov tray using a microscope. Dominant members were identified to genus or species with assistance from trained plankton taxonomists at the CSIRO Plankton Ecology Laboratory (Eriksen et al. 2019).

Zooplankton size structure

A size distribution of the sample particles, known as a Normalised Biomass Size Spectra, was produced to analyse the size structure of the zooplankton community (Vandromme et al. 2012). Spherical biovolume was calculated from the size measurements obtained from ZooScan. Each particle was assigned to one of 50 logarithmic size categories based on its spherical biovolume. The sum of the spherical biovolume of the particles in each size class (mm^3) was standardised by the fraction of sample scanned and the volume of water filtered (m^3), and normalised by dividing this value by the width of the size class measured in biovolume (mm^3). Both axes of the Normalised Biomass Size Spectra use a logarithmic scale.

Drivers of zooplankton biomass and manta numbers

To investigate potential drivers of zooplankton abundance and *M. alfredi* visits to Hanifaru Bay, we constructed generalised linear models (GLMs) using R (R Core Team 2019). Separate analyses were conducted for two response variables: (i) Zooplankton biomass ($mg\ m^{-3}$), with a Gamma error structure and log-link function; and (ii) Manta ray abundance (number of *M. alfredi* observed during zooplankton sampling), with a negative binomial error structure and log-link function (Poisson error structure was overdispersed). We visually inspected diagnostic plots to assess assumptions of homogeneity of variance and normality. Predictors in both models were Tide (hours from high tide) and Behaviour (Feeding, Non-feeding and Absent). To account for the circular nature of Tide (~12-hr cycle), the variable was transformed using a truncated Fourier series (a harmonic function of sines and cosines). This ensures that the cyclical nature of this predictor is captured, while guaranteeing that the response values predicted at the extremes of the predictor range are the same (i.e., the same prediction for Zooplankton biomass or Count at times of 0 and 24 hours). For the Manta ray abundance model, the Behaviour predictor was reduced to two categories – namely Feeding and Non-feeding, and Zooplankton biomass (mg m^{-3}) was included as a predictor. Models were plotted on the response scale using the package "visreg" in R (Breheny and Burchett 2017).

Critical thresholds for feeding behaviour and strategy

We assessed whether there might be a critical threshold for *M. alfredi* feeding – i.e., a level above which the likelihood of feeding increases dramatically. We thus used a GLM with a binomial error structure to analyse manta ray behavioural response (Non-Feeding = 0, Feeding = 1) in relation to zooplankton biomass (mg m^{-3}) as a predictor. The critical density threshold was taken as the zooplankton biomass at which the proportion of feeding was 0.5. A theoretical prey density threshold was plotted for comparison, based on findings by Armstrong et al. (2016). Their study assumed general morphometrics (average disc width of 3.5 m, mouth opening of 0.3 m, and weight of 100 kgs) and swim speeds (2 knots when feeding) for *M. alfredi*, which are applicable in the current study.

Feeding samples were categorised into either Solo feeding (Straight, Surface and Somersault) or Group feeding (Piggy-back and Chain) based on the most common strategy observed in the manta rays (Stevens 2016). A GLM with a binomial error structure was used to analyse manta ray feeding strategy response (Solo = 0, Group = 1) in relation to zooplankton biomass (mg m^{-3}) as a predictor. The critical density threshold was taken as the zooplankton biomass at which the proportion of Group feeding was 0.5.

Zooplankton community analysis

To determine how different the zooplankton communities were for the *M. alfredi* behaviours (Feeding or Non-Feeding), non-metric multidimensional scaling was used based on abundance counts of the different taxonomic groups from the Zooscan analysis. The Bray Curtis distance measure was used because it is unaffected by joint absences of taxonomic groups in samples. To account for abundance of certain taxa, data were transformed using a root transformation. To test for differences in community composition between *M. alfredi* behaviours (Feeding and Non-Feeding), we performed an adonis analysis, a multivariate analysis of variance. Both the adonis and non-metric multidimensional scaling were conducted using the "vegan" package in R (Oksanen et al. 2007).

Results

A total of 77 zooplankton samples were collected (Feeding = 33, Non-feeding = 22, and Absent = 22) over a period of nine days. Overall zooplankton biomass ranged between 0.7- and 643.1 mg m^{-3} (mean = 90.7, SD = 130.9). For manta ray behaviours, zooplankton biomass for Feeding samples ranged between 7.3 and 593.6 mg m^{-3}, for Non-feeding samples between 1.1 and 175.6 mg m^{-3}, and for absent samples between 0.7 and 643.1 mg m^{-3}.

GLM analyses showed that Tide and Behaviour were significantly related to Zooplankton biomass in Hanifaru Bay (Figure 3.3). Zooplankton biomass was greatest just following high tide (t = -3.83, p = 0.0003, Figure 3.3a), and *M. alfredi* were more commonly observed feeding when zooplankton biomass was higher (t = -2.83, p = 0.006, Figure 3.3b).

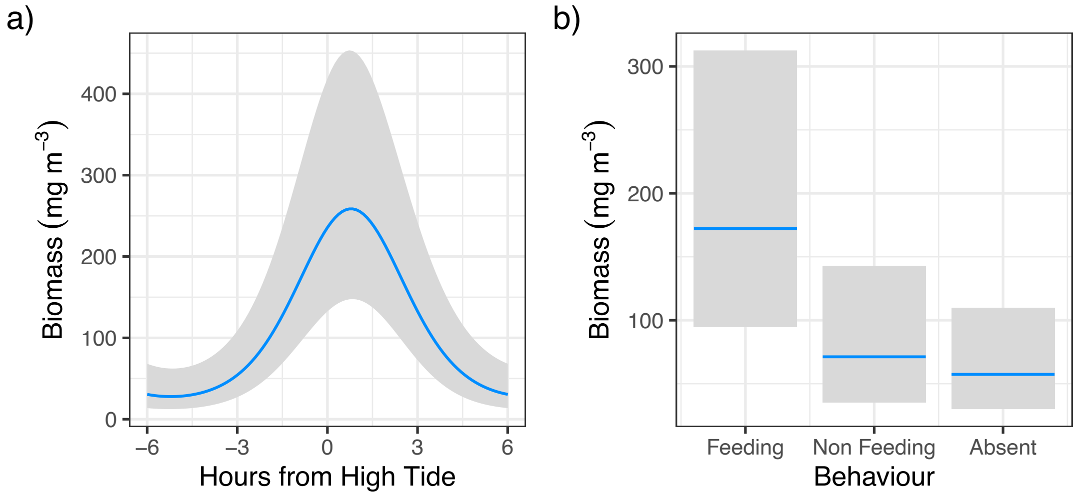


Figure 3.3. Predictors of Zooplankton biomass in Hanifaru Bay, Maldives. Significant predictors of Zooplankton biomass included a) Tide (hours from high tide), and b) Manta ray behaviour (Feeding, Non-feeding and Absent). Biomass is on the response scale, with 95%

confidence intervals.

Manta ray behaviour was significantly related to zooplankton biomass ($z = 3.08$, $p = 0.002$), with a prey density threshold of 53.7 mg m^{-3} calculated for feeding *M. alfredi* (Figure 3.4).

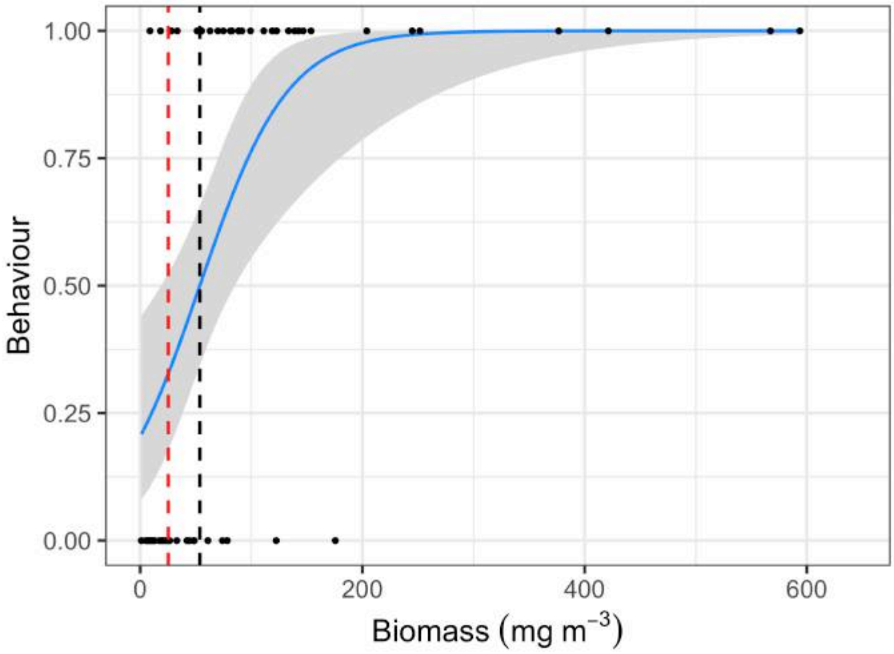


Figure 3.4. Critical prey density foraging threshold. Logistic regression of reef manta ray *Mobula alfredi* behaviour (Feeding = 1, Non-feeding = 0) in relation to zooplankton biomass (mg m^{-3}). The black dashed line represents the critical prey density threshold of zooplankton biomass required to trigger manta ray feeding from *in situ* sampling (53.7 mg m^{-3}), and the red dashed line represents the theoretical prey density threshold calculated to meet the metabolic requirements of foraging *M. alfredi* (25.2 mg m^{-3}; Armstrong et al. 2016).

Manta ray abundance was significantly related to Behaviour ($z = -5.55$, $p < 0.0001$; Figure 3.5), with more *M. alfredi* present when they were feeding in Hanifaru Bay. Tide and Biomass did not significantly relate to manta ray abundance. Manta ray abundance ranged between 0 and 25 individuals.

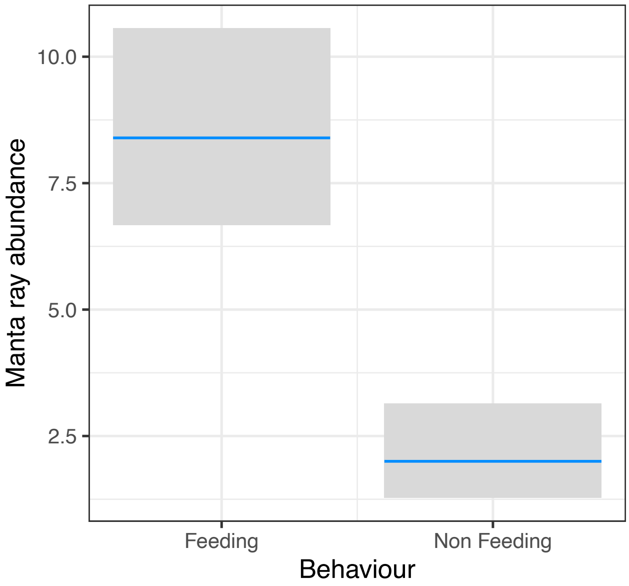


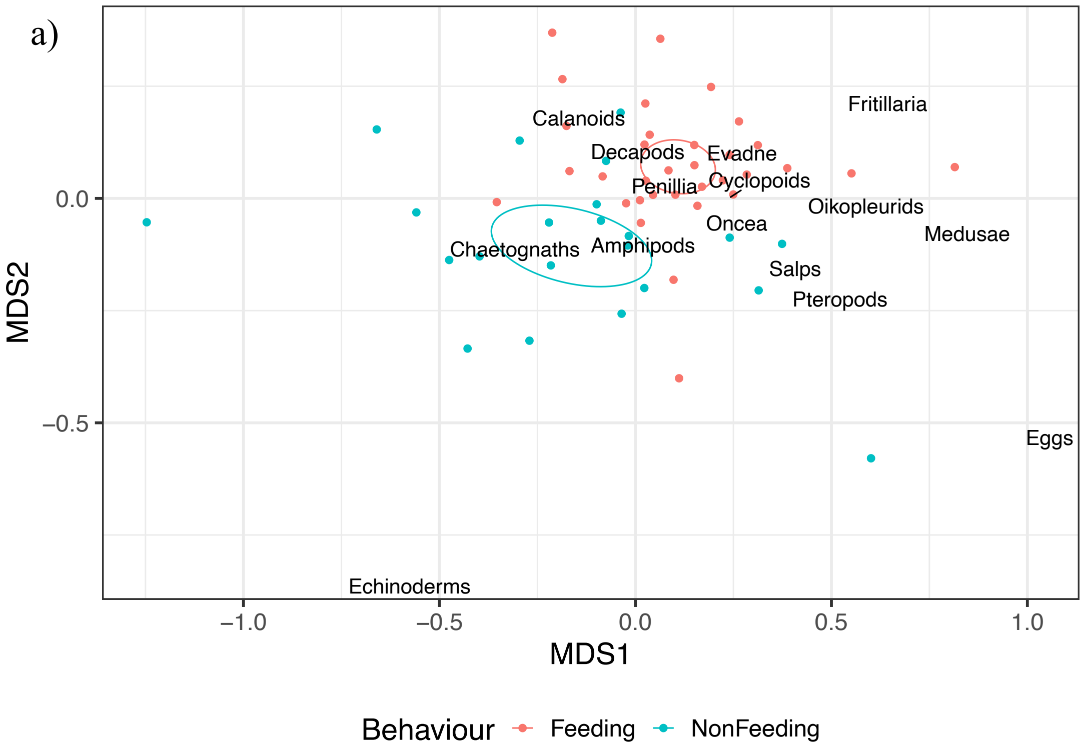
Figure 3.5. Predictors of reef manta ray *Mobula alfredi* abundance in Hanifaru Bay, Maldives. Feeding Behaviour was a significant predictor of greater manta ray numbers in Hanifaru Bay. Manta ray abundance is on the response scale, with 95% confidence intervals.

There was no significant difference among different feeding strategies in relation to zooplankton biomass during the study (ANOVA: F = 1.02, df = 4,28, p = 0.41). In addition, when samples were pooled into Solo feeding strategies and Group feeding strategies there was no significant difference in zooplankton biomass (z = 0.98, p = 0.33). However, only groups were observed feeding when biomass concentrations exceeded 200 mg m^{-3} (Figure 3.4).

Zooplankton community composition

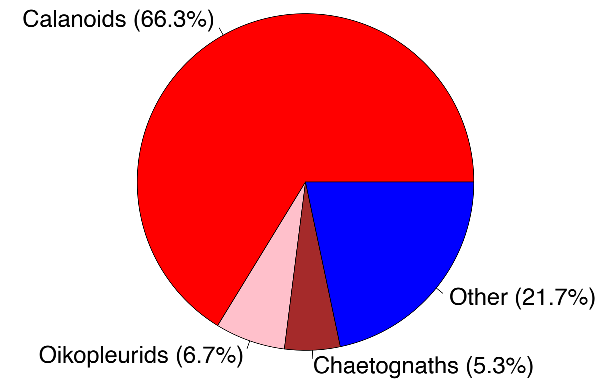
There were differences in the zooplankton community composition between Feeding and Non-feeding samples when analysed using non-metric multidimensional scaling on the Zooscan taxonomic counts (Figure 3.6a). The 95% confidence ellipses for Feeding and Non-feeding were not overlapping, implying that they were significantly different zooplankton community compositions, and this was confirmed by the adonis analysis (F = 9.42, df = 1,53, p = 0.001). Feeding samples were more dominated by Crustaceans (such as copepods), compared to Non-feeding samples which were more associated with gelatinous taxa (such as chaetognaths and eggs).

a)



b)

c) Feeding



d) Non-feeding

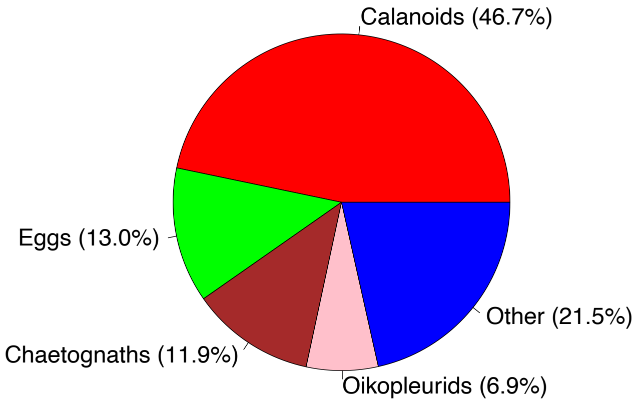


Figure 3.6. Zooplankton composition and reef manta ray *Mobula alfredi* behaviour. a) Non-metric multidimensional scaling analysis of zooplankton community composition. Ellipses represent 95% confidence intervals and broad taxonomic groups are labelled as per their association with manta ray behaviours. b) *Undinula vulgaris* specimens (credit: Julian Uribe-Palomino). Percentages of zooplankton community composition in Hanifaru Bay in relation to manta ray behaviour: c) Feeding; and d) Non-feeding. 'Other' comprises taxonomic groups that contributed less than 5% to the total community composition.

Calanoid copepods comprised 66.3% of Feeding samples compared to 46.7% of Non-feeding samples (Figure 3.6c and 3.6d respectively). Chaetognaths were 5.3% of Feeding samples, and 11.9% of Non-feeding samples. Fish eggs were less than 2% of Feeding samples, and 13.0% of Non-feeding samples. Based on microscopy, juvenile and adult *Undinula vulgaris* were the dominant calanoid copepod species in both Feeding and Non-feeding samples (25.0% and 30.7% respectively, Figure 3.6b).

Zooplankton size structure

Analysis of the size structure of zooplankton from Hanifaru Bay revealed that the biovolume of zooplankton increased in the majority of size categories when *M. alfredi* were feeding (Figure 3.7). The biovolume of zooplankton was significantly higher across particle size categories during *M. alfredi* Feeding events than Non-feeding events (Mean total standardised biovolume: Feeding = 288.4 and Non-feeding = 172.1; t = -2.66, df = 51.38, p = 0.01). Feeding and Non-feeding samples had similar biovolumes of small and large particles, but Feeding had significantly more moderate-sized particles (from $10^{-1.2}$ to $10^{0.5}$ mm^3).

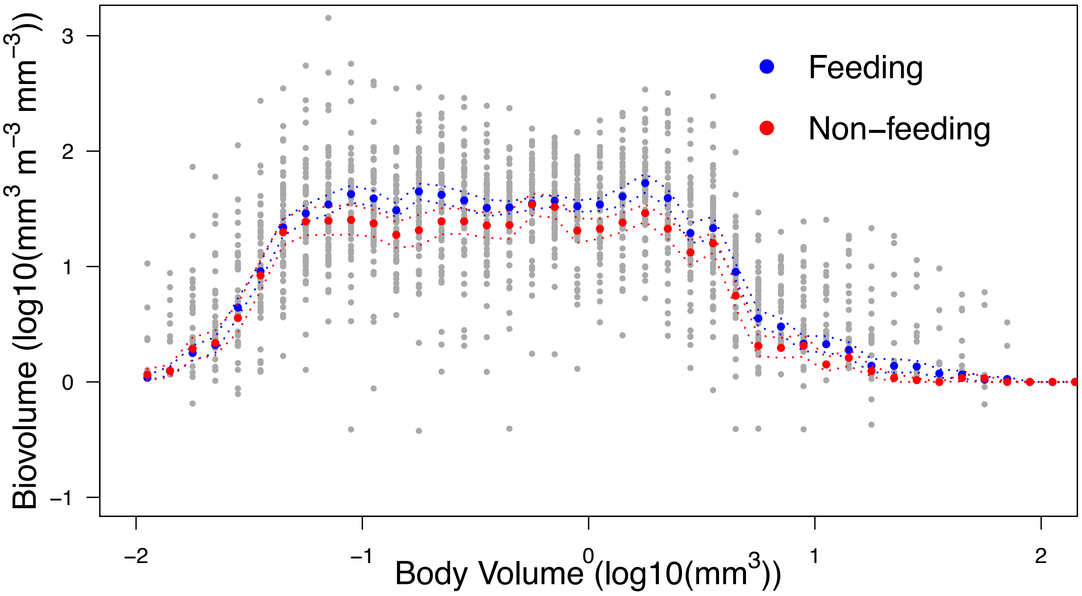


Figure 3.7. Zooplankton size structure analysis. Normalised Biovolume Size Spectra of the zooplankton community when reef manta rays *Mobula alfredi* are Feeding (n = 33) and Non-feeding (n = 22). Dashed lines represent standard error.

Discussion

Zooplankton concentrations influence the number of *M. alfredi* present and their observed behaviour in Hanifaru Bay. Rapid changes in zooplankton are observed across the tidal cycle, and *M. alfredi* feed when biomass reaches a critical density which is higher than predicted to meet their theoretical metabolic requirements. *Mobula alfredi* forage when the zooplankton community is dominated by calanoid copepods, and are less likely to feed when there are greater numbers of gelatinous taxa (such as chaetognaths or eggs). Taxonomic analysis reveals large-bodied copepods, *Undinula vulgaris*, dominate the zooplankton environment at Hanifaru Bay, suggesting oceanic incursions may play an important role in bringing zooplankton to this small reef inlet.

Tidal influence on zooplankton density and manta ray foraging

Manta rays feed when zooplankton biomass is high, which is typically observed on the high to ebbing tide at Hanifaru Bay. Oceanographic investigations in Hanifaru Bay suggest tidal currents draw zooplankton into the shallow reef systems of the atoll, where they become trapped inside due to a back-eddy mechanism created by the unique shape of the reef system and the combination of the lunar and monsoon currents (Hosegood *pers comms*). Tides are known to influence the distribution and abundance of zooplankton around island inlets in the Great Barrier Reef (Alldredge and Hamner 1980), and have been shown to influence manta ray feeding behaviour at aggregation sites in Indonesia (Dewar et al. 2008), eastern Australia (Armstrong et al. 2016), and the Chagos Archipelago (Harris et al. 2021). Therefore, short-term *in situ* observations of zooplankton concentrations in relation to tidal cycles and manta ray behaviour can help inform when *M. alfredi* are likely to be observed in Hanifaru Bay.

Animal movements and productivity hotspots

Large planktivores seeking to exploit ephemeral food sources in surface waters are likely to respond to currents and water movements that concentrate zooplankton. *Cetorhinus maximus* forages along thermal fronts (Sims and Quayle 1998), *R. typus* targets regions of upwelling (Ryan et al. 2017), and surface foraging in *M. alfredi* is often tidally driven (Dewar et al. 2008, Armstrong et al. 2016). In conjunction with responding to physical oceanographic cues, animals that seek patchily distributed prey sources are also likely to congregate in areas where they have previously encountered energetically rewarding prey abundances, resulting in larger numbers of animals in reliable foraging regions. For example, *M. alfredi* predictably switch to the down-current side of the atolls in the Maldives in response to monsoonal winds and primary productivity (Harris et al. 2020). Area-restricted search theory predicts that animals will remain localised in areas where they have a higher probability of encountering prey (Bailey et al. 2019), and this perhaps explains why some *M.*

alfredi individuals remain in Hanifaru Bay when not feeding. This location also has two cleaning stations used by *M. alfredi* (Stevens 2016), and it is hypothesised manta rays will frequent cleaning stations in close proximity to foraging opportunities (Armstrong et al. 2021). Manta rays in Palmyra Atoll used area-restricted searching when adjacent to ledges or channels with high plankton concentrations, but their movements were more random at larger spatial scales (Papastamatiou et al. 2012). Area-restricted searching has also been observed in two dolphin species (*Tursiops truncatus* and *Delphinus delphis*) in areas of high prey availability, and where they have had previous successful foraging experience, suggesting memory plays a role in their movement ecology (Bailey et al. 2019). For *M. alfredi*, their apparent preference for returning to the same cleaning stations over time (Armstrong et al. 2021), suggests they may form a cognitive map of shallow reef environments, and this is likely the case for known productivity hotspots as well.

High critical feeding threshold for manta rays at Hanifaru Bay

The critical prey density threshold for *M. alfredi* feeding in Hanifaru Bay (53.7 mg m^{-3}) is more than four times higher than that in east Australia where *M. alfredi* feeds (11.2 mg m^{-3}; Armstrong et al. 2016), and in east Africa where *R. typus* feeds (12.4 mg m-3; Rohner et al. 2015). It is also double the theoretical prey density threshold calculated to meet the metabolic requirements for *M. alfredi* (25.2 mg m^{-3}; Armstrong et al. 2016), which may explain why this site hosts such a large feeding aggregation of this species. However, these large planktivorous elasmobranchs are assumed to feed in the mesopelagic layer (Couturier et al. 2013, Burgess et al. 2016), so an understanding of the prey densities available at these depths is required to gauge the relative importance of aggregations sites such as Hanifaru Bay in meeting these species' energetic requirements. Sampling zooplankton at depth remains a logistical challenge for researchers, but with technological advances, such as satellite tags equipped with accelerometer data loggers, and unmanned video submersibles (Stewart et al. 2018a), these inferences can be better investigated.

Manta ray feeding strategies

In the current study, plasticity in *M. alfredi* feeding strategies in response to changes in prey biomass in Hanifaru Bay is not supported. This contrasts with work previously conducted in Hanifaru Bay that found manta rays were significantly more likely to employ group feeding strategies as prey density increased (Stevens 2016). The previous work was based on a qualitative visual index for prey density, with data obtained over a long time period (> 5 years) and included aggregations upwards of 150 animals. However, we did see that when zooplankton biomass values were very high, over 200 mg m^{-3}, that only Group feeding strategies were used, and no Solo feeding was seen. But in either scenario, it is uncertain whether the observations are due to true cooperative feeding strategies, or simply that coordinate movements reduce collisions with other manta rays (Stevens 2016). It is likely our relatively short sampling duration failed to detect this phenomenon, and our results suffer from small sample size. More work needs to be done to quantitatively assess whether the presence of higher zooplankton biomass is positively correlated with group feeding events.

Zooplankton composition and size

Differences in the composition of the zooplankton community were observed between *M. alfredi* feeding and non-feeding events, and *M. alfredi* was observed feeding when the overall biovolume of zooplankton was greater. Calanoid copepods dominate the zooplankton community for manta rays at Hanifaru Bay, with *U. vulgaris* the most prominent species. *Undinula vulgaris* is a key species in tropical areas due to its large size and tendency to swarm in high numbers, making it a good food resource for planktivorous fishes (Alvarez-Cadena et al. 1998). This species has been observed at numerous large planktivore feeding aggregation sites, including those visited by *M. alfredi* in eastern Australia (Couturier et al. 2013, Armstrong et al. 2016), *R. typus* in the Gulf of Tadjoura, Djibouti (Boldrocchi et al. 2018), both *M. birostris* and *R. typus* in the Gulf of California (Notarbartolo-di-Sciara 1988, Lavaniegos et al. 2012), and both *M. alfredi* and *R. typus* in the Philippines (Canencia and Metillo 2013, Yap-Dejeto et al. 2018). *Undinula vulgaris* is considered an indicator of the influence of neritic-oceanic waters in reef environments, and its local distribution can suggest oceanic water sources (Alvarez-Cadena et al. 1998). Further investigation into the ecology of *U. vulgaris* in tropical environments may aid our understanding of how vital swarms of this species are for supporting large tropical planktivores, and whether their distribution and abundance is likely to be impacted by a rapidly changing climate.

Caveats to zooplankton sampling

There were two main limitations in the sampling design of the current study. First, hand towing of the plankton net (because boat engines are prohibited in the area) resulted in tow speeds slower than recommended (22 m vs 60 m per minute) for minimizing avoidance by larger zooplankton (Harris et al. 2000). However, the use of a net with a relatively large mouth area (50 cm diameter) should help mitigate this. Further, the use of slower towing speeds (Davies and Beckley 2010) and a relatively large mesh size for tropical waters (200 µm; Eriksen et al. 2019) should reduce the pressure wave from the towed net, and thus minimise concerns about net avoidance by zooplankton. Second, zooplankton sampling was limited to surface waters. This could influence the findings, particularly in relation to investigating the zooplankton dynamics when manta rays are employing different feeding strategies. For example, somersault feeding and cyclone feeding strategies do not necessarily occur in surface waters. This issue could be overcome with the use of drop nets, vertical free-fall nets that sample on the way down with weighted rings for propulsion (Eriksen et al. 2019), to provide coverage throughout the water column.

Conclusions

Identifying important foraging opportunities for vulnerable species such as manta rays remains a goal for implementing effective conservation strategies for the species. Here, we conducted the first analysis of the food environment for *M. alfredi* at Hanifaru Bay, and highlighted the importance of tidal regimes and high zooplankton density in driving *M. alfredi* aggregations at this site. Conducting high resolution investigations into the dietary basis of aggregations can help inform drivers of species movements and habitat preferences, as these movements are frequently related to prey availability and productivity. This can be challenging in remote locations where resources are sparse, and where fieldwork is logistically difficult (i.e., hand-towing for zooplankton is seldom recorded in methods), which may provide an explanation as to why most feeding studies for marine megafauna have only superficially investigated zooplankton dynamics. The methods from this current study could be replicated at other large planktivore feeding aggregations to ascertain whether these findings are broadly applicable to planktivore feeding ecology elsewhere. This study failed to record the zooplankton dynamics during a mass feeding aggregation at this site, and so our findings are suggestive of what can be observed at this location, but they may not provide the whole picture. Longer term sampling, and more targeted methodologies that allow for sampling of zooplankton throughout the water column, will help elucidate what leads to mass feeding aggregations and the role of different feeding strategies for *M. alfredi* at this site. Nevertheless, the findings here have emphasised the foraging importance of this site for large aggregations of *M.*

alfredi. To maintain this natural phenomenon, we suggest a number of management considerations. For example: (1) Implementing a code of conduct for tourism interactions with manta rays would help ensure human activities do not interfere with their foraging aggregation at the site; and (2) To preserve the zooplankton community, the oceanographic conditions of the region should not be altered (i.e. no dredging or alterations to natural sand movements). Climate change also poses an unknown risk to this aggregation site, as our findings suggest the zooplankton are coming from elsewhere and could be altered by predicted temperature shifts. Here, we have determined the importance of zooplankton dynamics in driving the aggregative behaviour of *M. alfredi* at their largest aggregation site at Hanifaru Bay, and this information can help inform management of this ecologically and economically important marine protected area and core zone within a UNESCO Biosphere Reserve.

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Data availability statement

The underlying raw data is available in the public eSpace repository:
https://doi.org/10.14264/98ddbde

Chapter 4

Movements and Distribution

Chapter 4.1

Photographic identification and citizen science combine to reveal long distance movements of individual reef manta rays *Mobula alfredi* along Australia's east coast

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Abstract

Research into the movement ecology of terrestrial and marine animals is growing globally, especially for threatened species. Understanding how far an animal can move and the extent of its range can inform conservation planning and management. On the east coast of Australia, reef manta rays *Mobula alfredi* are the subject of a photographic identification study, *Project Manta*. In June 2018, videos of reef manta rays from the SS Yongala (19.31^{o} S, 147.62^{o} E), were submitted to the *Project Manta* east coast sightings database. The videos were of two individuals previously identified from North Stradbroke Island (27.42^{o} S, 153.55^{o} E), about 1150 km to the south of the SS Yongala. This represents the greatest point-to-point distance travelled by individual *M. afredi* and extends the latitudinal range for this sub-population on the east coast. This study highlights that citizen science input can provide valuable data to address knowledge gaps in the distribution and population range of marine species. Knowledge of the 1000+ km range movement potential of individual *M. alfredi,* highlights the possibility that regional sub-populations may span jurisdictional zones of more countries than previously considered likely, complicating conservation management of this species.

Key words

home range, animal movement, population connectivity, photo-ID, Great Barrier Reef, elasmobranch, dispersal capacity

Introduction

Knowledge of the movement ecology of species, and how far individuals move is important for understanding population structure and for conservation planning and management (Hays et al. 2016a). The movements and distributions of non-commercial fish species are generally poorly-understood when compared to commercially important fishes, and other marine megafauna such as sea turtles (Houghton et al. 2006, Fossette et al. 2010, Schofield et al. 2013), cetaceans (Christal and Whitehead 1997, Williams et al. 2009, Cheney et al. 2013) and seabirds (Ludynia et al. 2012, Péron et al. 2013, Hennicke and Weimerskirch 2014).

The reef manta ray *Mobula alfredi,* is a large, pelagic elasmobranch (disc width up to 5 m) found in tropical and subtropical waters of the Indo-Pacific Oceans, often associated with coastlines and coral or rocky reef habitats. This species displays aggregative behaviours at predictable locations that have provided good opportunities to investigate their biology and ecology (Dewar et al. 2008, Marshall et al. 2011b, Kitchen-Wheeler et al. 2012). The application of photographic identification (photo-ID) and electronic tagging has shown a relatively high degree of site fidelity to meso-scale geographical regions (Braun et al. 2015, Couturier et al. 2018, Setyawan et al. 2018). One outcome of such site fidelity, could be the formation of local sub-populations, particularly if there are barriers to interchange of individuals with adjacent populations. Currently, there is little evidence of contemporary connectivity among regions across the species' distribution (Stewart et al. 2018a).

To date, the largest point-to-point movements by individual *M. alfredi* are around 650 km, based on movements along the east coast of Australia (Couturier et al. 2014a) and between atolls in the Maldives (Stevens and Peschak 2016). Several other studies have demonstrated shorter movements between locations (Table 4.1.1), and studies have yet to demonstrate movements across international boundaries (Stewart et al. 2018a).

Table 4.1.1. Greatest point-to-point distances moved by individual *Mobula alfredi.*

Location	**Species**	**Method**	**Distance (km)**	**Study**
East Coast, Australia	*M. alfredi*	Photo-ID	650	(Couturier et al. 2014a)
Maldives	*M. alfredi*	Photo-ID	650	(Stevens and Peschak 2016)
East Coast Australia	*M. alfredi*	Satellite tag	520	(Jaine et al. 2014)
Komodo NP, Indonesia	*M. alfredi*	Photo-ID	450	(Germanov and Marshall 2014)
Japan	*M. birostris**	Photo-ID	350	(Homma 1997)
Red Sea, Saudi Arabia	*M. alfredi*	Satellite tag	169	(Braun et al. 2015)
Maldives	*M. birostris**	Photo-ID	160	(Kitchen-Wheeler 2008)
Raja Ampat, Indonesia	*M. alfredi*	Acoustic track	100	(Setyawan et al. 2018)

Hawai’i	*M. alfredi*	Acoustic track	63	(Clark 2010)
Hawai’i	*M. alfredi*	Photo-ID	40	(Deakos et al. 2011)

* Indicates the species was likely to be *M. alfredi*, but was published as *M. birostris* as these papers pre-date the redescription of the former (Marshall et al. 2009).

Mobula alfredi occurs around the Australian coastline in warm temperate and tropical waters, spanning >7000 km from Shark Bay, WA (25.99 S, 113.79° E), across northern Australia, to the Solitary Islands, NSW (30.21° S, 153.27° E) (Armstrong unpublished data). The longest documented point-to-point movement of an individual reef manta ray from these coastlines was on the east coast, between North-West Solitary Island, NSW to Lady Elliot Island, Qld (24.11° S, 152.71° E), a distance of 650 km (Couturier et al. 2014a). This coastline is characterised by shallow continental shelf waters with rocky and coral reefs, forming semi-continuous habitat. Here, through a combination of citizen science and researcher-focused photo-ID surveys, we report on the movements of *M. alfredi* in waters along Australia’s east coast and consider how the species’ use of what is in effect linear suitable habitat may result in the formation of regional sub-populations.

Methods

Photo-ID is an approach that can be applied to any species whose individuals have unique skin patterns or other features that are stable over time (Wiirsig and Jefferson 1990, Bansemer and Bennett 2008, Reisser et al. 2008, Marshall and Pierce 2012). Photographs of animals can provide discrete information about individuals’ locations in time and space. Each manta ray has a ventral body surface that has light and dark pigment distributed in a unique pattern, that varies in the number, size, shape, position and density of spots and patches (Kitchen-Wheeler 2010, Marshall et al. 2011b). On the Australian east coast, *Mobula alfredi* has been the focus of a photo-ID program (*Project Manta*) since 2007. Images of manta rays from along the coast are submitted for inclusion in the *Project Manta* database by trained researchers performing repeated, intense surveys at specific locations and, more opportunistically, by citizen scientists, such as recreational SCUBA divers, snorkelers, and people within the dive industry (Dudgeon et al. in press). Images, along with various metadata (including the date and location of image capture), are entered into a searchable database that allows the history of individual rays to be explored.

On 1 June and 29 June 2018, videos of *M. alfredi* were submitted to *Project Manta* by citizen scientists. Two individual reef manta rays were filmed at the wreck of the SS Yongala, a 107 m long, 3,364 t steamer that sank in 30 m of water in 1911 off Townsville, Queensland (19.31° S, 147.62° E). Still images of the two rays from the videos were matched against images in the *Project Manta* east coast sighting database. This northern site of the SS Yongala, and the site to the south (Manta Bommie, North Stradbroke Island) where the individuals had previously been seen, were used to estimate the greatest point-to-point distance moved by each ray, using the marmap package in R (Pante et al. 2018, R Core Team 2019). This estimate excluded possible passage of manta rays through the 70 km long Great Sandy Strait that separates Fraser Island from the mainland, as this includes narrow and very shallow sections, and there have been no records of manta rays in the strait.

Results

As of 29 June 2018, the east coast sighting database contained verified photographic records of 1235 individual *Mobula alfredi*, from 6375 encounters recorded at 31 unique locations, between the Solitary Islands, NSW in the south, and Tijou Reef, Qld (13.16° S, 143.97° E) in the north. This included 69 individuals identified from north of the SS Yongala (75 encounters), 10 individuals recorded at the SS Yongala site itself (12 encounters), and 1156 individuals from south of the wreck (6288 encounters); with no prior overlap of sightings among these locations (Figure 4.1.1).

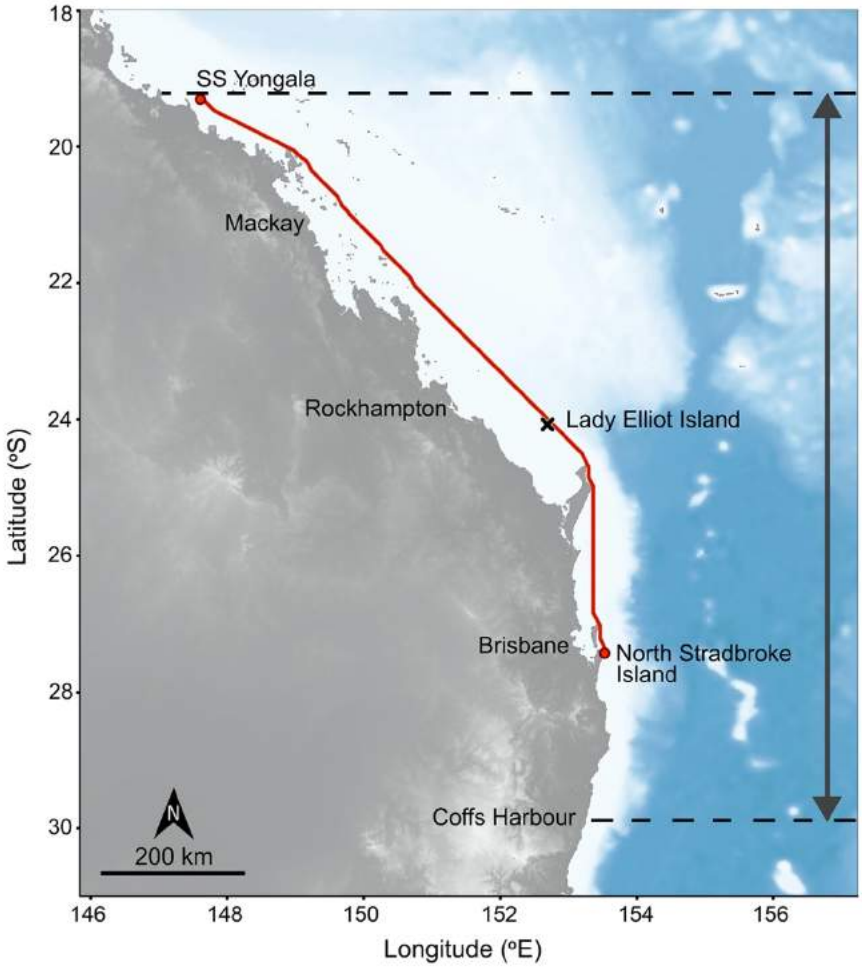

Figure 4.1.1. The largest point-to-point movement for an individual reef manta ray *Mobula alfredi.* The red line presents the shortest straight-line movement between the wreck of the SS Yongala and North Stradbroke Island (1150 km). The arrow on the right indicates the latitudinal range of the regional sub-population of M. alfredi on this coastline as at 29 June 2018.

Citizen science images of *M. alfredi* from the SS Yongala site taken on 1 June 2018 were matched to reef manta ray individuals coded #736 and #1153 in the database (Figures 4.1.2 and 4.1.3). The subsequent sighting at the SS Yongala on 29 June 2018 also matched individual #736, an immature male (Figure 4.1.2). This individual had been sighted 20 times before at Manta Bommie, North Stradbroke Island (27.42° S, 153.55° E) between April 2013 and December 2017. Individual #1153, a sexually mature male (Figure 4.1.3), was previously sighted twice off North West Island (23.30° S, 151.70° E) in April 2017, and once at Manta Bommie in March 2018.

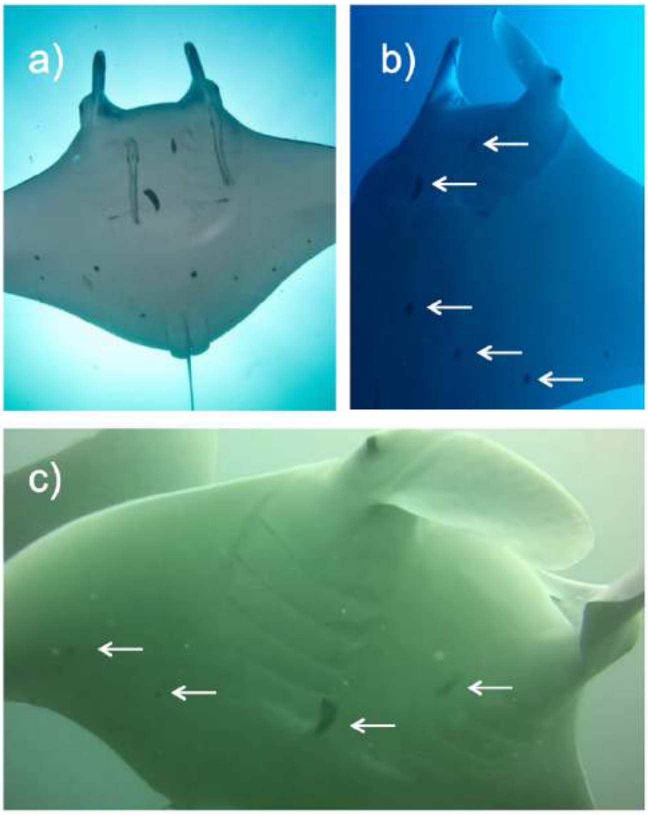

Figure 4.1.2. Images of reef manta ray #736, an immature male; a) Reference image; and images submitted from SS Yongala on b) 29 June 2018 and c) 1 June 2018. Arrows indicate matching spot pattern (photo credit: a) John Gransbury, b) Cam Risbey and c) Laura Billett).

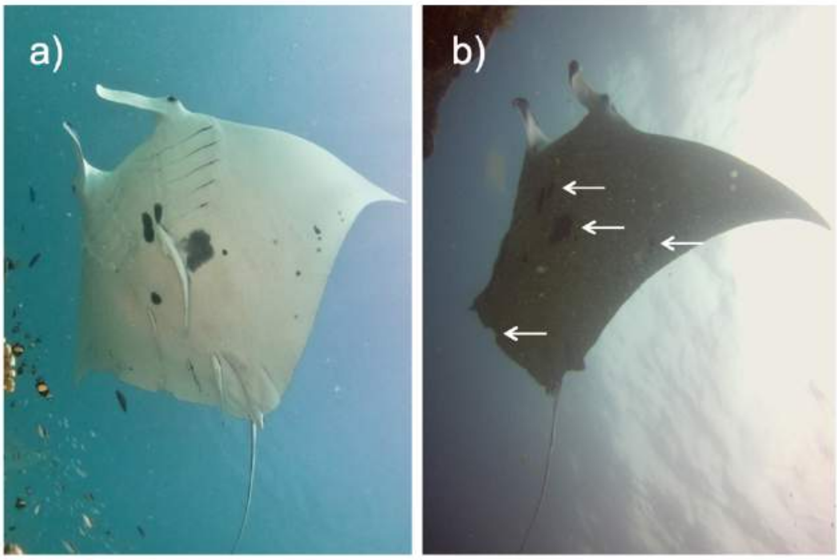

Figure 4.1.3. Images of reef manta ray #1153, a sexually mature male; a) Reference image and

b) image submitted from SS Yongala on 1 June 2018. Arrows indicate matching spot pattern and tissue loss (likely bite wound) to right pectoral fin (photo credit: a) John Gransbury and b) Ariela Schnitman).

Images of these two reef manta ray individuals in the database showed that they had been at Manta Bommie, North Stradbroke Island prior to their sighting at the SS Yongala. The shortest distance between the two sighting locations, without crossing land, is 1150 km (Figure 4.1.1). Manta ray #1153 moved between these two sites within a 3-month period at a speed of at least 12.7 km.d^{-1}, and manta ray #736 within a 7-month period at a speed at least 5.5 km.d^{-1}.

Discussion

The observations here extend the longest verified movement of an individual *Mobula alfredi* between two locations, from 650 km (also from the east coast of Australia; Couturier et al. 2014) to 1150 km. Interrogation of the east coast photo-ID database had revealed a well-defined sub-population of *M. alfredi* between the Solitary Islands in the south and the Capricorn Bunker Group in the southern Great Barrier Reef (Couturier et al. 2011). The latitudinal extent of this group of rays now extends to the SS Yongala in the north (Figure 4.1.1).

As reef manta rays are large-bodied, pelagic rays with a wide tropical and subtropical distribution in the Indo-Pacific, they could potentially move vast distances. However, previous studies have documented maximum point-to-point movements of <650 km (Table 4.1.1), and demonstrated high site fidelity and restricted movements (Dewar et al. 2008, Kessel et al. 2017, Couturier et al. 2018, Setyawan et al. 2018). In contrast, other pelagic elasmobranchs have longer point-to-point movements, such as the tiger shark *Galeocerdo cuvier* (3,500 km) (Lea et al. 2015), blue shark *Prionace glauca* (4,500 km) (Vandeperre et al. 2014), white shark *Carcharodon carcharias* (10,000 km) (Bonfil et al. 2005), and whale shark *Rhincodon typus* (15,000 km) (Guzman et al. 2018). A satellite tagged individual of the larger manta ray species, *M. birostris* (disc width up to 7 m) moved 1500 km between mainland Ecuador and the Galapagos Islands (Hearn et al. 2014). However, similar to the reef manta ray, the majority of studies for *M. birostris* have found this species undertakes relatively short distance point-to-point movements (<300 km; Graham et al. 2012, Stewart et al. 2016a).

Why *M. alfredi* appears to have relatively small directional excursions, and consequently small population ranges is uncertain. Soft barriers to dispersal, such as deep water, may play a role. For example, the volcanic islands of Hawai'i are separated by deep waters, and there is no evidence of connectivity between populations that are only ~150 km apart (Deakos et al. 2011). However, in the Maldives, individual *M. alfredi* have transited deep waters (~2000 m; Stevens and Peschak 2016) and as such, depth alone does not appear to be a barrier to movement. A recent report of the first *M. alfredi* to be seen in the eastern Pacific Ocean, off the coast of Costa Rica, extends the known geographical distribution for the species (Arauz et al. 2019). It is unknown whether this individual migrated from islands 6,000 km to the west, or whether it is part of a previously undocumented population, as the individual had not been identified anywhere else before this sighting (it was not in the global MantaMatcher database of ~9839 individual rays; Town et al. 2013). In the current study, the northward range extension of a regional (eastern Australian) *M. alfredi* sub-population is supported by the positive match via photo-ID of two individuals from North Stradbroke Island sighted at the SS Yongala.

This study highlights the utility of citizen science contributions to photo-ID databases. Using citizen scientists, we identified the unusual movements of two individuals from within a large population of many hundreds of *M. alfredi*. Citizen scientist involvement offers a way to increase observer effort in remote locations, such as the extensive coastal waters of northern Australia. In total, 67% of the photographic records in the *Project Manta* database in eastern Australia have been contributed by citizen scientists. In less populated and more remote regions, including northern QLD, up to 100% of sightings are sourced from citizen scientists (Dudgeon et al. in press). Photo-ID databases are commonly used to track the movement of animals, including manta rays (Homma 1997, Kitchen-Wheeler 2010, Couturier et al. 2011, Deakos et al. 2011, Marshall et al. 2011b), but there are several challenges associated with their use. Photo-ID is restricted to *in situ* observations, and the geographic and temporal cover provided by citizen scientists is unregulated, which can result in data collection bias. In northern Australia, the *Project Manta* database has opportunistic sightings of *M. alfredi* (75 encounters north of the SS Yongala, in comparison to 6300 encounters further south). The under-representation of sightings in the north is due to a lack of researcher directed surveys in this region, sparse human population, and prevalence of salt-water crocodiles *Crocodylus porosus* and box jellyfish *Chironex fleckeri* (Harrison et al. 2004, Caldicott et al. 2005). Electronic tagging offers an alternative approach to tracking animals, but generally provides short-term information and is expensive, limiting the number of animals that can be tracked (Stewart et al. 2018a). However, molecular approaches based on analysis of tissue biopsies have the potential for exploring connectivity between different regions (Dudgeon et al. 2012).

Conclusions

For reef manta rays, this extended movement capability adds complexity to the management of this threatened species, as it may cross jurisdictional boundaries. In Indonesia, individuals have travelled between locations up to ~450 km apart, moving between protected waters and regions of higher fishing risk (Germanov and Marshall 2014). Given the long-distance movement of reef manta rays observed in this study, there is likely to be increased population connectivity among regions. In northern Australia, relatively shallow coastal waters could potentially allow unobstructed movement of *M. alfredi* across international jurisdictions that offer different levels of protection and exposure to targeted fisheries. Greater harnessing of citizen science, in conjuction with international collaboration and data sharing, could provide valuable information to assess these long distance multi-jurisdiction movements.

Declarations

Ethics approval and consent to participate

Approval was obtained from The University of Queensland Animal Ethics Committee (SBS/319/14/ARC/EA/LEIER and SBS/342/17)

Consent for publication

Consent has been provided from contributing photographers: John Gransbury, Cam Risby, Ariela Schnitman and Laura Billett.

Availability of data and material

Data is contained in the *Project Manta* east coast database which is not currently available online. This information can be provided by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

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Chapter 4.2

The geographic distribution of reef and oceanic manta rays (*Mobula alfredi* and *Mobula birostris*) in Australian coastal waters

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Abstract

The known distribution of manta rays in Australian waters is patchy, with records primarily centred around tourism hotspots. We collated 11,614 records of *M. alfredi* from photo-ID databases (*n*= 10,715), aerial surveys (*n*= 378) and online reports (*n*= 521). The study confirms an uninterrupted coastal distribution from north of 26°S and 31°S on the west and east coasts respectively. More southerly *M. alfredi* records relate to warm-water events with a southernmost extent at 34°S. Coastal sightings of *M. birostris* were rare (*n*= 32), likely reflecting a preference for offshore waters, but encompass a wider latitudinal extent than *M. alfredi* of 10-40°S.

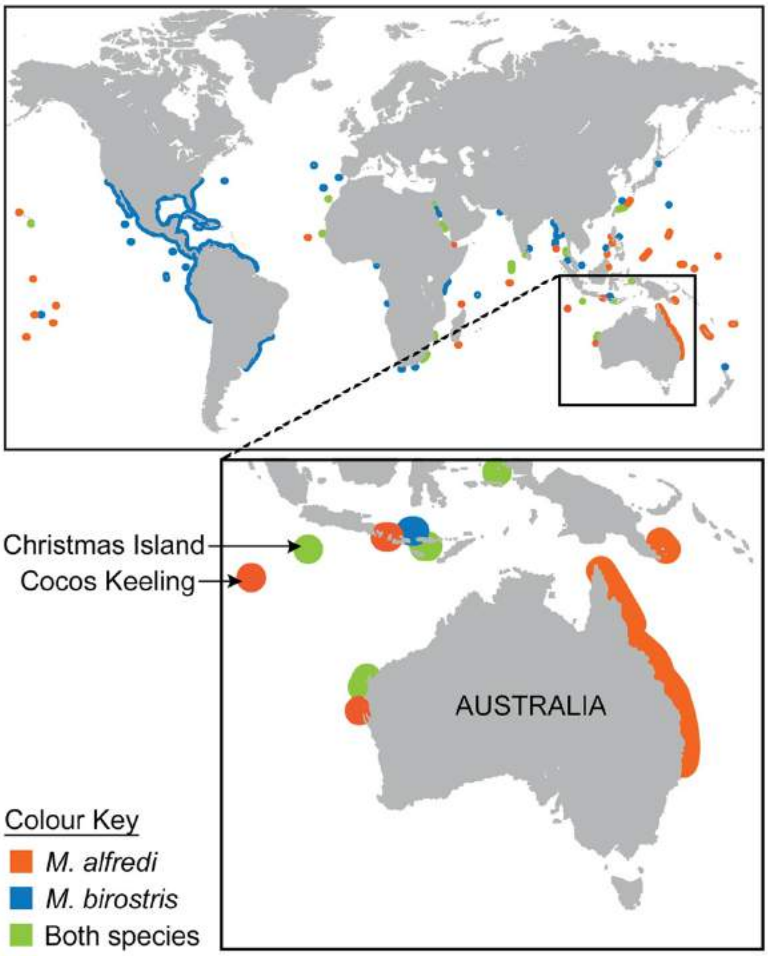
Keywords

reef manta ray, citizen science, connectivity, photo-ID, species distribution range.

Main Text

There are currently two recognised species of manta ray (order Myliobatiformes), which are large, pelagic, planktivorous rays with broadly overlapping, near global distributions in tropical and warm temperate oceanic waters. The reef manta ray, *Mobula alfredi* (Krefft, 1868), has a geographical distribution throughout much of the tropical and subtropical Indo-Pacific region, with the majority of records from relatively shallow waters associated with mainland coastlines, offshore islands, and reefs (Marshall *et al.* 2009, Couturier *et al.* 2012; Figure 4.2.1). However, the known distribution of *M. alfredi* is extremely patchy, with most records from dive ecotourism hotspots in Mozambique, South Africa, Maldives, Japan, Guam, the Red Sea, Philippines, New Caledonia, Indonesia and Australia (Couturier *et al.* 2012, O'Malley *et al.* 2013; Figure 4.2.1). This could be indicative of environmental preferences of this species, or a consequence of effort-mediated sampling bias.

In Australian waters, *M. alfredi* is reported to occur to at least ~26°S on the west coast and to ~30°S on the east coast (Last and Stevens, 2009; Couturier *et al.* 2012). The majority of sightings are from subtropical locations where the species aggregates seasonally coinciding with regular ocean-based tourism activities. The occurrence of the species elsewhere remains mainly anecdotal. Large *M. alfredi* aggregations are typically associated with feeding behaviours and higher localised zooplankton concentrations, although the species also shows high site fidelity to patches of reef used for cleaning (Armstrong *et al.* 2016, Couturier *et al.* 2018). On the east coast, seasonal, southward movements coincide with a strengthening of warm southerly flowing East Australia Current (EAC) during the Austral summer (Ridgway & Godfrey, 1997). A strong EAC results in productive shelf-edge upwelling (Bakun 1996), and provides a southerly expansion of thermally suitable habitat for *M. alfredi*. Investigation into seasonal movements along the west coast are in process, and are also likely to be influenced by a warm southerly moving ocean current – the Leeuwin Current (Godfrey & Ridgway 1985; A.J. Armstrong *unpublished data*). There are few records of *M. alfredi* from tropical Australian waters due to relatively sparse human coastal populations, a large coastline with limited accessibility, regions of high turbidity, and a lack of in-water diving activities due to risks posed by the salt-water crocodile *Crocodylus porosus* and the box jellyfish *Chironex fleckeri*. However, given the lack of barriers to movement along Australia's northern coastal habitats, it is possible that *M. alfredi* has an uninterrupted distribution between the sub-tropical regions of both coastlines.



Adapted from Couturier *et al.* (2012) Fig.2a

Figure 4.2.1 Previously published distribution for *Mobula alfredi* 🟧 (orange) and *Mobula birostris* 🟦 (blue) or both 🟩 (green), globally (Main) and for Australia (Inset). Note the species' patchy distributions, including the discontinuity between the west and east coasts of Australia. Adapted from Couturier *et al.* (2012).

In comparison, knowledge of the Australian distribution of the oceanic manta ray, *Mobula birostris* (Walbaum, 1792), is based on relatively few photographs of individuals from scattered locations (Figure 4.2.1; Couturier *et al.* 2015). In contrast to *M. alfredi*, *M. birostris* has a wider global distribution of between 41° N and 40° S. Its distribution appears highly patchy with most individuals seen at aggregation sites around cleaning stations adjacent to deep-water. Studies on the species in the Indo-Pacific suggest restricted movements and fine-scale population structure (Stewart *et al.* 2016), and to date there has been no photographic evidence of wide-scale movement of individual *M. birostris* based on photographic-identification (photo-ID) databases. Clarification

of our understanding of the spatial distribution of both manta ray species provides a basis for conservation, management and research into regional connectivity (Stewart *et al.* 2018a).
The aim of this study was to address the gaps in published distributions of *M. alfredi* and *M. birostris* due to effort-mediated sampling bias. To address the species' apparent patchy distributions, we collated records from photo-ID databases, aerial surveys, online records (including museum records) and the scientific literature. All research complied with The University of Queensland's Animal Ethics Committee approvals SBS/319/14/ARC/EA/LEIER and SBS/342/17, and was conducted under Great Barrier Reef Marine Park Authority Permits G12/35136.1 and G16/37856.1.

Two photo-ID databases for the east and west coasts of Australia are maintained by an Australian-wide collaborative research program, *Project Manta* (McGregor *et al.* 2008; Couturier *et al.* 2011, Dudgeon *et al.* 2019), and contain verified images of individual manta rays, identified by the unique spot pattern on their ventral body surface. Images are collected on dedicated research trips, or submitted from citizen scientists, with sighting date and location, and behavioural observations. Aerial surveys that specifically targeted manta rays (*n*= 51) were conducted on near-shore reefs in tropical north Queensland. Between the 2 and 20th November 2017, research aerial survey flights (*n*= 3) followed the outline of the major reefs off Cairns and Port Douglas covering approximately 300 km (16.308°S to 16.790°S; 145.681°E to 146.131°E). Surveys were flown in a Cessna 206 high-wing aircraft at 300-600 m altitude, with a wind speed of ~8 kt. Two researchers recorded the presence of animals and GPS coordinates of the sightings. Additionally, GPS coordinates of manta ray sightings were recorded by pilots conducting tourist flights covering the same region (*n*= 48 between 03/11/17 and 25/02/18). Additional aerial records were collated from the scientific literature or government reports, with surveys conducted for the purposes of population monitoring of marine megafauna such as dugongs *Dugong dugon* and various dolphin species. Trained observers included sightings of manta rays during aerial surveys of the coastline of the Northern Territory (Groom *et al.* 2015, Palmer 2015, Groom *et al.* 2017), and of Ningaloo Reef and Shark Bay, Western Australia (WA; Preen *et al.* 1997, Hodgson 2007). However, as the surveys from WA pre-date the resurrection of *M. alfredi* as a valid species (Marshall, Compagno & Bennett 2009) all manta rays were reported as *M. birostris*. More recent sightings of manta rays along this coastline using other methods, including photo-ID, indicate that *M. birostris* is rarely seen, and thus we assumed the majority of aerial sightings were likely to have been *M. alfredi*. Low altitude aerial surveys for marine megafauna do allow for the identification of manta rays when they are near to the water's surface through a combination of their size and dorsal colouration, the latter of which typically features species specific distinct, high-contrast white blazes on a black background (not featured in other *Mobula* species; Marshall, Compagno & Bennett 2009).

Online searches yielded additional manta ray location records found through Google (www.google.com) and YouTube (www.youtube.com) with species keywords (e.g. manta, reef manta) in combination with specific locations (e.g. Karratha, Rowley Shoals, Broome, Darwin, Weipa, among others), regions (e.g. Northern Territory, far North Queensland; Figure 4.2.2) or activities (e.g. snorkelling, fishing, fly fishing). The online search returned results from a variety of sources including the scientific literature, databases (GBRMPA Eye on the Reef) business and personal blogs, fishing forums, local news reports, videos and online image galleries. Reports were classified as those with or without supporting imagery. If imagery was lacking, reports were only included if accompanying descriptions (e.g. swimming at the surface with mouth open, circling in dense patches of plankton; or body size estimates, e.g. 2 – 4 m disc width) matched known behaviours and/or species morphology. Image verification was completed by a *Project Manta* researcher, experienced in *Mobula* identification.

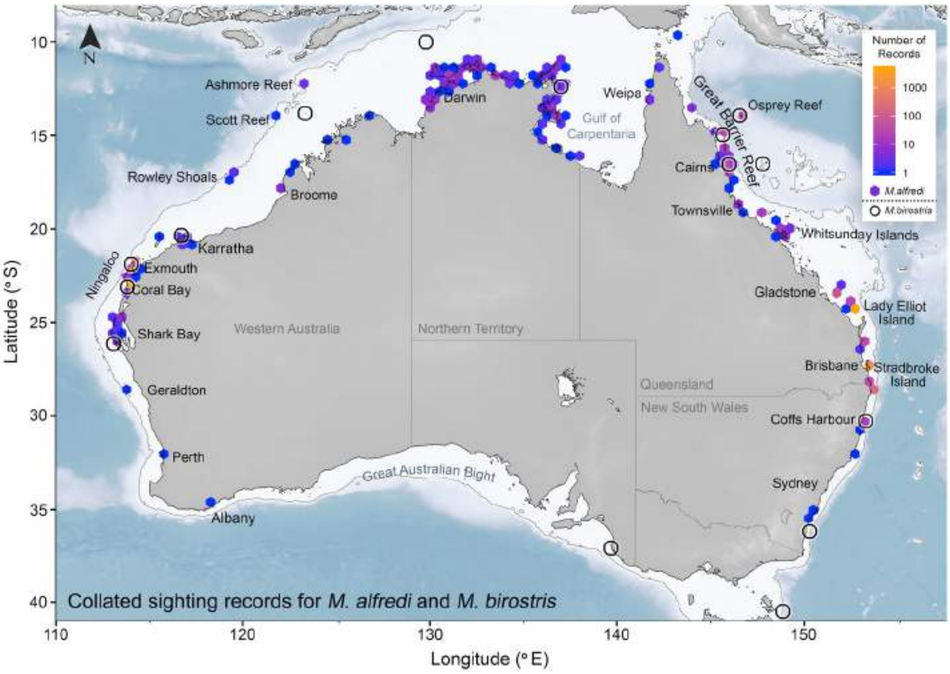


Figure 4.2.2. Collated sighting records for *Mobula alfredi* and *Mobula birostris* in Australia, with data sourced from scientific literature, image databases, aerial surveys, museum records, and online reports. *M. alfredi* sightings are aggregated across a 0.5 ° gridded area and are represented by hexagonal cells, where colour is indicative of the sighting count (from 1 to >1000) per cell. Sightings of *M. birostris* are represented by open black circles and are indicative of location only (not count, due to the limited number of observations). Note that data from Cocos Keeling Island and Christmas Island are not shown. The unbroken grey line off the coast of Australia represents the 500 m isobath.

We collated 11,614 Australian sightings of *M. alfredi*, showing a near-continuous distribution across northern Australian coastal waters from Shark Bay, WA (26°S) to the Solitary Islands Marine Park in New South Wales (NSW; 31°S; Table 4.2.1; Figure 4.2.2). Over 92% of *M. alfredi* records were sourced from the two photo-ID databases maintained by the *Project Manta* research group (*n*= 10,715). The east coast database contained 55% (*n*= 6,480) of the sightings and the west coast database contained 37% (*n*= 4325). Records from the east coast database spanned 13°S to 31°S, with 91% from just two locations (Lady Elliot Island = 71% and North Stradbroke Island = 20%). Records from the west coast database spanned 21.6°S to 23.5°S, with almost all sightings encompassed by the Ningaloo Reef World Heritage Area, WA. Citizen science submissions comprised 64% (*n*= 4200) and 50% (*n*= 2108) of east and west coast database entries respectively.

In comparison, there were very few photo-ID database records for *M. birostris* (*n*= 18), with the majority from the Ningaloo Reef region (*n*= 16), and the remainder from the Great Barrier Reef (*n*= 1) and Coral Sea (*n*= 1).

Table 4.2.1. Collated sightings for Mobula alfredi and *M. birostris* in Australian waters summarised by source.

Sighting Record Source	*Mobula alfredi*	*Mobula birostris*
Manta ray identification database	**10,715**	**18**
Project Manta East coast database	**6480**	**2**
Researcher	*1826*	
Citizen Scientist	*4200*	
Researcher & Citizen Scientist	*454*	
Project Manta West coast database	**4235**	**16**
Researcher	*2127*	
Citizen Scientist	*2108*	
Aerial Observations	**378**	**2**
Marine Mammal Survey	*255*	*2*
Manta Survey	*120*	
Online sighting reports	**521**	**9**
No supporting media	42	2
Supporting media (e.g. image, footage)	479	7
Museum records	**0**	**1**
Total	**11,614**	**32**

Aerial sightings represented just 3% (*n*= 378) of the total Australian manta ray records, but filled much of the previously under sampled northern Australia. For aerial surveys targeting manta rays (Great Barrier Reef), 95 *M. alfredi* individuals were observed by pilots trained in manta ray identification on 48 scenic reef flights and 28 *M. alfredi* from 3 research specific flights covering near-shore reefs of Cairns and Port Douglas. Northern Territory marine megafauna monitoring surveys between 2014 and 2016 contributed 211 sighting records, of which 2 were reported as "likely to be *M. birostris*" and were accepted as such, although there was no mention of the features used to determine species identity. Similar megafauna monitoring aerial surveys covered Ningaloo Reef and Shark Bay regions, WA (1989, 1994, 2007) and contributed 44 records.

Online sighting reports (4%; *n*= 521) provided evidence of *M. alfredi*'s presence in areas that were not encompassed by the photo-ID databases or aerial surveys. Associated imagery confirmed species identity for 91% of sightings, with the remainder originating from trained observers in government reports, or from sightings in which behaviour or appearance was described. Online reports were generally concentrated near 'remote' towns (e.g. Weipa, Queensland; Karratha, WA), where on-water activities such as fishing occur. Eleven records of *M. birostris* from multiple locations were found online, nine of which had supporting media to allow for confirmation of the species.

The southern latitudinal extent for *M. alfredi* is similar on both east and west coasts. The most southerly confirmed records on the west coast were from Coogee Beach, Perth (32.1°S) and anecdotally from Cheynes Beach, Albany (34.9°S). On the east coast, the most southerly confirmed photographic records are from South West Rocks, NSW (30.9°S). Photographic images from Australia's offshore remote territories in the Indian Ocean confirmed the presence of *M. alfredi* (*n*= 80) and *M. birostris* (*n*= 1) at Cocos Keeling Islands, and only *M. birostris* at Christmas Island (*n*= 2).

Australia is the only continent with warm poleward-flowing currents on both coasts. These currents influence the southern range of various mobile species (Couturier *et al.* 2011*,* Dudgeon *et al.* 2013, Payne *et al.* 2018), with many tropical marine species reaching their southern range limit, and many temperate species reaching their northern limit, at latitudes of ~25°S to ~30°S on Australia's east and west coastlines (Gomon *et al.* 2008, Last & Stevens, 2009, Blair *et al.* 2014). Given the few records of *M. alfredi* at locations further south than Shark Bay (~30°S) or Coffs Harbour (~25°S) on the west and east coasts respectively, it is unlikely that sightings south of these regions represent their normal distribution. For example, manta rays reported as far south as Cheynes Beach (34.9°S, Albany, WA) coincided with an exceptional 'marine heatwave', where sea surface temperatures peaked at up to 5°C warmer than normal, and temporary range extensions of many marine fishes were recorded (Pearce *et al.* 2011, Pearce & Feng 2013). The *M. alfredi* distribution reported here is in agreement with computer modelled distributions that show a decrease in occurrence probability at more southerly latitudes (Aquamaps; Scarponi, Coro, & Pagano 2018). Aquamap modelled distributions suggest that under climate change, *M. alfredi* will experience a more permanent southerly range extension in line with those reported during recent marine heatwaves. Future studies would benefit from the inclusion of a temporal analysis of records to better resolve trends in seasonality outside of tourism monitored locations.

This study identified locations within Australia's northern waters where *M. alfredi* has not previously been reported, including the coastline of the Northern Territory, and the northern coasts of WA and Queensland. Confirmed sightings of *M. alfredi* at Cocos Keeling Islands and *M. birostris* at Christmas Island, located ~2000km and ~1500 km from the Australian mainland respectively, are consistent with Kashiwagi *et al.* (2011). We could not find evidence to support the presence of both species at Christmas Island as reported in Couturier *et al.* (2012).

The near-continuous distribution for *M. alfredi* raises questions about regional population structure. It is well-established that *M. alfredi* exhibits strong migratory behaviour and movements of up to 500 km are not uncommon (Couturier *et al.* 2011, Germanov & Marshall 2014, Jaine *et al.* 2014), with few confirmed dispersals in Australia up to 1150 km (Armstrong et al. 2019). Individual rays have demonstrated affinity for particular sites and regions (Braun *et al.* 2015, Couturier *et al.* 2014, Kessel *et al.* 2017, Marshall *et al.* 2011), although most photo-ID and tagging studies have focused on populations separated by large distances and/or by regions of deep water (Deakos *et al.* 2011). For *M. alfredi*, the absence of dispersal barriers along the shallow and expansive continental shelf of northern Australia may facilitate broad-scale connectivity. In *Dugong dugon*, Australian coastal distribution, features of site fidelity, and movement capacity are similar to *M. alfredi* (Hobbs *et al.* 2007, Sheppard *et al.* 2006). Although signals of gene flow between coastlines depend on the molecular marker used (McDonald 2005, Blair *et al.* 2014), results across markers appear to be suggestive of an isolation by distance trend and genetic exchange with neighbouring regions is likely (McDonald 2005). Whether *M. alfredi* moves between Australian coastlines or northern neighbouring countries is currently unknown. Our study found single manta ray records for Ashmore and Scott Reefs (Supplementary Figure C), located ~135 km south of Indonesia's southernmost islands, Rote and Palau Ndana, however it is not clear whether the records refer to *M.*

alfredi or *M. birostris.* Distances between these offshore reefs, the southernmost Indonesian islands, and the Australian mainland, fall within the known movement range (<500 km) of both manta ray species. The newly confirmed continuous Australian tropical distribution suggests that for *M. alfredi* previously reported distribution breaks cannot accurately inform assumptions of population structure. Further studies using methods capable of delineating cryptic population structure throughout a continuous range (e.g. molecular approaches) will be required to ensure the population level effectiveness for any conservation or management actions in *M. alfredi* (Stewart *et al.* 2018a). By combining multiple data types, we were able to produce a significant update on the former published species distribution for manta rays in Australian waters. *M. alfredi* has a near-continuous distribution spanning almost two-thirds of Australia's coastline and adjacent islands and reefs, predominantly restricted to warm waters north of ~30°S. Although records are scarce, we also provide the first Australia-wide map of *M. birostris* occurrence, confirming species presence on all coastlines, including temperate waters.

Acknowledgements

The authors wish to acknowledge the Northern Territory Department of Environment and Natural Resources for providing access to the dugong aerial survey data; GSL Aviation, Cairns, for assisting with the targeted aerial survey for manta rays in the Great Barrier Reef; the Great Barrier Reef Marine Park Authority for access to manta ray data mined from the Eye on the Reef database, and finally we would like to thank the hundreds of citizen scientists from across Australia who contribute data to the *Project Manta* photo-ID databases.

Chapter 5

Spot the difference: Using artificial intelligence on individual markings to delineate global populations of manta rays

Abstract

Unique individual markings are common in many species and have been used to understand aspects of their movement ecology and population dynamics. Numerous elasmobranch species display persistent individual markings through time, including manta rays *Mobula alfredi* and *M. birostris*. Here we apply convolutional neural networks (CNNs) to test whether unique markings of manta rays can be used to infer relatedness among individuals, and whether these markings can provide insights into connectivity or isolation among populations. We collated 52 photographic identification (photo-ID) databases from around the globe, representing separate populations of manta rays (27 for *M. alfredi* and 25 for *M. birostris*), totaling 17,135 individual rays. We then applied CNNs to these photo-IDs to analyse pattern expression of individuals. We found pattern expression was different between the two species of manta rays (adonis: $n = 11{,}151$, $F = 663.63$, $df = 1$, $p < 0.001$); and both species showed significantly more variation in pattern expression among than within populations (*M. alfredi* adonis: $n = 8{,}188$, $F = 50.10$, $df = 23$, $p < 0.001$; *M. birostris* adonis: $n = 2{,}963$, $F = 10.52$, $df = 18$, $p < 0.001$). We found differences in the pattern expression were significantly associated with geographical distances between populations for *M. alfredi*, but not for *M. birostris* (ANCOVA: $F = 22.78$, $df = 3{,}425$, $p < 0.001$). This is likely a consequence of their different ecology, whereby *M. birostris* is a wider-ranging species than *M. alfredi*, and their different space-use is likely to impact the contemporary and/or historical connectivity of populations. This study has highlighted that pattern expression in manta rays is likely to be a heritable trait and that CNNs for pattern recognition may prove to be useful for addressing ecological questions.

Keywords

machine learning, population structure, animal patterns, spatial ecology, elasmobranch

Introduction

There is a rich history of research into biological pattern expression in animals. Darwin's theory of natural selection holds that animal colouration and patterning evolves due to reproductive advantage or survival benefits (Darwin 1859). Pattern expression is observed throughout the animal kingdom and has many benefits, including intra- and interspecies recognition, warning, mimicry, camouflage, and attracting a mate. For example, Lake Malawi cichlid species use colour to recognise conspecifics (Couldridge and Alexander 2002); the strawberry poison frog *Oophaga pumilio* exhibits an aposematic signal that warns potential predators that they are unpalatable (Maan and Cummings 2008); many palatable syrphids (hoverflies) use black and yellow patterns to mimic unpalatable species such as wasps *Vespidae* spp. (Marchini et al. 2017); ground nesting birds such as nightjars use their plumage pattern to avoid detection from potential predators (Troscianko et al. 2016); and ornamentation in male king penguins *Aptenodytes patagonicus* influences female mate selection (Pincemy et al. 2009).

In terms of intraspecies recognition of pattern expression, individuals of the same species have unique, persistent pattern expression over time. In terrestrial environments, leopards have their spots (Miththapala et al. 1989), tigers their stripes (Karanth and Nichols 1998), and giraffes their pelage patterns (Shorrocks and Croft 2009), all unique to individuals and persistent through time. In East Africa, despite access to continuous habitat and the ability to move long distances, genetic analysis of giraffes has shown that pelage patterns have diverged into different lineages, suggesting potential isolation due to pelage-based mate recognition (Thomassen et al. 2013). In the marine environment, numerous elasmobranch species display persistent individual markings through time, including grey nurse sharks *Carcharias taurus* (Bansemer and Bennett 2008), whale sharks *Rhincodon typus* (Araujo et al. 2017), and manta rays *Mobula alfredi* and *M. birostris* (Marshall et al. 2011b). Compiling population databases of individuals based on their markings has allowed researchers to investigate species' population dynamics.

The unique natural markings of individuals allows for a minimally-invasive mark recapture approach to monitor various aspects of elasmobranch biology and ecology, including population size (Couturier et al. 2014b), seasonal movements (Couturier et al. 2011, Harris et al. 2020), demographic parameters (Bansemer and Bennett 2011), social behaviours (Corcoran and Gruber

1999, Jacoby et al. 2012) and residency (Araujo et al. 2017). For manta rays, this approach has been used to update distribution records (Couturier et al. 2011, Armstrong et al. 2020a), document long distance movements of individuals (Armstrong et al. 2019), and confirm connectivity between locations (Germanov and Marshall 2014). To date, there has been no global analysis undertaken with these datasets. This current study analyses the unique markings of manta rays discerned from photographic identification (photo-ID) databases to test whether pattern expression differs among different geographical regions globally.

Manta rays are large planktivores with a circumglobal distribution (Couturier et al. 2012). On the IUCN Red List of Threatened Species, *M. alfredi* is listed as Vulnerable, and *M. birostris* Endangered (Marshall et al. 2019, Marshall et al. 2020). Manta rays are species of economic importance for two very different reasons: they have great ecotourism value in many places around the world (O’Malley et al. 2013), and they are also a targeted fish species due to the value of their gill rakers in traditional Chinese medicine (O'Malley et al. 2017). They have conservative life history traits with slow growth rates, late age of maturity, and low reproductive output (Croll et al. 2016). Therefore, they are unable to sustain substantial fisheries pressure. Both *M. alfredi* and *M. birostris* are relatively rare globally, but they do form mono-specific aggregations at various locations, primarily for feeding and visiting cleaning stations (Armstrong et al. 2016, Setyawan et al. 2018, Germanov et al. 2019, Peel et al. 2019b, Armstrong et al. 2021). Aggregation sites have allowed researchers to collect baseline data about these threatened species.

Broad-scale analyses using photo-ID for manta rays can be hampered due to the use of different approaches for data collection and image quality (Marshall and Pierce 2012). However, some of the challenges associated with image quality (e.g., angle of capture, illumination, water clarity etc.) may be overcome through recent advances in machine learning. Convolutional neural networks (CNNs) provide an efficient way of processing images in a fast and consistent manner, with high rates of accuracy (Brodrick et al. 2019). They are a class of deep neural networks used for analysing visual images by placing importance on certain aspects or objects in the image and differentiating between them (Valueva et al. 2020). These neural networks have commonly been applied to facial recognition, and challenges related to high-level variation of face geometry and facial appearance have driven technological advances in this field (Tan et al. 2017, Li et al. 2018, Zhao et al. 2018, Mane and Shah 2019). A recent application of CNNs to assist in automating photo-ID of manta rays has shown 90% accuracy in predicting the top-3 matches between individual manta rays (Moskvyak et al. 2019), providing a reliable platform to address broad-scale questions about manta ray pattern expression.

Here the CNNs outlined in Moskvyak et al. (2019) are used on photo-ID databases to examine connectivity and isolation of manta ray populations globally. Three hypotheses are tested about heritability of manta ray pattern expression: 1) *Mobula alfredi* and *M. birostris* have different pattern expression; 2) Different populations of each species have different pattern expression; and 3) Pattern expression becomes more dissimilar with geographic distance. Testing these hypotheses provide novel insights into the connectivity and isolation of manta ray populations.

Methods

Photo-ID databases

Photo-ID databases from across the global range of *Mobula birostris* and *M. alfredi* were collected (Figure 5.1), and the best ventral image of each individual was collated and assigned to species and location. Some locations throughout the species range were unable to be included in the analysis as photo-ID databases were either not readily available during the data collection period, or data have not been compiled at all aggregation sites (e.g., *M. alfredi* in the Red Sea and *M. birostris* off Ecuador).

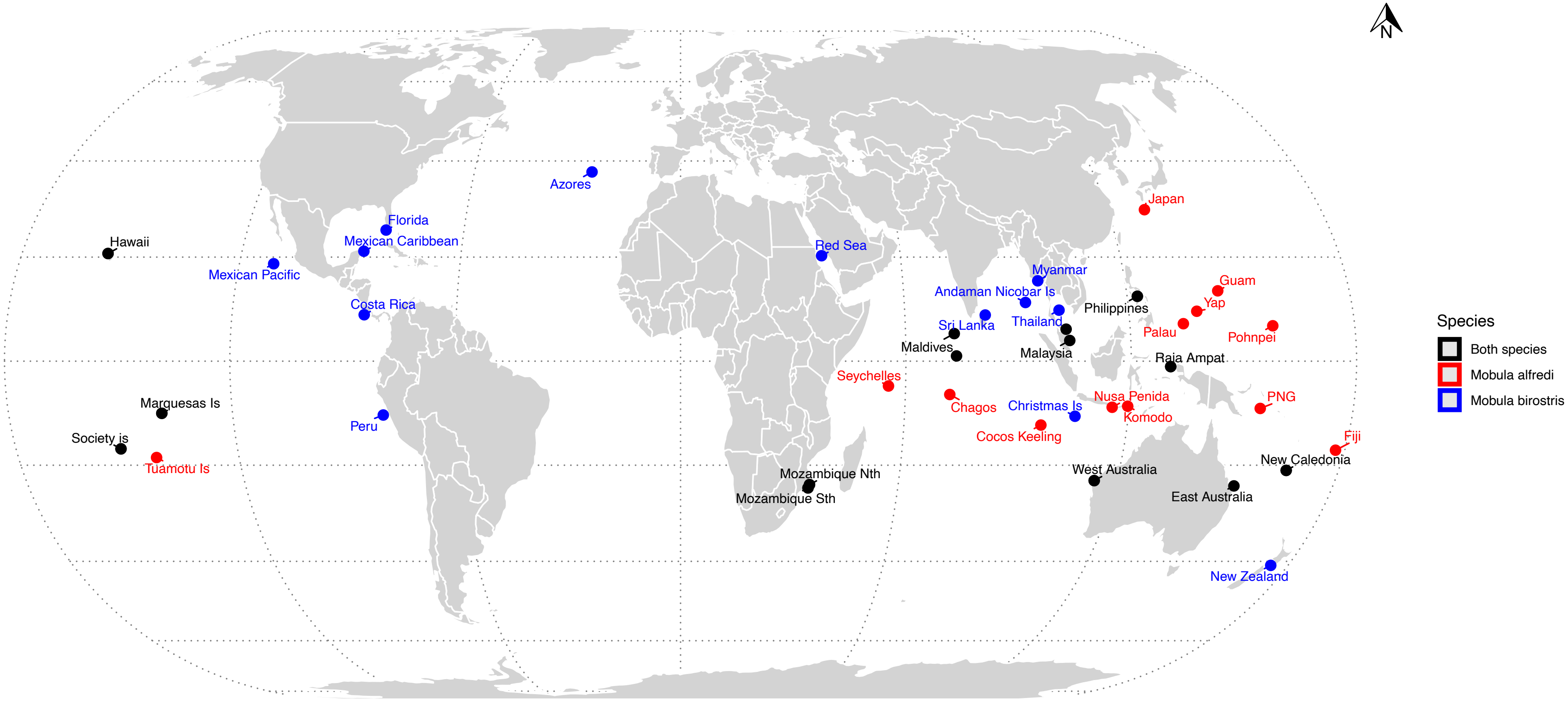


Figure 5.1. Global population database locations for *Mobula alfredi* and *M. birostris*. Circles represent the major aggregation locations for each population of manta rays in the study (27 for *M. alfredi* and 25 for *M. birostris*, totaling 17,135 individual rays).

Images were processed for use in the automated photo-ID tool from Moskvyak et al. (2019). This tool adapts methods developed for facial recognition technology and implements a deep CNN to learn embeddings for images of natural markings. The result is an attribute table of 256 numerical embeddings relating to each image, which can then be used in multivariate analysis to examine the similarity of pattern expression among individual rays (Figure 5.2).

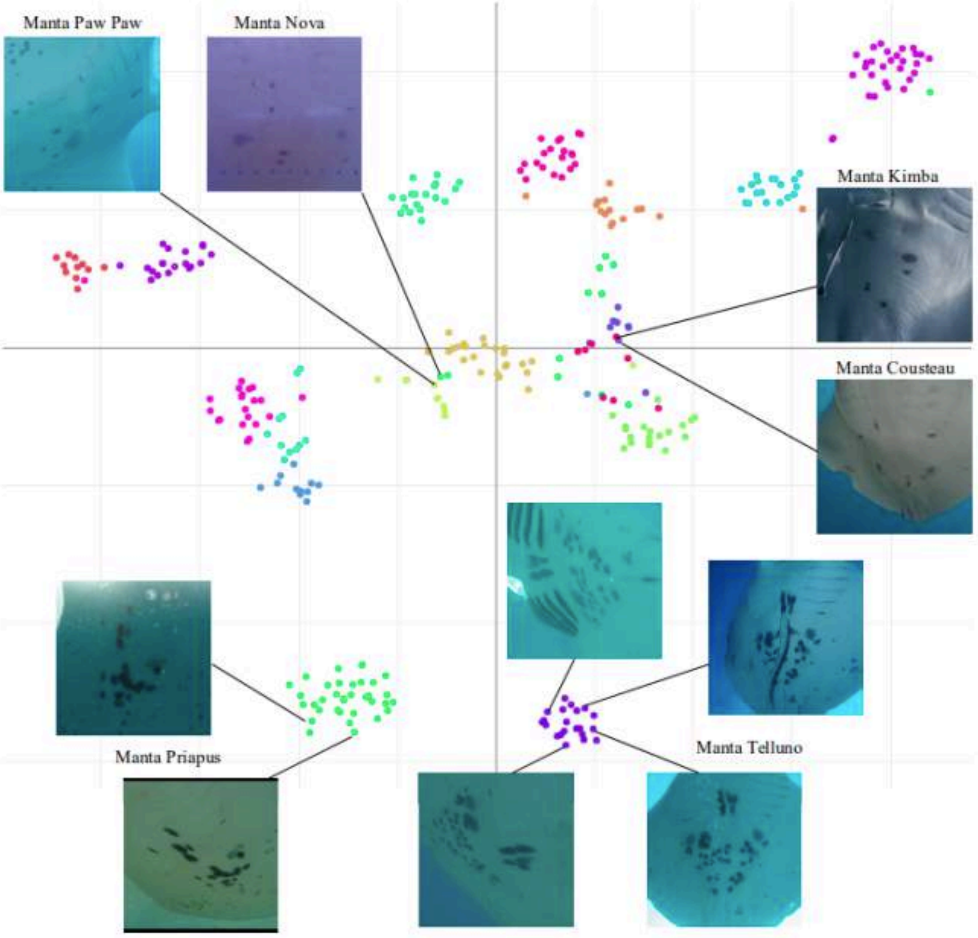


Figure 5.2. Example output from the automated photo-ID tool (Moskvyak et al. 2019). Visualisation of embeddings computed for the *Mobula alfredi* test set using multivariate analysis. Embeddings for multiple photos of individual manta rays form tight clusters and show that the learned representation is invariant to rotations, viewing angle and small occlusions (e.g., Manta Telluno and Manta Priapus). Mixing between individuals happens when there are sparse dot patterns (e.g., Manta Paw Paw and Manta Nova; Manta Kimba and Manta Cousteau).

Each image of the ventral surface was cropped manually for analysis, with particular focus on the area from the mouth to the pelvic region, and from the area lateral to the gill slits on each side of the animal. Manta rays exhibit at least two main colour morphs: typical (whereby the dorsal surface is predominately black, and the ventral surface is mostly white with differing degrees of dark spots and patches); and melanistic (whereby both the dorsal and ventral surfaces are black with varying degrees of white spots and patches on the ventral surface; Marshall et al. 2009). Currently the tool has been trained for typical colour morphs of manta rays, and thus melanistic individuals were excluded from the pattern analysis. Images were further excluded if they were of low resolution, had the pattern obscured, or presented with an extreme acute angle of view.

The first step was to ascertain whether there was any overlap of individuals among any of the population databases, to confirm the assumed geographical isolation of the populations. To do this, images were analysed using the automated photo-ID tool, and a distance value between 0 and 1 was assigned for pairs of images, where the lower distance indicates higher likelihood of a match. Given the high accuracy of this tool (90%; Moskvyak et al. 2019), only the first 100 predictions were scrutinised (manually checked) in the population databases for potential matches. Confirmed matches were only recorded between locations where previous overlap in movement had been recorded (e.g., Germanov and Marshall 2014, Venables et al. 2020), and these matches were within the first 10 predictions, providing confidence in the validity of this approach.

Pattern expression analysis

To test whether the analysis could determine differences between pattern expression on the two described species of manta rays, Principle Components Analysis (PCA) on the numerical embeddings was conducted using the R package “vegan” (Oksanen et al. 2007). PCAs were plotted with standard error (SE) ellipses around the centroid, as non-overlapping ellipses imply statistical significance. To test differences between species, a one-way multivariate analysis of variance (MANOVA) was conducted using an adonis analysis in the R package “vegan” (Oksanen et al. 2007). PCAs based on the numerical embeddings were also constructed for populations of *M. birostris* and *M. alfredi* separately, with a one-way MANOVA adonis analysis used to test differences among populations.

To test whether the pattern expression in manta rays diverged with increasing geographical distance among populations, we related the distance between population centroids on the PCA to the geographical distance between populations. If populations are geographically isolated, then increased distance between population centroids on the PCA should reflect increased geographical distance between populations. We performed an analysis of co-variance (ANCOVA), with the response being PC distances (distance between each set of population centroids), and the predictors being Geographical distance (kms between each set of population locations) and Species as main effects and as an interaction. For the Geographical distance, the location of the core aggregation site was chosen for each population database, and the least cost distance between aggregation sites (i.e. the shortest distance across water, unconstrained by depth), was calculated using the R package "marmap" (Pante and Simon-Bouhet 2013).

To assess whether results from the CNN approach here is consistent with current understanding of connectivity of manta ray populations, we compared our results with three relevant genetic studies that explored population structure and connectivity (A.J. Armstrong *unpublished*, Hosegood 2020, Venables et al. 2020b). As per the previous investigations into differences in pattern expression between populations, the analyses here consisted of subsetting the relevant populations of *M. birostris* and *M. alfredi* from each study and conducting a PCA analysis and one-way MANOVA based on the numerical embeddings.

Results

Photo-ID databases

We examined 13,816 images of *Mobula alfredi* from 27 populations, and 3,319 images of *M. birostris* from 25 populations (Table 5.1). The number of images for each population database ranged from 2 to 4,262 individuals for *M. alfredi*, and from 1 to 719 for *M. birostris*.

Table 5.1. Population database sizes for *Mobula alfredi* and *M. birostris*. Latitude and longitude represent locations for major aggregations sites for each population, from which the majority of images were collected.

Location	Latitude	Longitude	*Mobula alfredi*	*Mobula birostris*
Andaman Nicobar Islands	11.18	92.53	4	6
Azores	37.57	-25.77	0	29
Chagos	-6.34	71.86	162	0

Christmas Island	-10.49	105.72	0	2
Cocos Keeling	-12.19	96.81	72	0
Costa Rica	8.84	-84.63	0	52
East Australia	-24.11	152.71	1,410	4
Fiji	-17.05	177.51	168	0
Florida	25.40	-81.59	0	55
Guam	13.43	144.60	44	0
Hawaii	20.71	-156.50	464	3
Japan	29.56	130.42	83	0
Komodo National Park *	-8.57	119.58	1,173	0
Malaysia	6.08	102.88	10	5
Maldives	5.24	72.99	4262	680
Marquesas Islands	-9.93	-138.94	25	1
Mexican Caribbean	21.17	-86.66	0	267
Mexican Pacific	18.69	-110.67	0	417
Mozambique North ^	-23.85	35.56	1,107	278
Mozambique South ^	-24.52	35.22	610	51
Myanmar #	15.37	96.48	0	203
New Caledonia	-21.02	165.68	360	6
New Zealand	-36.18	175.97	0	46
Palau	7.13	134.28	350	0
Nusa Penida *	-8.77	115.45	665	0
Peru	-10.25	-79.65	0	31
Philippines	12.38	122.76	185	54
Papua New Guinea	-9.00	155.10	47	0
Pohnpei	6.73	158.11	36	0
Raja Ampat	-1.03	130.51	1,021	719
Red Sea	20.28	38.51	0	29
Seychelles	-4.72	55.43	174	0
Society Islands	-16.79	-151.56	150	3

Sri Lanka	8.78	81.50	2	18
Thailand #	9.71	101.34	0	373
Tuamotu Islands	-18.49	-142.45	74	0
Western Australia	-21.91	113.92	1,108	21
Yap	9.51	138.19	50	0
Total			**13,816**	**3,319**

* ^ # Symbols indicate pairs of populations where overlap in photo-ID matches have been recorded.

The automated photo-ID tool generated predictions for matches between *M. alfredi* individuals and *M. birostris* individuals. Matches between individuals for each species were not observed among populations where no previous overlap had been reported (existing overlap is indicated in Table 5.1). Existing matches were observed in the first 10 predictions, after which no matches were observed for either species. This provided confidence that scrutinising 100 predictions was adequate, and the majority of population databases can be considered separate units for analysis.

Hypothesis 1: Mobula alfredi and M. birostris have different pattern expression

The PCA of the 256 numerical embeddings relating to manta ray ventral images showed extensive scatter, with considerable overlap between the two species of manta rays, suggesting that pattern expression is variable for each species (Figure 5.3a). However, there were clear differences in the centroids, as the SE ellipses did not overlap and the density contours for each species were different (Figure 5.3b), implying significant difference between pattern expression in these species. The adonis analysis examining the pattern expression of *M. alfredi* and *M. birostris* found significant differences between the two species of manta rays (n = 11,151, F = 663.63, df = 1, $p < 0.001$). Of the 11,151 individual manta rays in the pattern analysis, 8,188 were *M. alfredi* individuals and 2,963 *M. birostris* individuals.

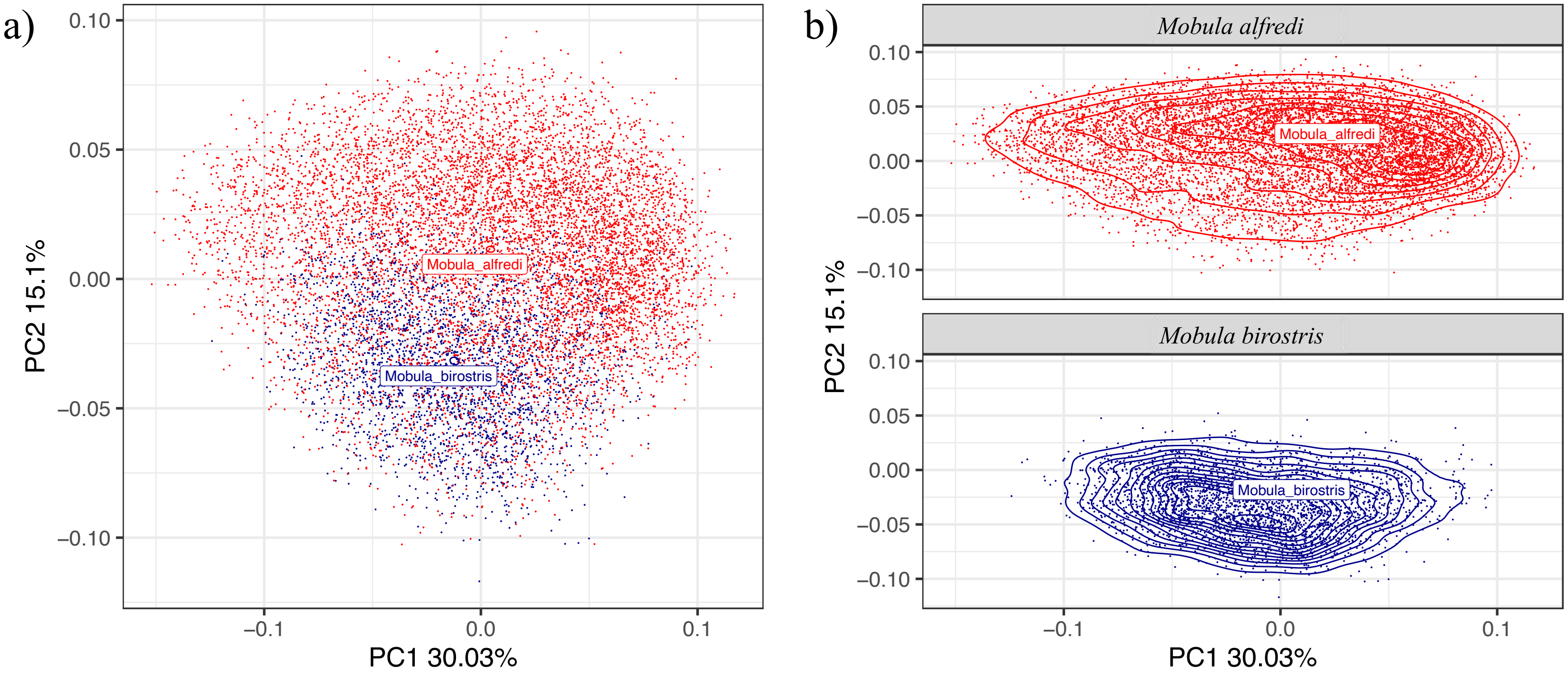


Figure 5.3. Principle Components Analysis of pattern expression in species of manta rays. Testing differences between manta ray species: a) *Mobula alfredi* (red, n = 8,188) and *M. birostris* (blue, n = 2,963), each point represents an individual ray and ellipses are standard error; and b) Density contours of PCA output to show the overlapping but different manta ray pattern expression, with species labels representing centroid location. Axes show the proportion of variance explained by the first two principal components.

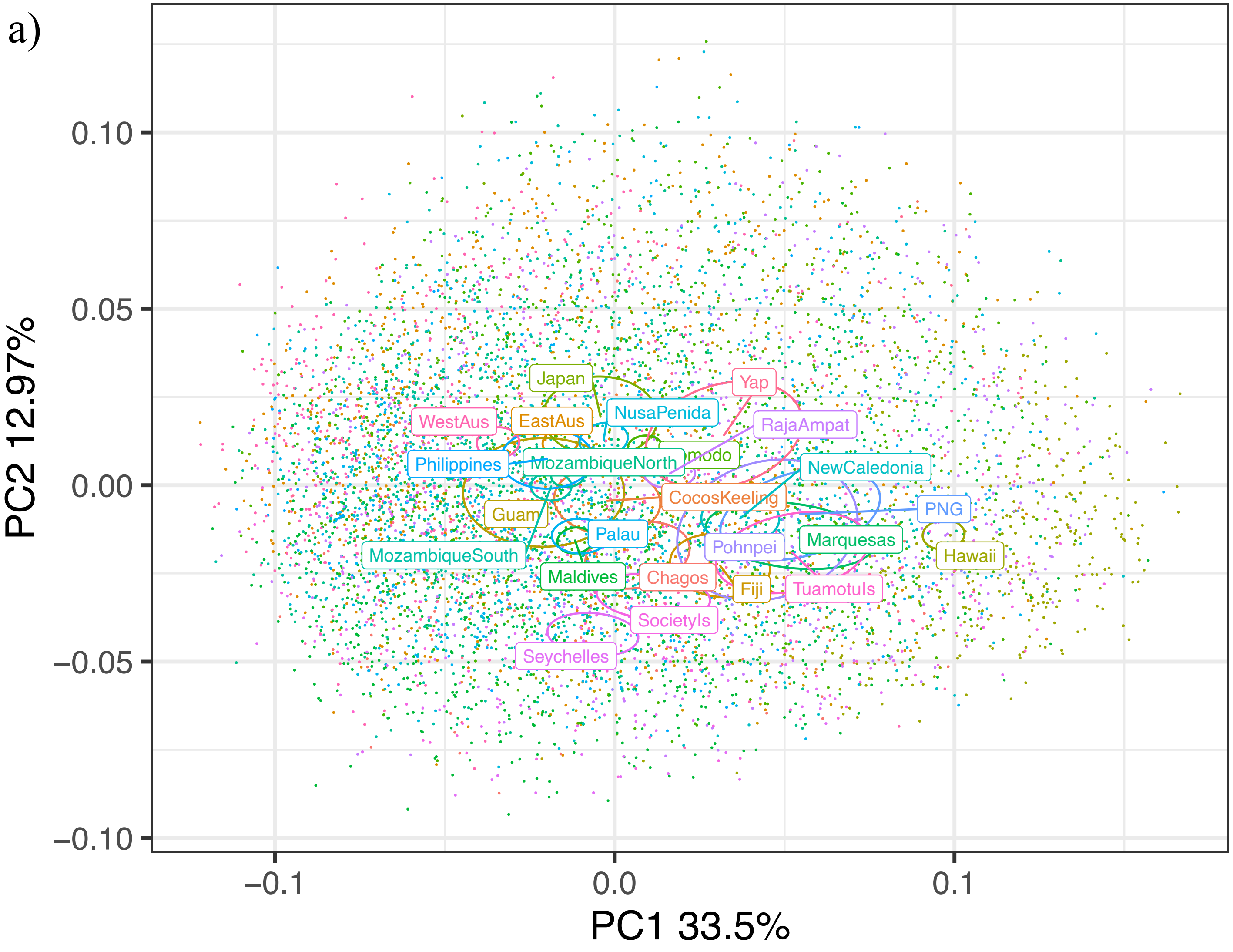
Hypothesis 2: Different populations of each species have different pattern expression

The PCA demonstrated pattern expression can significantly differentiate among populations of *M. alfredi,* where non-overlapping SE ellipses imply significant differences (Figure 5.4a). There is considerable scatter, and not all populations are isolated, but there is clear separation among many populations (adonis analysis: n = 8,188, F = 50.10, df = 23, p < 0.001).

The PCA also demonstrated pattern expression can significantly differentiate among populations of *M. birostris,* although there were fewer non-overlapping SE ellipses than for *M. alfredi* (Figure 5.4b). The adonis analysis found overall significant differences in pattern expression among some populations of *M. birostris* (n = 2,963, F = 10.52, df = 18, p < 0.001). The greater F-value for *M. alfredi* than *M. birostris* (50.10 vs 10.52) suggests less overlap among *M. alfredi* populations. This is explored further in Hypothesis 3.

To alleviate concerns around the vastly different number of images from each population, the PCAs were repeated with n < 100 for *M. alfredi* and *M. birostris* populations. The position of the mean remained the same, although the precision around the mean was reduced (see appendix, Figure A7).

a)


0.10
0.05
0.00
−0.05
−0.10
PC2 12.97%
−0.1
0.0
0.1
PC1 33.5%
Japan
Yap
NusaPenida
RajaAmpat
WestAus
EastAus
modo
Philippines
MozambiqueNorth
NewCaledonia
CocosKeeling
PNG
Guam
Palau
Marquesas
Pohnpei
Hawaii
MozambiqueSouth
Maldives
Chagos
Fiji
TuamotuIs
SocietyIs
Seychelles

b)

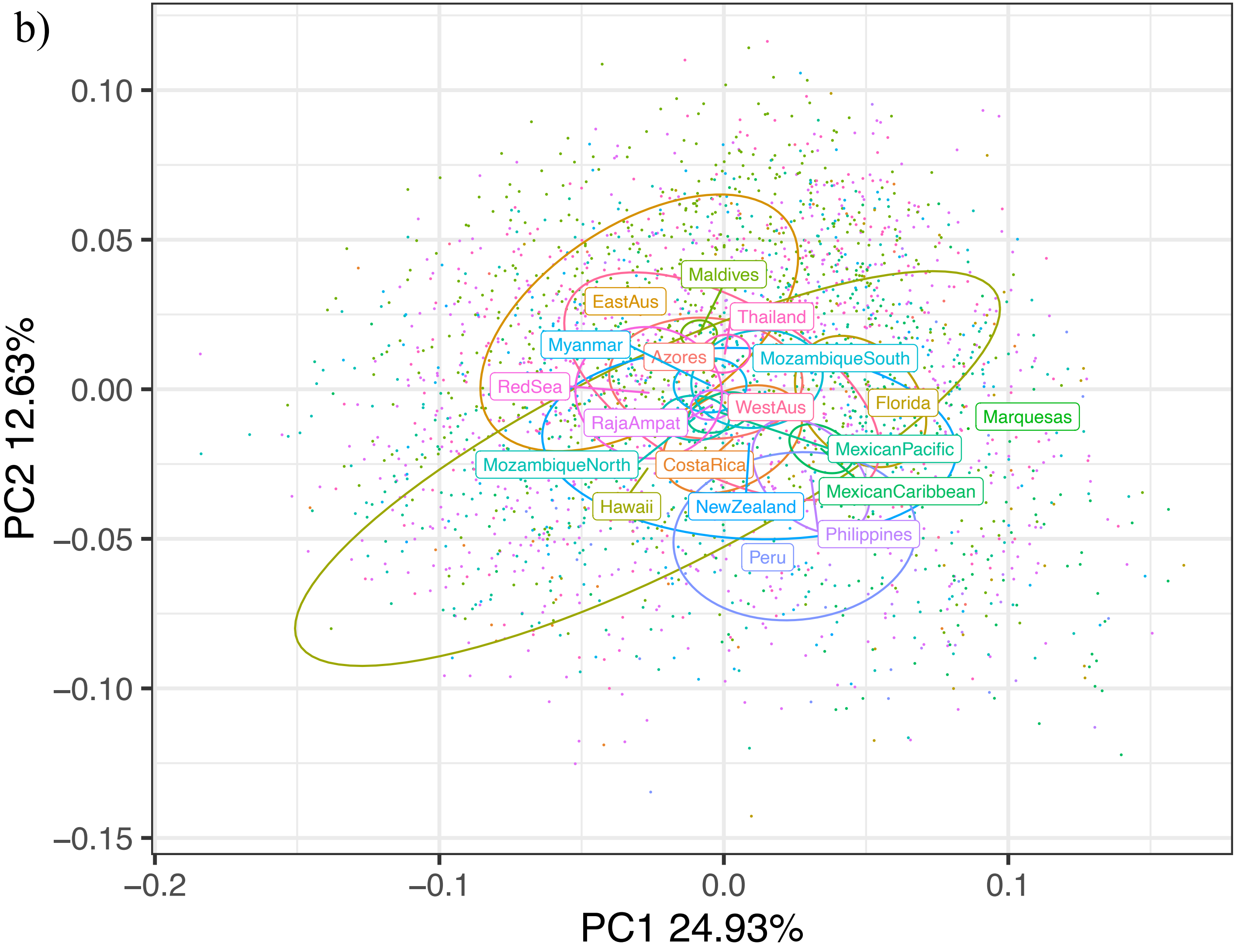

PC2 12.63%
PC1 24.93%
0.10
0.05
0.00
−0.05
−0.10
−0.15
−0.2
−0.1
0.0
0.1
Maldives
EastAus
Thailand
Myanmar
Azores
MozambiqueSouth
RedSea
Florida
WestAus
Marquesas
RajaAmpat
MexicanPacific
MozambiqueNorth
CostaRica
MexicanCaribbean
Hawaii
NewZealand
Philippines
Peru

Figure 5.4. Principle Components Analysis of pattern expression in populations of manta rays. a) *Mobula alfredi* (n = 24) and b) *M. birostris* (n = 19). Ellipses represent the standard error around the mean for each population, each population is represented by a different colour, and each point represents an individual ray (*M. alfredi* n = 8,188; *M. birostris* n = 2,963). Axes show the proportion of variance explained by the first two principal components.

Hypothesis 3: Pattern expression becomes more dissimilar with geographic distance

The ANCOVA of PC distances revealed a significant interaction between Species and Geographical distance (kms), with similar intercepts but significantly different slopes between the two species (Figure 5.5; F = 22.78, df = 3,425, p < 0.001). The degree of dissimilarity of individual markings in *M. alfredi* populations increased with Geographic distance between sites, with closer sites showing similar pattern expression and sites further apart showing dissimilar markings (Figure 5.5). By contrast, among populations of *M. birostris,* there was no relationship between PC distances of pattern expression and Geographical distances (km), which implies there is relatively more mixing among *M. birostris* populations.

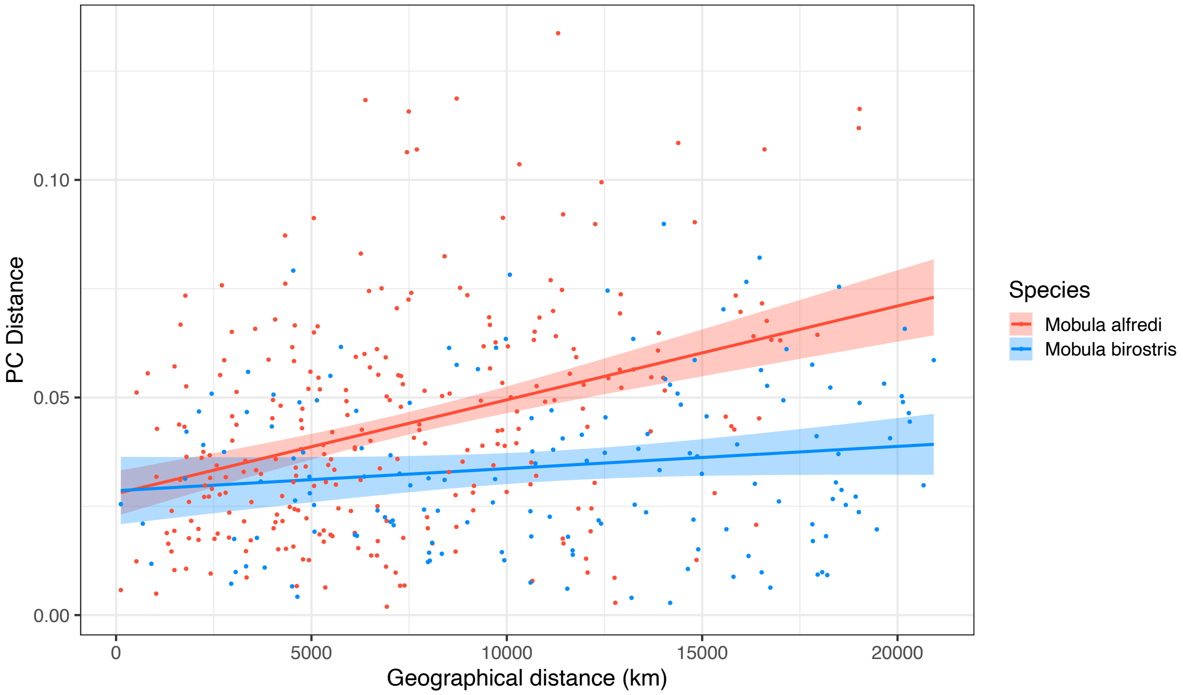
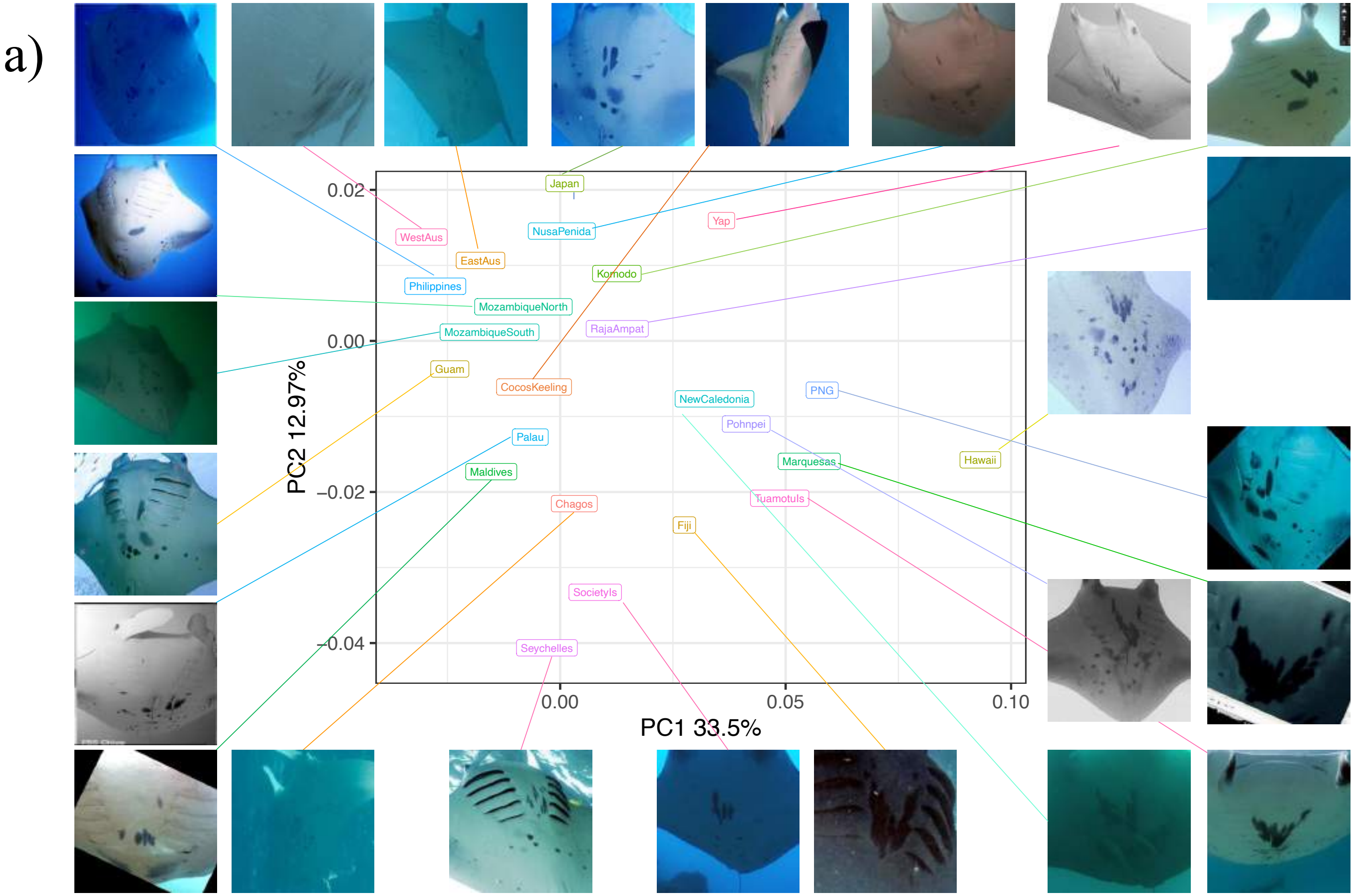


Figure 5.5. Comparing distance matrices for populations of manta rays. ANCOVA of PC distances of pattern expression and Geographical distance (kms) for *Mobula alfredi* and *M. birostris* populations. Each point represents distances between each manta ray population. 95% confidence intervals shown.

Typical individuals show greater differentiation among M. alfredi populations

Differences in pattern expression among populations of the two species were also responsible for the greater differentiation among *M. alfredi* populations (Figure 5.6). The typical individual from each population, i.e. the individual images closest to each centroid, showed that *M. alfredi* individuals were more distinct from one another (Figure 5.6a) than those of *M. birostris* (Figure 5.6b). *Mobula alfredi* populations in the bottom right of the PCA had stronger markings between the gill slits, whereas populations on the top left of the PCA had less distinct markings between the gills and darker smudges on the abdomen. By contrast, *M. birostris* populations on the left of the PCA tended to have slightly more distinct markings than populations on the right, but this pattern was not consistent.


a)
Japan
Yap
NusaPenida
WestAus
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Komodo
Philippines
MozambiqueNorth
MozambiqueSouth
RajaAmpat
Guam
CocosKeeling
NewCaledonia
PNG
Pohnpei
Palau
Maldives
Marquesas
Hawaii
Chagos
Tuamotuls
Fiji
Societyls
Seychelles
0.02
0.00
−0.02
−0.04
PC2 12.97%
0.00
0.05
0.10
PC1 33.5%

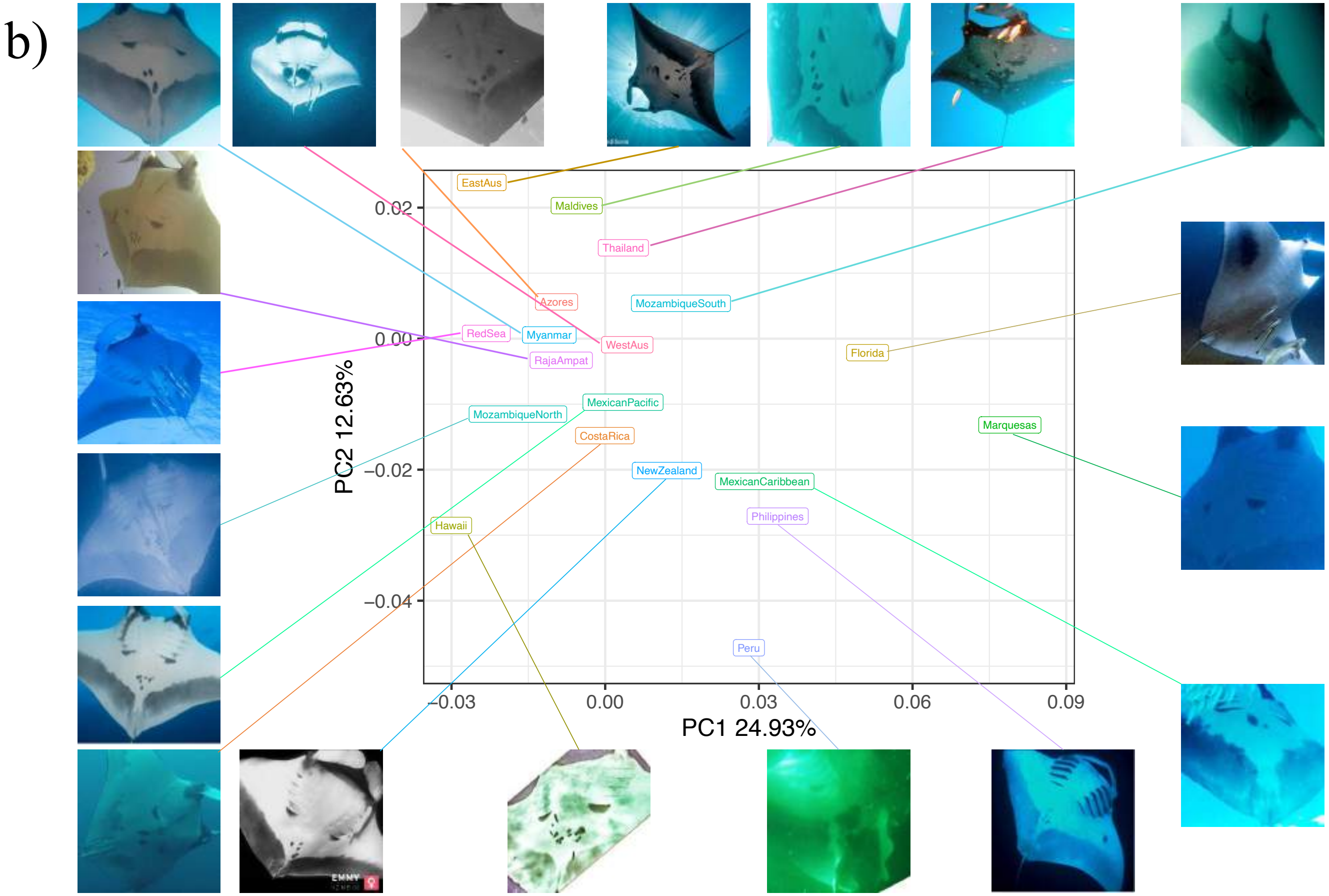


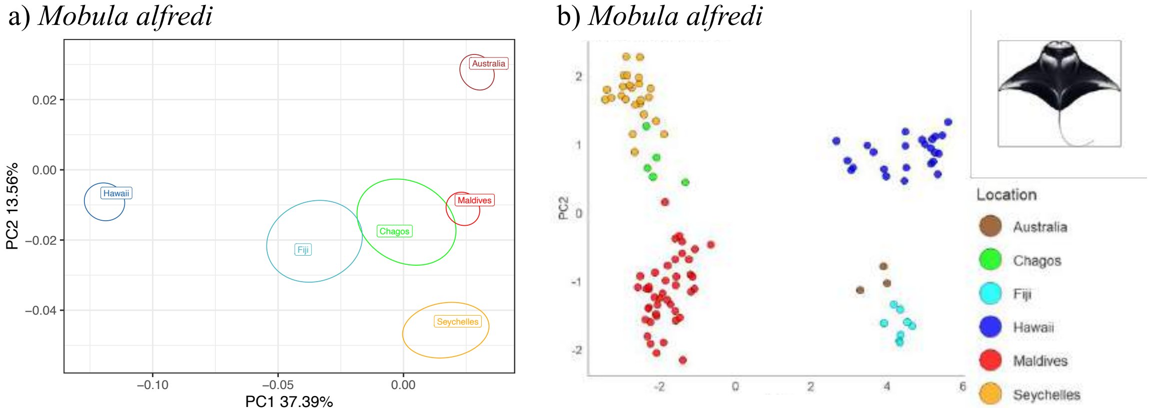
Figure 5.6. Representative individuals from manta ray populations. Each image represents the typical-looking animal from each population, by using the image closest to the centroid of each population: a) *Mobula alfredi* and b) *M. birostris*. Refer to figure 4 for standard error ellipses.

Differences in pattern expression among populations are consistent with genetic analysis results

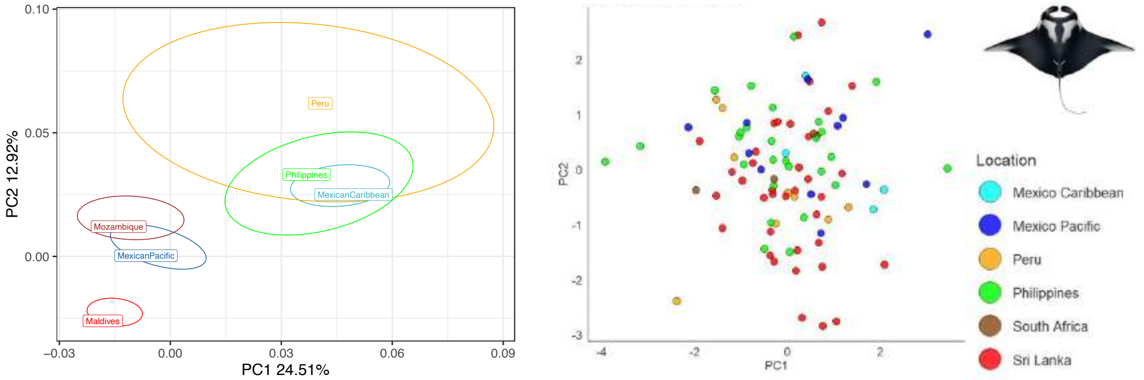
The first comparison was with a genetic study that analysed single nucleotide polymorphisms (SNPs) between geographically separated populations from across the global distributions of *M. alfredi* and *M. birostris* (Hosegood 2020). Pattern expression analysis in the current study determined that there was greater structure among populations of *M. alfredi*, with non-overlapping SE ellipses (Figure 5.7a), than among populations of *M. birostris*, which appeared to have less overall structure (Figure 5.7c), which is consistent with Hosegood's genetic analysis that found greater structure in *M. alfredi* populations than in *M. birostris* populations (Figure 5.7b and 5.7d). Both approaches found clear separation of Hawaiian populations of *M. alfredi* (Figure 5.7a and 5.7b), potentially indicative of its relative (or complete) geographical isolation from other populations. Further, the genetic analysis revealed separation of intra-Indian Ocean populations of *M. alfredi*, with Chagos, Maldives and Seychelles individuals forming clusters to the left of the PCA. The pattern expression analysis showed the SE ellipses of these same populations fell to the right of the PCA with minimal overlap. However, the SE ellipse for Fiji was also close to these populations, suggesting caution should be applied when interpreting the output as independent convergent evolution of pattern expression may occur. The pattern expression analysis for *M. birostris* lacked data from Sri Lanka and South Africa, and instead *M. birostris* populations from the Maldives and Mozambique respectively were used, due to the relatively close geographical proximity of their locations. For populations of *M. birostris*, the genetic approach revealed mixing among all populations analysed (Figure 5.7d), and the pattern expression analysis similarly revealed little structure in the complimentary populations (Figure 5.7c). The Maldives was an exception in this output, with a non-overlapping SE ellipse, however given no genetic data are available for this population a direct comparison was not possible.

Pattern analysis

Genetics analysis


a) *Mobula alfredi*

b) *Mobula alfredi*


c) *Mobula birostris*

d) *Mobula birostris*

Figure 5.7. Similar results between pattern expression analysis and genetic analysis for populations of *Mobula alfredi* and *M. birostris*. Results from principle components analysis for pattern expression analysis: a) *M. alfredi* and c) *M. birostris*. Centroids are labelled by population, with standard error ellipses. Principle components analysis for genetic analysis based on single nucleotide polymorphisms: b) *M. alfredi* and d) *M. birostris*. Points represent individuals from each population as per legend details (adapted from Hosegood 2020). Original labels from Hosegood (2020) were used, but differences equate to populations of *M. birostris* where pattern expression data was not available.

The second pattern expression comparison was with two genetic studies from southern Africa and the Australian coastline that analysed SNPs from *M. alfredi* (A.J. Armstrong *unpublished*, Venables et al. 2020b). The pattern expression analysis based on PCA of populations from southern Africa and Australia supported findings from genetic studies (Figure 5.8), whereby the SE ellipses from the Mozambique population databases overlapped suggesting connectivity, and the SE ellipses of eastern Australia and Western Australia did not overlap suggesting isolation. The Venables et al. (2020) study showed connectivity between *M. alfredi* in southern Africa and isolation from animals across the Indian Ocean in western Australia, and unpublished data from A.J. Armstrong showed isolation between populations from *M. alfredi* in western Australia and eastern Australia.

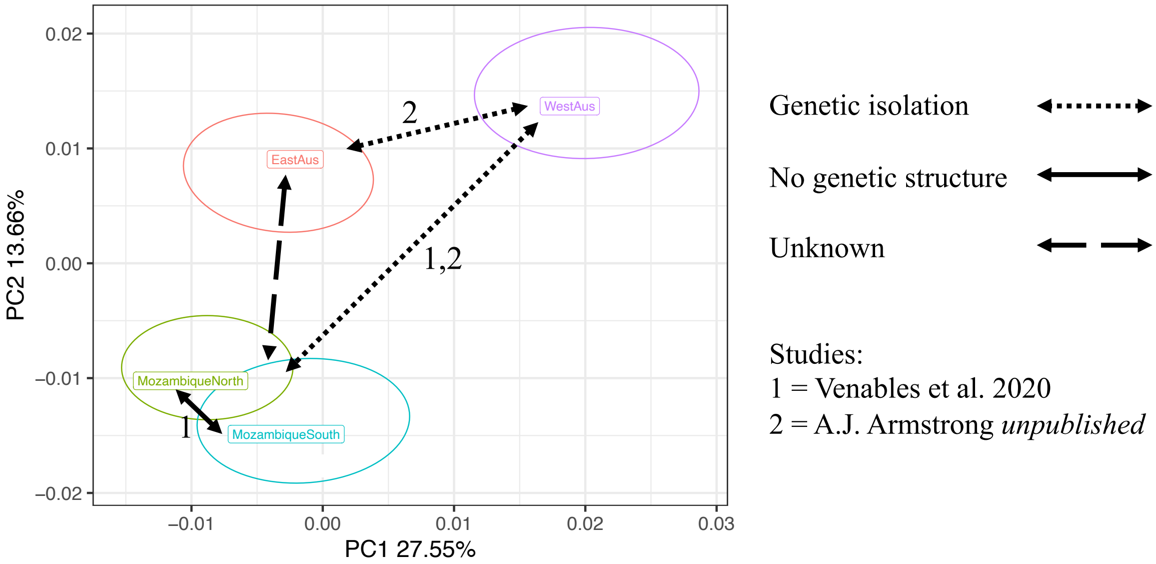


Figure 5.8. Comparison between pattern expression analysis and genetic analysis for *Mobula alfredi* populations. Principle components analysis for pattern expression analysis with standard error ellipses and centroids labelled by population (EastAus = eastern Australia, WestAus = western Australia).

Discussion

The use of artificial intelligence to analyse pattern expression in manta rays reveals differences between the two species, and among populations of *Mobula alfredi* and *M. birostris*. There is more variability in the pattern expression in *M. alfredi,* and differences among populations are associated with the geographical distance between locations. Whereas variability in pattern expression in *M. birostris* is lower, and differences are not associated with geographical distances between populations. This is potentially indicative of the species' ecology, suggesting *M. alfredi* populations are more isolated from one another, and there is more mixing in *M. birostris* which is the wider-ranging species. The use of pattern expression analysis to indicate heritability of unique markings in manta rays is supported by comparisons with genetic studies, where evidence of connectivity and isolation in populations of manta rays has complementary findings from both approaches.

Hypothesis 1: Mobula alfredi and M. birostris have different pattern expression

Analysis of pattern expressions was able to differentiate between the two species *M. birostris* and *M. alfredi*. One of the reasons for this differentiation may be the strong pigmentation that *M. birostris* displays along the trailing edge of their pectoral fins, which may help the convolutional neural networks (CNNs) to delineate between these species. This trait is one of the morphometric characteristics used to distinguish between the two species of manta rays in their relatively recent formal description just over a decade ago (Marshall et al. 2009). However, there is a considerable amount of variation in pattern expression within each species, and consequent overlap in the points of the PCA. This is potentially due to the recent evolutionary history of these species, with divergence estimates of <0.5 Ma (Kashiwagi et al. 2012). Such recent speciation of species capable of long-distance movements, and in the absence of physical barriers to gene flow, suggests that ecological forces such as habitat choice have likely played a role in speciation in manta rays (Kashiwagi et al. 2012). This spatial ecology and habitat choice between the species has implications for the connectivity of their respective populations, where *M. alfredi* is more closely associated to inshore reef and coastal habitats so is less likely to undergo broad scale movements, and the use of offshore environments such as seamounts by *M. birostris* means this species may be more likely to cross deep water and mix with other populations.

An additional consideration when examining pattern differences between manta ray species, is that of the putative third species in the western Atlantic *M. cf. birostris* (Marshall et al. 2009). During initial analysis, populations that were considered likely to contain individuals of the third species were grouped together, and a PCA for the three species was constructed. The output revealed that the third species was significantly different from the other two, but that the scatter of points largely overlapped the area of the PCA that *M. birostris* individuals occupied. These findings have the potential to support recent work that found genetic and morphological distinctions between these species (Hinojosa-Alvarez et al. 2016), however may be confounded by a lack of *a priori* knowledge about which species each individual from the input populations were identified as. Without this additional information, it was decided that this aspect of the analysis be postponed until more clarity on this putative third species has been gained.

Hypothesis 2: Different populations of each species have different pattern expression

Pattern expression analysis found significant differences among populations within both *M. birostris* and *M. alfredi,* revealing that among population variation was greater than within population variation for both species. For example, populations of *M. alfredi* from Hawaii and Seychelles were clearly separated by their pattern expression from all other populations analysed. This is likely to be due to their relative (or complete) geographical isolation from other regions, in support of the hypothesis that pattern expression is heritable and may be used to distinguish separate populations. Satellite tagging studies have shown relatively small home ranges for these large animals (maximum individual movements reported to be between 112 km and 700 km; Graham et al. 2012, Jaine et al. 2014, Braun et al. 2015, Armstrong et al. 2020b), and acoustic tracking work has revealed high site fidelity to particular habitats such as cleaning stations or productive foraging grounds (Dewar et al. 2008, Couturier et al. 2018, Setyawan et al. 2018, Harris et al. 2021). Presumably the small home ranges exhibited by these animals, that are otherwise capable of long distance movements (>1000 kms; Armstrong et al. 2019), infer their biological and ecological needs can be met locally, or that deep water presents a significant barrier to species movement.

Hypothesis 3: Pattern expression becomes more dissimilar with geographic distance

A comparison of the pairwise distances between populations and their geographic distances showed that pattern expression became more dissimilar with increasing distance for populations of *M. alfredi* but not for *M. birostris.* This is potentially indicative of differences in the movement ecology of the two species, as individual *M. birostris* generally move further than those of *M. alfredi* (~1500 kms vs <1000 kms; Hearn et al. 2014, Armstrong et al. 2019). Current evidence suggest that *M. alfredi* has greater associations and site fidelity to reef structures (Couturier et al. 2012, Armstrong et al. 2021), and tends to migrate seasonally over relatively small distances (Couturier et al. 2011, Setyawan et al. 2018). Whereas *M. birostris* is considered more of an oceanic wanderer, although definitive evidence of this is currently sparse (Stewart et al. 2018a).

Differences in pattern expression between the species are supported by global analysis of genetic structure, which found more structure observed between *M. alfredi* populations than *M. birostris* populations (Hosegood 2020). Analysis of *M. alfredi* populations around the Indian Ocean and Australian coastlines also supported the findings of isolation or connectivity observed in the genetic analyses of these populations (A.J. Armstrong *unpublished*, (Venables et al. 2020b). The current study highlights the utility of pattern expression analysis to complement the genetic findings, and may provide insight into population connectivity/isolation in regions where genetic information is not available, or difficult to acquire.

Determining population structure in threatened species is important for management and conservation, particularly for populations subject to habitat loss or fragmentation, isolation, and/or exploitation (Baguette et al. 2000, Scalici et al. 2008, Hughes et al. 2015). Tools such as genetics are useful for determining population structure (Galindo et al. 2006, Clark et al. 2008, Tenaillon et al. 2010); however, difficulty in acquiring samples can make this approach prohibitive. Whereas access to photo-ID databases can provide a means to acquire many samples relatively easily, and with the development of CNNs to address ecological problems, this approach is worth pursuing further. The findings from the current study suggest geographically separated populations of *M. alfredi* can likely be treated as separate units for conservation efforts, but that populations of *M. birostris* likely have greater mixing or potential to mix, and may benefit from broader protection measures that account for the likely connectivity between some populations.

Drivers of pattern expression

The function of pattern expression in manta rays remains an outstanding ecological question. One hypothesis is that if pattern expression is indeed heritable, perhaps it serves to prevent inbreeding (Van Oosterhout et al. 2003). This would require that a manta ray has an innate sense of what it looks like, in order to avoid mating with a close-kin relative. Work on parasitic brood species, such as cuckoos, has shown that these birds have to learn what conspecifics sound and look like, rather than having an innate recognition of conspecifics (Soler and Soler 1999). Observations where both manta ray species share habitat in sympatry have confirmed that *M. alfredi* responds to the presence of *M. birostris* by retreating from a shared resource such as a cleaning station (Marshall 2008a), suggesting that same/different species recognition is possible. However, there are also rare records that confirm hybridisation of both species in the wild (Walter et al. 2014), which may suggest there is something more complex at play. An investigation into the visual acuity system and behaviour of these species may aid our understanding as to whether pattern expression plays a role in species recognition or pattern recognition between conspecifics.

The explanation for the broader colouration pattern in manta rays, whereby they are generally lighter on the ventral surface and darker on the dorsal surface, is easier to determine. There is a reproductive advantage if animals are well camouflaged to their environment, and Thayer's Law of countershading is an example (Thayer 1918). It is observed in numerous terrestrial and marine species, in which an animal's colouration is lighter on the underside and darker on the upper side of the body to blend in with the background light conditions. However, the underlying driver(s) of individual pattern expression that manta rays display on their ventral surface is difficult to determine. The Turing pattern concept was developed to provide a mechanism for pattern development and shows that some pattern expression can be mathematically predicted (Turing 1952). However, these mechanisms fail to explain the variety of pattern expression observed on the ventral surface of manta rays.

Some animals produce disruptive patterns to break up the otherwise continuous surface of their body to confuse predators about their body shape and/or size (Cott 1940). Recent investigations into the pelage patterns of wild giraffes *Giraffa camelopardalis*, found spot patterns were likely heritable from mother to calf, and different phenotypes (based on spot size and shape) impacted neonate survival (Lee et al. 2018). Lee et al. (2018) suggested that survival in this species is related to camouflage, but could potentially reflect pleiotropy of pattern expression with other traits impacting fitness. Given manta rays are large and conspicuous animals, with few natural predators and high survivorship rates (Couturier et al. 2012), their unique markings are unlikely to undergo selective pressure, and thus mutations in patterning and shading are likely to persist in the gene pool. Thus, a more likely explanation for observed differences in pattern expression in manta rays, is that regional differences are a byproduct of the level of inbreeding at particular geographic locations, and that the persistence of mutations in pattern expression over time, has likely led to the regional distribution of distinct patterns and colour morphs observed throughout their range. However, this discussion could benefit from regional exploration of these trends to investigate whether selection pressure (sexual or otherwise) operates at a regional or ocean basin level.

These findings provide insights into underlying evolutionary processes, and raise questions about the impact of historical dispersal or contemporary connectivity on populations pattern expression. The concept of character displacement may be of relevance to differences observed in pattern expression in reef manta rays and oceanic manta rays where their distributions overlap. Character displacement is a term used to describe when two similar species inhabit the same environment and an evolutionary change occurs (Brown and Wilson 1956). Under certain conditions, a divergence in a species characters, such as behaviour, morphology, or physiology may be favoured by natural selection (Losos 2000). For example, research into two species of African tinkerbirds, found character displacement in phenotypic traits facilitated species recognition (Kirschel et al. 2009). This mechanism could provide a contemporary, rather than historic, explanation for the observed distribution of pattern expressions in the two species of manta rays.

Utility of convolutional neural networks

The CNN used in this analysis was shown to have 90% accuracy in matching images of individual manta rays (Moskvyak et al. 2019), which for our ecological question provides confidence in the pattern being detected, without the inherent concerns of some other applications of CNNs (i.e., facial recognition for border security, or driverless cars). In ecology, CNNs have broadly been used to assess landscape features (Brodrick et al. 2019); find and count animals in remote videos (Weinstein 2018); detect sea turtles from drone imagery (Gray et al. 2019); and assess vegetation types for weed management (Wang et al. 2018). Here, the use of CNNs to detect patterns in unique markings from images of manta rays is a novel and effective way to provide insights into population connectivity. This approach could be adapted to investigate the population connectivity and isolation on a range of species that exhibit unique natural markings that are persistent through time, and where effort has already resulted in photo-ID databases throughout these animals' distribution. In the marine environment for example, large global databases exist for the endangered whale shark *Rhincodon typus* (Araujo et al. 2020a); and regional databases for the critically endangered grey nurse shark *Carcharias taurus* (Bansemer and Bennett 2008), and this application of CNNs may provide a non-invasive approach for assessing these at-risk populations.

Yet there are certain aspects of how and when CNNs work, that remain unclear (Liu et al. 2017). For example, with facial recognition, there is a wide range of identification information that must be processed and thus a single feature cannot be the focus, and instead a fusion of multiple features is required for achieving robust facial recognition performance (Li et al. 2018). However, this can lead to erroneous outputs. For example, high quality face morphing or image slicing can fool facial recognition technology, presenting a risk to border security at automated immigration check points (Wandzik et al. 2017). In certain scenarios, even small errors can represent an unacceptable risk. The CNNs behind driverless cars, with an accuracy of 99.3% of detecting the correct traffic signal, are still in development due to the danger of that 0.7% inaccurate prediction (Ouyang et al. 2020). For ecologists that are interested in measuring and describing patterns in nature, if a CNN is successful at achieving this, then the underlying mechanics, and small chances of error, are less important; i.e., CNNs are useful to sort through the variation in patterns in nature, and what the individual morphometric values represent are not necessarily important.

Limitations and future directions

There was considerable variation within populations, and inferences about connectivity and isolation need to be made with caution. For example, this approach is not currently able to answer questions about whether the distribution of certain pattern expressions is due to recent evolutionary history, or contemporary movement of the animals. Nor can it distinguish whether certain pattern expressions represent lineages within populations and are representative of inherited characteristics. To overcome these issues, more development of the current approach is required. A useful development would be to train the CNN to certain landmarks on the rays (e.g., pectoral fin shading, gill slits etc.) to improve our interpretation of the weighting on particular features of the animals. Further, a missing component in the analysis is the inclusion of melanistic individuals, which may provide important insights into the observed patterns. Future work could explore how morphometric variations such as pattern expression reflect spatial distributions, by investigating associations in colour polymorphism frequencies and environmental parameters (such as latitude, longitude and temperature) in global populations. Where spatial information is available about the sighting history of individuals from within populations, investigations could also be undertaken to explore whether fine-scale structure exists within populations.

Conclusions

This work presents a novel approach of artificial intelligence to provide evidence of heritability of pattern expression in manta rays. The findings of higher variability in pattern expression among populations of *M. alfredi* in comparison to populations of *M. birostris* has important implications for the management of these species, suggesting *M. alfredi* may be afforded protection by local management approaches, but that *M. birostris* likely requires broader level management to ensure adequate protection of this endangered species. The outcome of this work highlights the importance of global collaborations in research efforts to effectively address questions at a broader scale. This work is important because it provides evolutionary insights into the radiation of these threatened species globally, and emphasises how spatial ecology can influence the distribution of natural pattern expression in animals.

Chapter 6

General Discussion

This thesis has answered key questions regarding the spatial ecology of manta rays. Firstly, cleaning stations were shown to influence the site selection and preference for manta rays in reef environments by providing an environment to promote mutualism between cleaner fishes and their clients. Secondly, investigations into the zooplankton dynamics in Hanifaru Bay in the Maldives revealed that tidally driven concentrations of these microscopic organisms are responsible for feeding aggregations of reef manta rays at the largest known aggregation site for this species. Next, the use of citizen science provided new records for long-distance movement of individual reef manta rays, and confirmed the species' occupancy of coastlines where such data were previously absent due to low or no human presence. Finally, an advance in convolutional neural networks (CNNs) was applied to photographic identification (photo-ID) databases of manta rays, to provide potential evidence of heritability of pattern expression in these animals, and connectivity or isolation of global populations. By working across various spatial scales, this thesis has contributed to our fine scale understanding of space use by manta rays in reef environments, as well as expanded our understanding on the connectivity of global populations of these large charismatic animals. Here I explore these findings in a broader context, explain caveats and limitations of the work, and provide suggestions for future research directions.

The importance of cleaning stations

Movement studies have primarily focused on two fundamental drivers of species movements, those that meet either reproductive or foraging needs. Chapter two of this thesis has challenged that idea, by providing evidence to support expanding the potential mechanisms investigated in movement studies and proposing an alternative driver of movement in the form of cleaning symbiosis for body maintenance. This investigation into manta rays use of cleaning stations found that mutualism can promote site selection in a large-bodied, mobile marine species. Fine-scale site use by manta rays was strongly associated with the distribution of the obligate cleaner *Labroides dimidiatus*, and the hard coral structures where this site-attached species has established cleaning stations. We found that interactions with cleaner species were the most commonly observed behaviour in the client, taking priority over other life history traits such as foraging and reproductive behaviour at these sites.

In chapter two we observed that manta rays preferred cleaning station areas consist of high cleaner wrasse densities and hard coral structures, but there are likely more factors that contribute to “quality” cleaning environments. For example, current regimes at these sites likely influence their cleaning station preferences. As in other pelagic elasmobranch species, manta rays need to keep swimming to ventilate their gills. They are unable to rest on the bottom to facilitate cleaning interactions, as do demersal lemon sharks or the Caribbean reef shark *C. perezi* for example (Keyes 1982, Sazima and Moura 2000). Grey reef sharks *C. amblyrhynchos* at Ningaloo Reef are observed to visit the only known shallow-water inshore cleaning station for this species (Wheeler et al. 2013). Here, sharks face into the current and pose for the cleaner wrasse, and it is suggested the fast currents at this site facilitate sharks hovering to clean and still meet their oxygen demands. Cleaning events for *M. birostris* at a seamount in the Philippines (Murie et al. 2020), and for *M. alfredi* in southern Mozambique (Rohner et al. 2013) were influenced by currents, as moderate currents are favourable to a manta ray’s ability to hover and facilitate cleaning. These findings suggest the preference to the prominent hard coral structures in chapter two may not just be related to suitable habitat for the cleaner wrasse, but also linked to flow dynamics that facilitate cleaning interactions.

The work in chapter two highlights that to understand the mechanisms behind spatial variations in ecological phenomena (i.e., movements and habitat selection of mobile species), it is vital to investigate relationships between organisms and their environments (de Knegt et al. 2010). Interestingly, we found that a community of small cleaner fishes (<12 cm TL) are implicated in driving the site selection of some of the larger fishes in the sea (<5 m DW), which demonstrates the disproportionate influence these small fish may have on reef environments (Waldie et al. 2011). Yet how manta rays remember particular cleaning environments remains to be determined. This is part of the big question about how many marine animals – fishes, reptiles, birds mammals – are able to navigate over vast distances to, often very specific areas for e.g. mating, nesting/egg-laying/spawning/ pupping, feeding, or cleaning (Hays et al. 2016b). The relative roles of an innate sense of direction, and memory of particular landmarks is unclear. The work in chapter two proposes that the reef association observed in these large mobile planktivores is driven by their mutualistic relationship with cleaner fish, and that their site choice may be the result of a fine-scale cognitive map of the reef environment.

Memory and cognition

Memory and learning likely play an important role in the efficient movements and foraging behaviours of marine megafauna (Regular et al. 2013, Scott et al. 2014). These can generally be inferred from site fidelity to certain locations, but in reality can be difficult to quantify (Fagan et al. 2016). In chapter two we observed high levels of site preference in manta rays to particular cleaning stations, however confirming manta ray memory or cognition in these circumstances – beyond a visual assessment based on obvious features or the presence of cleaners – is challenging. One way to test their cognitive abilities could be to conduct a manipulative exclusion experiment, whereby cleaner wrasse are removed from high-use cleaning stations. Sites could then be monitored to record whether manta rays return to these previously "preferred" locations or whether they seek cleaning services from elsewhere. Previous research in laboratory settings have revealed that sharks and rays do have spatial memory, can learn and habituate, and solve cognitive tasks (Schluessel 2015). Experiments have confirmed they have the ability to solve spatial tasks and retain information for extended periods of time (Schluessel and Bleckmann 2012). In particular, freshwater rays have the ability to construct a cognitive map of their environment, and use complex orientation strategies to solve problems (Schluessel and Bleckmann 2005). These findings may help explain how manta rays can navigate large complex habitats and remember important locations for fulfilling life history tasks.

Limitations of scale

Gaining information on the spatial and temporal scales at which animals access particular locations can help identify critical habitats and important sites to inform on species' life history characteristics (Wiley and Simpfendorfer 2007). Chapter two utilised passive acoustic telemetry for continuous monitoring of tagged manta rays. However, some drawbacks to this approach are that only tagged animals are monitored, and only animals moving within the range (<800 m) of acoustic listening stations will be detected (Heupel et al. 2004), which presents a real challenge for monitoring highly mobile elasmobranch species. This study used an alternative to single acoustic listening stations, in the form of a VEMCO Positioning System (VPS). While such systems produce high-resolution tracking data that relates to animals' fine-scale movements (Espinoza et al. 2011, Furey et al. 2013), they generally only provide restricted spatial coverage. The natural world is patchy (Perry et al. 2002), and cleaning stations exist in a patchwork mosaic across the reef. Setting up this study at a fine-scale makes it difficult to understand how much of the daily energy budget of manta rays is spent at these cleaning station sites. Hence there is a need to expand these investigations to a larger scale, to examine how *M. alfredi* budgets its time, habitat occupancy, and behaviour in the near reef environment.

Quality cleaners and community diversity

Numerous questions relating to cleaning stations remain. For example, establishing what comprises a "quality" cleaning environment is key to understanding the movements and site preference of manta rays for certain locations. A detailed investigation into the cleaner fish community, and whether different species of cleaner fish preference particular regions of manta rays would help address the question of "quality". Optimal forage theory dictates that feeding is likely to occur where resources are highest (Stephens 1986) and abundance of ectoparasite loads has been shown to correlate with client size (Grutter and Poulin 1998). Cleaner wrassees (*Labroides dimidiatus* and *Thalassoma lunare*) preferentially forage in the pelvic region of pelagic thresher sharks *Alopias pelagicus* (Oliver et al. 2011), and dead specimens confirmed high numbers of digenean flatworms in this region, providing an explanation for the body site preference in the cleaner wrasses (Cadwallader et al. 2014). Owing to the absence of dead specimens, and the large-bodied nature of manta rays, their ectoparasite loads would be difficult to quantify. However, exploring cleaner fish abundance, diversity and resource partitioning on cleaning manta rays would help answer these questions. Niche partitioning has been observed in the community of cleaner fishes that attend to giant sunfishes *Mola mola* (now *M. alexandrini*) attending cleaning stations in Indonesia (Konow et al. 2006). For manta rays, investigations into the community of cleaner fishes in Mozambique found that cleaner fishes preferenced manta ray clients over smaller resident clients (Marshall 2008a), and that cleaner fishes demonstrated resource partitioning when multiple species were present, but would become more generalist when species diversity was lower. However, the cleaner fish community showed significant variation, even within this study site in the western Indian Ocean. This indicates a need to investigate evidence of niche partitioning and cleaner fish diversity at multiple aggregation sites. Further, there is a paucity of data in this regard for other more cryptic elasmobranch species that may also rely on shallow reef environments for aspects of their body maintenance. Examining the functional diversity in the cleaner fish community for a range of taxa would help us understand what impacts declining reef health may have on the site selection and health of these large threatened species.

Foraging ecology of large tropical planktivores

The findings of chapter three in this thesis demonstrate that manta rays target recurring high density zooplankton patches that are likely to exceed their energetic requirements. Manta rays are filter feeders that rely solely on some of the smallest marine animals, zooplankton, to meet their energy requirements (Paig-Tran 2012). Like the great whales, manta rays feed on the primary and secondary consumers of the marine biosphere (Murison and Gaskin 1989, Couturier et al. 2013), restricting themselves to lower trophic levels in the food chain (Lawson et al. 2019). The largest elasmobranch species, basking sharks *Cetorhinus maximus*, whale sharks *Rhincodon typus* and megamouth sharks *Megachasma pelagios*, are also primarily zooplanktivores (Sims and Merrett 1997, Nelson and Eckert 2007, Nakaya et al. 2008). Numerous studies have investigated the energetic requirements of these large animals, in an attempt to understand how they make a living off such patchily distributed resources (Sims 1999, Rohner et al. 2015, Lawson et al. 2019, Fortune et al. 2020). For the great whales, many have the thermal capacity to undergo large migrations and access highly productive polar waters (Murison and Gaskin 1989, Nowacek et al. 2011, Burkhardt and Lanfredi 2012). But for elasmobranchs like manta rays, they need to target ephemeral productivity hotspots in generally warmer and nutrient-poor waters. Chapter three demonstrates that the prey density threshold for manta rays foraging in Hanifaru Bay in the Maldives is more than four times what has been observed at other large planktivore feeding aggregation sites (Rohner et al. 2015, Armstrong et al. 2016, Burgess 2017), and more than double what has been calculated to meet their theoretical energy requirements (Armstrong et al. 2016). The ten percent law of ecological efficiency in energy transfer states that only about ten percent of the transferred energy between trophic levels is retained by the organism (Lindeman 1942). Perhaps the nutrient uptake from eating many thousands of small organisms, rather than the energetic cost of hunting one large organism, is more rewarding for these large planktivores. The aggregation of reef manta rays foraging in Hanifaru Bay in the Maldives is the largest on record and occurs seasonally (Harris et al. 2020), so potentially these large planktivores take advantage of the tidally-driven high density food environment described in chapter three to supplement lower prey availability at other times of year. A global synthesis of planktivore prey availability and composition would provide valuable insights into the importance of prey concentrations at key foraging sites.

Challenges of measuring the food environment for mobile species

In chapter three, the approach used to examine the zooplankton feeding environment of *M. alfredi* suffered some methodological issues. The spatial issue for this study was less about scale, and more about accessing the vertical space. There is a need to access the zooplankton community throughout the water column, which is logistically challenging, especially in a location where boat traffic is not allowed. Zooplankton species migrate vertically in response to predation (Gibson et al. 2009), and the variety in feeding strategies that the manta rays employ may be in response to this. However, in the current study we only focused on the surface layer due to logistical limitations. The temporal scale of this work was also an issue, as we were not able to sample *M. alfredi* feeding aggregations with over 25 individuals, and so may have only gained some insight into the zooplankton dynamics at large planktivore feeding sites, but not the whole picture.

Quality food for large planktivores

An important concept relating to foraging for large planktivores is whether their behaviour is the relative importance of quality or quantity in their food source. Optimal foraging theory dictates that they should be targeting not just abundant food sources, but also energetically rewarding sources in terms of calorific content (Stephens 1986). This concept needs to be explored in more detail and future work would benefit from calorific exploration of zooplankton at important foraging sites such as that of Hanifaru Bay in the Maldives. It may be that manta rays in tropical waters cannot be highly selective about where their next meal comes from, and so they opt for quantity over quality. In addition, a more detailed investigation into the vertical structure of the zooplankton community would help elucidate the feeding strategies employed at Hanifaru Bay in the Maldives, and whether these strategies are adaptive to the prey environment or in response to shared space use with other animals (Stevens 2016).

Feeding-cleaning hypothesis

From the findings in chapter two, together with other recent work (Stevens 2016, Murie et al. 2020), we have put forward a hypothesis that preferred cleaning station sites are likely paired with rich feeding grounds nearby. The study location in chapter two is approximately seven kilometres from the continental shelf edge, and the mesoscale oceanographic feature of the Capricorn Eddy (Weeks et al. 2010). The productivity of the Capricorn Eddy is a result of increased frontal activity and upwelling, providing foraging opportunities to seabirds such as wedge-tailed shearwaters *Puffinus pacificus* (McDuie et al. 2018), and has been associated with foraging of manta rays (Jaine et al. 2014). Cleaning station environments, at which manta rays may stay in close proximity for long periods (i.e., weeks to months), would need to be in the vicinity of places that fulfill the multiple biological and ecological functions of these animals. The study site of Hanifaru Bay in chapter three, where we explored the feeding environment at the largest known aggregation of manta rays, also contains two prominent cleaning stations, and manta rays are frequently observed being cleaned at these sites before and after foraging events (Stevens 2016). A study from 2019 proposed a "Feeding-Cleaning Trade-off" for manta rays moving between these two activities (Barr and Abelson 2019), however the study lacked the empirical evidence to back up its claims regarding manta ray "decision making" in this regard. Here, I propose the feeding-cleaning hypothesis – where mobile species such as manta rays select cleaning sites close to productive foraging opportunities – and I posit this hypothesis may also explain the habitat preferences of other large, mobile client species. In order to address this question, it would be necessary to track animals, both during their fine-scale habitat use at cleaning sites, and at broader scales, such as frontal systems or continental shelves, where they likely access productive foraging conditions. This could be achieved by dual tagging animals with both acoustic and satellite transmitters to provide coverage across these spatial scales.

The utility and drawbacks of citizen science

To investigate aspects of manta ray ecology such as feeding, we need to know where these animals are found, and the displacement distances they are capable of undergoing in order to exploit such productivity hotspots. Chapter four of this thesis has provided evidence of the largest point-to-point movements for reef manta rays, and has also updated their known distribution around the Australian coastline. Gaps in baseline information for such large animals may seem surprising, but for non-airbreathers these data are likely decades behind that of other marine megafauna taxa such as sea turtles and cetaceans, where distributions and movements are better understood (Henwood and Ogren 1987, Polacheck 1987, Wade and Gerrodette 1993, Monzón-Argüello et al. 2009, Casale 2010, Hammond et al. 2013). This is likely due to the challenges involved in tracking animals that do not need to surface to breath or visit land to reproduce. For example, the ability to access sea turtles at nesting beaches facilitates filling knowledge gaps such as “the lost years” (Putman and Naro-Maciel 2013, Mansfield et al. 2017). The work in chapter four of this thesis has highlighted that for non-airbreathers, alternative research approaches can provide insights where traditional research methods may be prohibitive due to cost or remote locations (Dudgeon et al. 2019a). Filling in knowledge gaps regarding the extent of these threatened rays in Australian waters is an important goal for their conservation. Manta rays are afforded protection in these waters via local legislation and international agreements such as the Convention of Migratory Species Act (Lawson et al. 2017), whereas their populations are threatened through much of their global range due to targeted fisheries (O'Malley et al. 2017). Consequently, knowing they are capable of long-distance movements, and inhabit areas along the northern Australian coastline in close proximity to other jurisdictional boundaries, raises concerns about how connected their global populations are.

The use of citizen science for collecting biological or ecological data is still controversial (López et al. 2020). There is some skepticism surrounding the robustness or rigor of this approach, which is warranted for accurately addressing certain questions regarding aspects of animal ecology, such as estimating populations size (Marshall and Pierce 2012). However, for improving records on distribution or long-distance movements such as those in chapter four, particularly in remote areas where field work can be costly or logistically challenging, public contributions can fill in gaps where other methods cannot. Using photographic identification (photo-ID) to update long distance travel is also biased by where photo-ID is most common, such as coastal versus offshore locations. Movement into ocean basins away from land reduces or eliminates the possibility of their being photographed and added to a photo-ID database, and thus the movement capabilities of these large animals may be underestimated. This may explain the paucity in sightings of *M. birostris* around the Australian coastline as this species is known to utilise offshore sites such as deep seamounts and oceanic fronts (Couturier et al. 2012), which may present a barrier to the average citizen scientist. As such, our understanding of its distribution and movements could be improved by input from a different category of citizen scientists, such as commercial fisheries, game fishers, oil and gas workers and those operating offshore. Other considerations include the need for trained megafauna spotters on aerial surveys, otherwise species-level identification may not be possible which may preclude use of those data in studies.

Machine learning can identify patterns in huge datasets

Assessing global population connectivity can present a challenge to researchers. Chapter five of this thesis presented a novel approach to this field by using artificial intelligence to provide evidence for heritability of phenotypic traits in manta rays. One of the interesting findings in this chapter was the suggestion that *Mobula alfredi* populations are less connected than *M. birostris* populations. This finding was supported by genetic studies that have indicated less structure between geographically separated populations of *M. birostris*. This is perhaps unsurprising given the larger body size of *M. birostris* (Disc width >7 m) over *M. alfredi* (Disc width <5 m). Meta-analysis has shown that swim speeds increase with body size, particularly for species capable of endothermy, such as tunas and white sharks *Carcharodon carcharias* (Watanabe et al. 2015). Mobulid rays, including manta rays, have a counter-current heat exchanger that warms the brain (Alexander 1996), which may suggest they are capable of muscle warming to enhance swim speeds (Dickson and Graham 2004). Although this has not been confirmed. Another meta-analysis of body size of numerous marine megafauna taxa (such as sea turtles, fishes, and mammals) has shown that migration distances scale with body size, wherein larger animals actively move further than smaller animals (Hays and Scott 2013). These studies provide support for the finding that *M. birostris* populations are likely more connected than *M. alfredi* populations as a product of their body size and how that influences the broad scale movement and habitat preference of animals.

There is a revolution in the application of machine learning tools such as CNNs. In the last few years CNNs have variously been used to: detect attention deficit hyperactivity disorder in adults using electroencephalography (EEG) data (Dubreuil-Vall et al. 2020); determine emotions in static facial images (Levi and Hassner 2015); diagnose breast cancer cells through histology images (Rakhlin et al. 2018); and annotate gene expression patterns in the mouse brain (Zeng et al. 2015). Here, this tool has been used to sift through the variation in pattern expression of thousands of manta ray images to detect differences between species and populations of these threatened rays. To our knowledge, this is the first such application of its kind, but has the potential to provide insights into population structure for many other animals that exhibit unique natural markings that are persistent through time. For example, the original purpose of the CNNs used in chapter five, was to distinguish between individual manta rays to automate photo-ID efforts (Moskvyak et al. 2019). For chapter five the output was adapted for a different application, to look for differences at the species or population levels. This same CNN has since been applied to the endangered whale shark *Rhincodon typus* to improve automated photo-ID of these large animals (WildMe 2021), but could potentially inform on differences among global populations of this species. Likewise this CNN could be adapted to work with other threatened species, such as the tiger *Panthera tigris* (Goodrich et al. 2015), zebra shark *Stegostoma tigrinum* (Dudgeon et al. 2019b), and African wild dog *Lycaon pictus* (Woodroffe and Sillero-Zubiri 2020), as a non-invasive way of assessing populations where photo-ID databases exist and where genetic sample collection can be challenging.

Trade-offs in machine learning

The uncertainty around what convolutional neural networks actually measure makes it difficult to interpret some of the output of the pattern analysis. Morphometric analysis has been used to address numerous questions about organismal biology, such as species identification (Cope et al. 2012), genetic mutations (Toussaint et al. 2021), and animal development and evolution (Lawing and Polly 2010). However, as with other fields of research, different approaches provide different information, and there are benefits and trade-offs. For example, there are numerous approaches when it comes to dietary studies. Stomach content analysis can provide detailed information about the recent food intake of an animal (Bennett et al. 2017), but cannot provide a temporal overview of a species' diet and often conducted after the death of the animal. In contrast, stable isotope analysis is minimally-invasive, that allows for inferences about an animals' diet over a longer time scale to be made, but does not provide direct information on the species consumed (Couturier et al. 2013). Here, pattern analysis has revealed differences between species or populations of manta rays when existing metadata is available, however it is important to point out that these findings are not predictive, and the approach will require more development to extend its utility.

Further applications of machine learning

A missing component in the current analysis from chapter five is the inclusion of melanistic individuals. Manta rays are quite unique amongst elasmobranchs, in that they exhibit polymorphic variations in colour expression, and their populations display varying frequencies of this trait (Venables et al. 2019). Further development of the CNN used in chapter five is required to process melanistic individuals, and determine whether this trait can provide additional insights into the observed patterns. Future work could explore how morphometric variations such as pattern expression reflect spatial distributions, by investigating associations in colour polymorphism frequencies and environmental parameters (such as latitude, longitude and temperature) in global populations. In addition, our current approach can be further tested in a number of ways. For example, as more genetic studies are undertaken more comparisons will be possible, and a synthesis of available satellite tracking data could also inform on likely areas of connectivity or isolation to be tested.

Investigations could also be undertaken to explore regional differences in pattern expression within populations and provide potential insights into barriers for movement. For example, on the east coast of Australia, individual manta rays sighted north of the Townsville region (~19°S) have not been sighted south of this area, and vice versa (A.O. Armstrong *pers. comms*.). This may be due to the bifurcation of the South Equatorial Current, as it approaches the coastal boundary of the Australian mainland at approximately 18°S (Ridgway and Dunn 2003). Boundary currents have the potential to interrupt the movements and dispersal of animals (Thompson et al. 2018). This mechanism is more apparent for larvae that rely on currents for dispersal, as currents can represent physical oceanographic barriers to dispersal for these species; for example coral larvae (Thompson et al. 2018); mollusc larvae (Rachel et al. 2007); and larval eels (Miller and Tsukamoto 2016). However, for more mobile species their distribution in relation to ocean currents is more likely related to foraging opportunities and energetics. For example, satellite tracking of sea turtles has revealed they time their movements with seasonal changes in the strength of currents to minimise the energetic cost of such movements, and maximise their access to productive frontal systems (Mansfield et al. 2017). For large-bodied animals such as manta rays, currents are unlikely to represent a physical barrier to movement, but rather influence the productivity hotspots that they exploit, and thus indirectly influence the structure of their populations. This hypothesis is worth exploring in populations of manta rays where spatial information on sightings that is relevant to oceanographic features is available.

In chapter 4.1 we reported the largest known distance movement for *M. alfredi* individuals. Two individuals travelled over 1,500 kms along the east coast of Australia, and were recorded at the *SS Yongala* south of Townsville and south of the bifurcation of the South Equatorial Current. Although this represents a substantial increase in movement distance for this species (formally ~650 kms), neither ray has subsequently been recorded travelling further north and thus the question about regional populations along this coastline remains. One of the individual rays has since been recorded attending the high-use cleaning station area at the southern aggregation site of LEI (in August 2021), which demonstrates remarkable site fidelity for this species, even in the face of such long migrations. Given the findings of chapter two, which indicate the importance of cleaning stations to manta ray body maintenance and their spatial ecology, it is likely that key cleaning stations and feeding sites exist for manta rays in the northern area of the GBR, but that current research has failed to identify them. These outstanding questions regarding connectivity and habitat use along Australia's east coast, provide an opportunity to test the power of combining citizen science methods for data collection, and machine learning techniques to analyse pattern expression between these potentially separate populations.

Management and conservation

Manta rays are highly valued economically on a global scale as a target for ecotourism activities (O'Malley et al. 2013). This has led to increased protections in some circumstances, for example the banning of targeted fisheries throughout the Indonesian archipelago (Setyawan et al. 2020). However, in other locations manta ray popularity has resulted in the destruction of benthic habitats at high traffic tourist sites, such as Kona in Hawaii (Clark 2010). Given the important ecological role of cleaning stations that has been discussed in chapter two, these habitats may provide candidate sites for protection, not just for megafauna like manta rays that frequent them, but also as umbrella sites for the diversity of species and the structural features that comprise these ecosystems (Roberge and Angelstam 2004). There are examples of the positive impact of implementing visitor management actions at high value sites for manta ray ecotourism, whereby tourism operators are required to manage their guests around cleaning station or foraging environments. For example, the dive site of "Manta Sandy" in Raja Ampat, Indonesia. Here, a line of coral rumble provides a visual boundary for SCUBA divers to stay behind, allowing the manta rays to carry out their normal behaviour and provides protection for the habitat itself (Setyawan et al. 2018). This is an example of a positive conservation outcome when marine conservation managers and ecotourism operators come together with the common goal of ensuring longevity in their industry and protecting their main attraction.

There remain several gaps in knowledge pertaining to the spatial ecology of manta ray species. Not least of which is an understanding of where they pup and the habitat preferences of juveniles. Numerous studies have reported on the courtship activities and late-stage pregnancy status of wild (Yano et al. 1999, Stevens et al. 2018) and captive manta rays (Murakumo et al. 2020), however understanding of their reproduction beyond this is largely limited to captive breeding programs (Nozu et al. 2017) or fisheries landed specimens (Marshall et al. 2008b). There have been a few sites nominated as potential nursery areas for these species (Stewart et al. 2018b, Pate and Marshall 2020, Werner 2020), but given their likely high yearling mortality (Smallegange et al. 2016), slow growth rates (Dulvy et al. 2014), and the difficulty to accurately size individuals and identify maturity in females (Marshall and Pierce 2012), there is still work to be done to confirm the status of these sites as true nursery areas. Future research should direct resources to addressing knowledge gaps pertaining to manta ray reproduction as a conservation priority for these threatened species.

Conclusions

This thesis addresses a number of outstanding questions regarding the spatial ecology of manta rays. It provides the first fine-scale and detailed investigation of cleaning station use in reef manta rays, and demonstrates the importance of mutualism for the site selection and movement to inshore reef environments of this otherwise pelagic species. This thesis has highlighted how important ephemeral resource patches are for driving aggregations of this largely solitary species, and quantified the required prey density for reef manta ray foraging events. In addition, the longest point-to-point movements for reef manta rays has been updated, and these findings have implications for cross-jurisdictional species management. The thesis has also filled knowledge gaps in the distribution of manta rays around the Australian coastline, to confirm semi-continuous space use through shallow tropical waters around the north of the country. Finally, this work has tested a novel approach in artificial intelligence to investigate heritable traits in manta ray species, and demonstrates how these traits may be used to infer connectivity or isolation among global populations. This work highlights the value of collaborations between global research teams to address broad ecological questions. By using a range of methodologies, this work demonstrates the benefits of a multifaceted approach in addressing questions relating to the spatial ecology of manta rays, and provides important contributions to the overall understanding of these large, threatened elasmobranchs.

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Appendices

Appendix A: Supplementary Tables and Figures

Table A1. Metadata of manta ray tag deployments. Locations: NSI = North Stradbroke Island, LEI = Lady Elliot Island. Manta ID refers to the number assigned to each individual *M. alfredi* based on a long-term photographic identification sighting database.

Date	Location	Tag serial #	Tag ID	Manta ID	Sex	Maturity	Size estimate
4-Feb-17	NSI	1256553	51375	#0460	Female	Mature	3.5 m
4-Feb-17	NSI	1256554	51376	#0512	Female	Juvenile	3 m
04-Feb-17	NSI	1256555	51377	#0815	Female	Mature	3.5 m
04-Feb-17	NSI	1256556	51378	#1139	Male	Mature	2.5 m
04-Feb-17	NSI	1256557	51379	#0476	Female	Mature	3.5 m
04-Feb-17	NSI	1256558	51380	#0601	Female	Juvenile	3 m
05-Feb-17	NSI	1256559	51381	#0622	Female	Mature	3.5 m
05-Feb-17	NSI	1256560	51382	#0875	Male	Juvenile	3 m
05-Feb-17	NSI	1256561	51383	#0724	Female	Juvenile	3 m
05-Feb-17	NSI	1256562	51384	#0516	Female	Juvenile	3 m
28-Feb-17	LEI	1256563	51385	#0445	Female	Mature	3.5 m
28-Feb-17	LEI	1256564	51386	#1129	Female	Mature	3.5 m
03-Jun-17	LEI	1256565	51387	#0831	Male	Mature	3 m
04-Jun-17	LEI	1256566	51388	#0076	Female	Juvenile	3 m
06-Jun-17	LEI	1256567	51389	#1103	Female	Mature	3.5 m
06-Jun-17	LEI	1266921	32432	#0648	Female	Mature	3.5 m

06-Jun-17	LEI	1266922	32433	#0201	Female	Mature	3.5 m
06-Jun-17	LEI	1266923	32434	#0029	Female	Mature	3.5 m
06-Jun-17	LEI	1266924	32435	#0003	Female	Mature	3.5 m
07-Jun-17	LEI	1266925	32436	#0537	Male	Mature	3 m
07-Jun-17	LEI	1266926	32437	#0816	Female	Mature	3.5 m
07-Jun-17	LEI	1266927	32438	#0551	Female	Juvenile	3 m
07-Jun-17	LEI	1266928	32439	#0524	Female	Mature	3.5 m
07-Jun-17	LEI	1266929	32440	#0778	Male	Juvenile	<3 m
07-Jun-17	LEI	1266930	32441	#0654	Female	Juvenile	<3 m
08-Jun-17	LEI	1266931	32442	#0172	Male	Mature	<3 m
08-Jun-17	LEI	1266932	32443	#0313	Female	Juvenile	3 m
08-Jun-17	LEI	1266933	32444	#0454	Female	Mature	3.5 m
09-Jun-17	LEI	1266934	32445	#0021	Male	Mature	<3 m
09-Jun-17	LEI	1266935	32446	#0304	Male	Mature	3 m
09-Jun-17	LEI	1266936	32447	#0541	Female	Mature	4 m
09-Jun-17	LEI	1266937	32448	#0010	Female	Mature	3.5 m
10-Jun-17	LEI	1266938	32449	#0305	Female	Mature	3.5 m
10-Jun-17	LEI	1266939	32450	#0203	Male	Mature	3 m
11-Jun-17	LEI	1266940	32451	#0118	Male	Juvenile	<3 m

12-Jun-17	LEI	1266941	32452	#0034	Female	Mature	3.5 m
12-Jun-17	LEI	1266942	32453	#1133	Male	Mature	3 m
12-Jun-17	LEI	1266943	32454	#0297	Female	Mature	3.5 m
13-Jun-17	LEI	1266944	32455	#0228	Male	Mature	3 m
13-Jun-17	LEI	1266945	32456	#0725	Male	Mature	3 m
29-Jun-18	LEI	1278726	15737 (V16P)	#1260	Male	Mature	3.5m
17-Jul-18	LEI	1278727	15738 (V16P)	#0419	Male	Mature	3 m

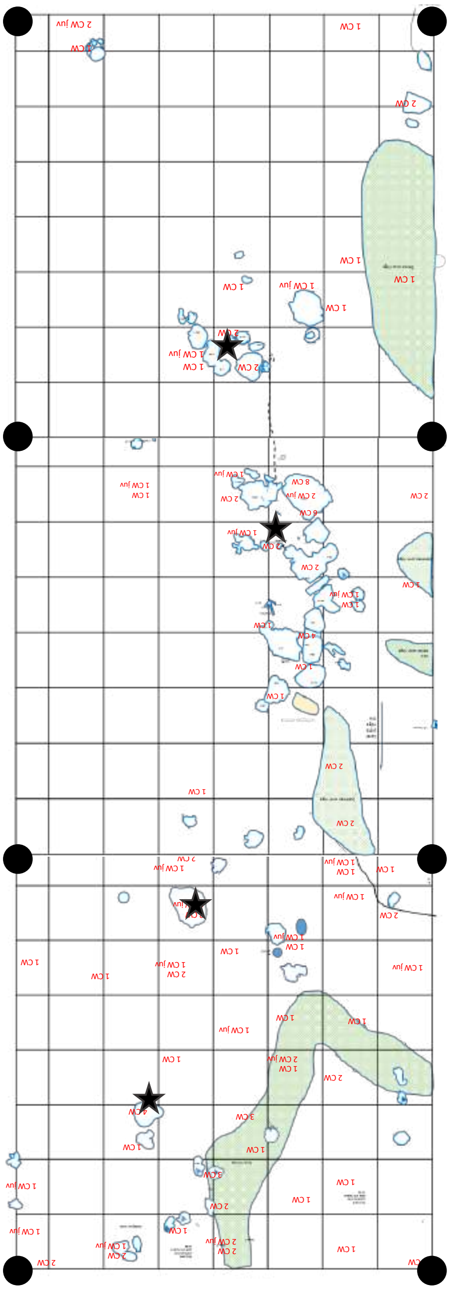

Figure A1. Mapped structural habitat within the Lady Elliot Island acoustic array. The mapped area was divided into three large squares (76 x 76 m) using the position of the acoustic receivers (seen here as black circles). Mapping was conducted using transect tape within 10 x 10 m gridded squares (size adjusted along the bounds of the larger 76 x 76 m square), and photographs of features were taken to confirm structural habitat. Features < 2 m were excluded from mapping. Stationary point surveys for cleaner species were conducted at locations marked with a black star.

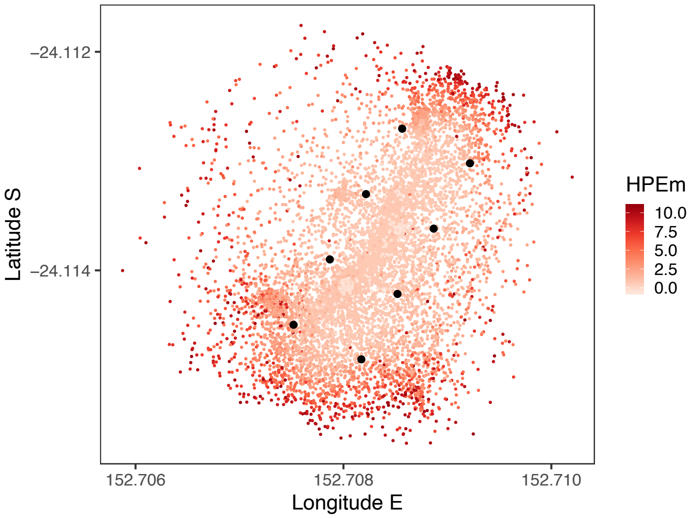

Figure A2. Error estimate of tagged *Mobula alfredi* positions in an acoustic array. The coloured dots represent 90% of tagged animal positions (n = 12,157) after removing positions with lower location accuracy (measured Horizontal Positioning Error (HPEm) over 10.82 m). Black circles indicate location of acoustic receivers.

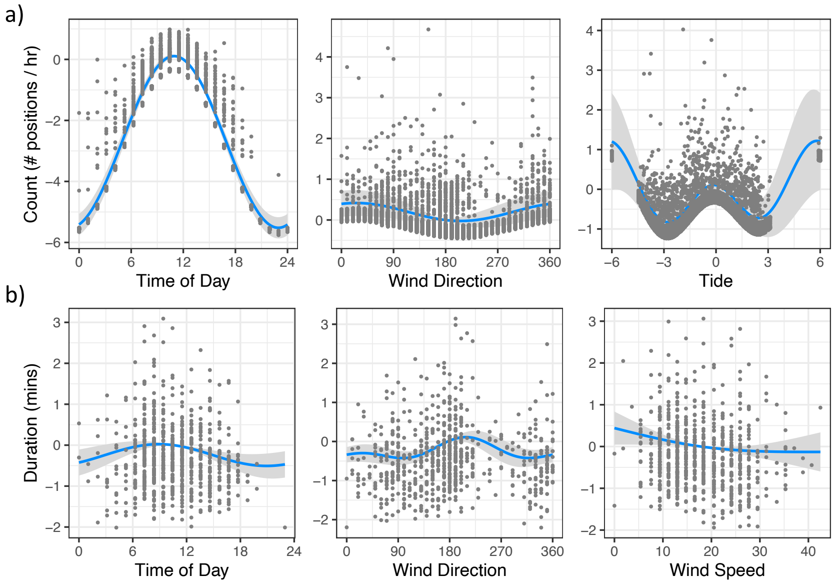

Figure A3. Generalised linear mixed effects models of tagged *Mobula alfredi* at Lady Elliot Island cleaning stations: a) Count per hour of detections by Time of Day (0 – 24 hrs), Wind Direction (South = 180°, North = 360°), and Tide (Hours from low tide at 0); and b) Duration in minutes of visitation by Wind Speed (km/hr), Wind Direction (South = 180°, North = 360°), and Tide (Hours from low tide at 0). Output is on the log-link scale.

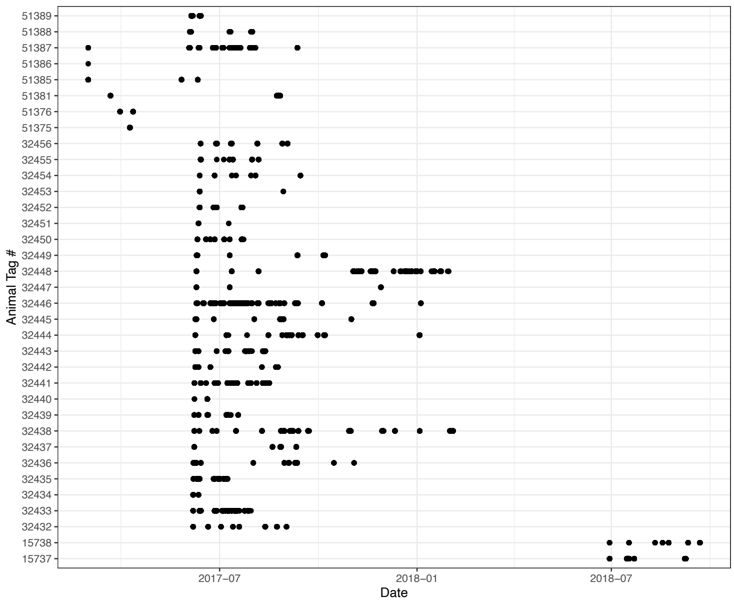

Figure A4. Abacus plot of tagged *Mobula alfredi* detections in an acoustic array off Lady Elliot Island. Each row represents a tagged animals detections over time.

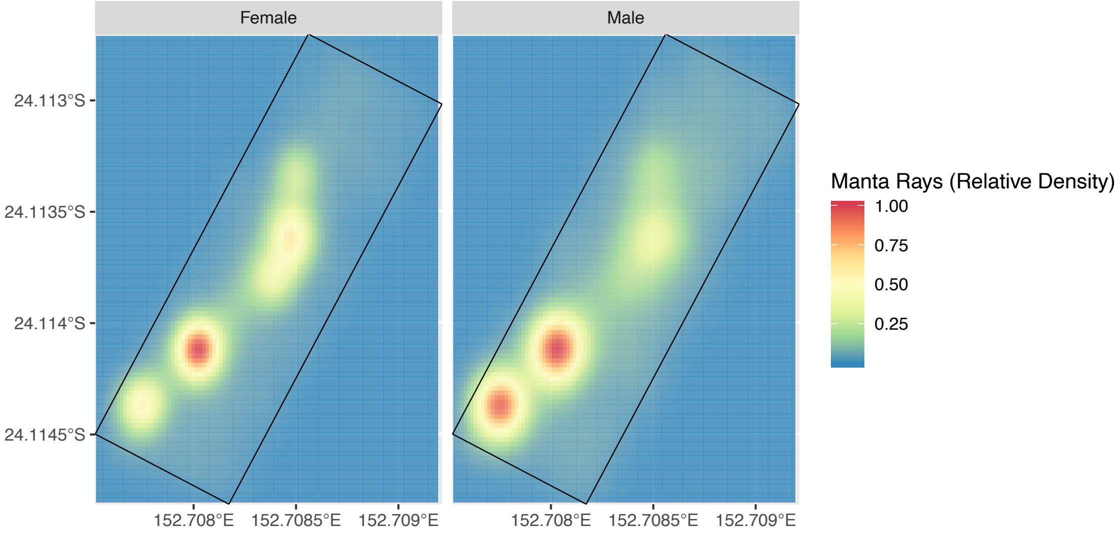

Figure A5. Habitat use of tagged *Mobula alfredi* in the Lady Elliot Island acoustic array between February 2017 and September 2018. Relative density of manta ray positions, with panels separated by sex of the tagged rays. The black rectangle in the figures represents the bounds of the acoustic array.

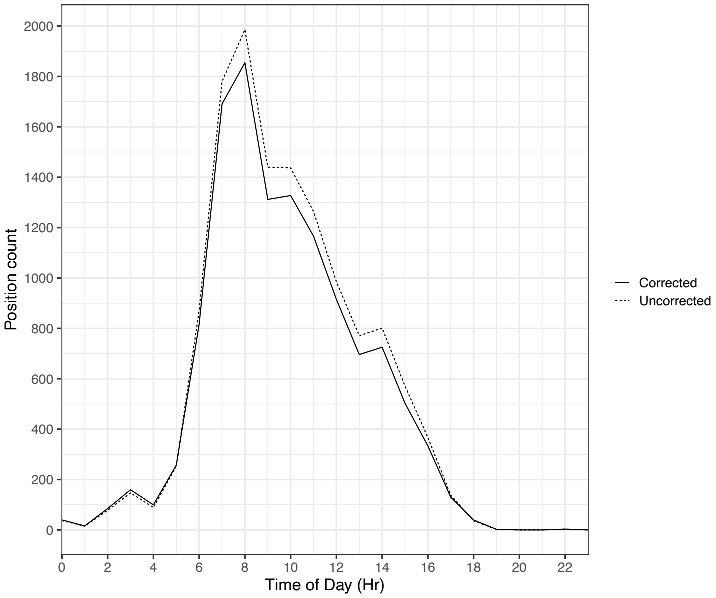

Figure A6. Corrected counts for tagged animal positions in an acoustic array off LEI. Using the standardised positioning frequency of sentinel transmitters, the solid line provides a corrected count per hour of animal positions from the acoustic array. The dashed line is the uncorrected count of animal positions from the array.

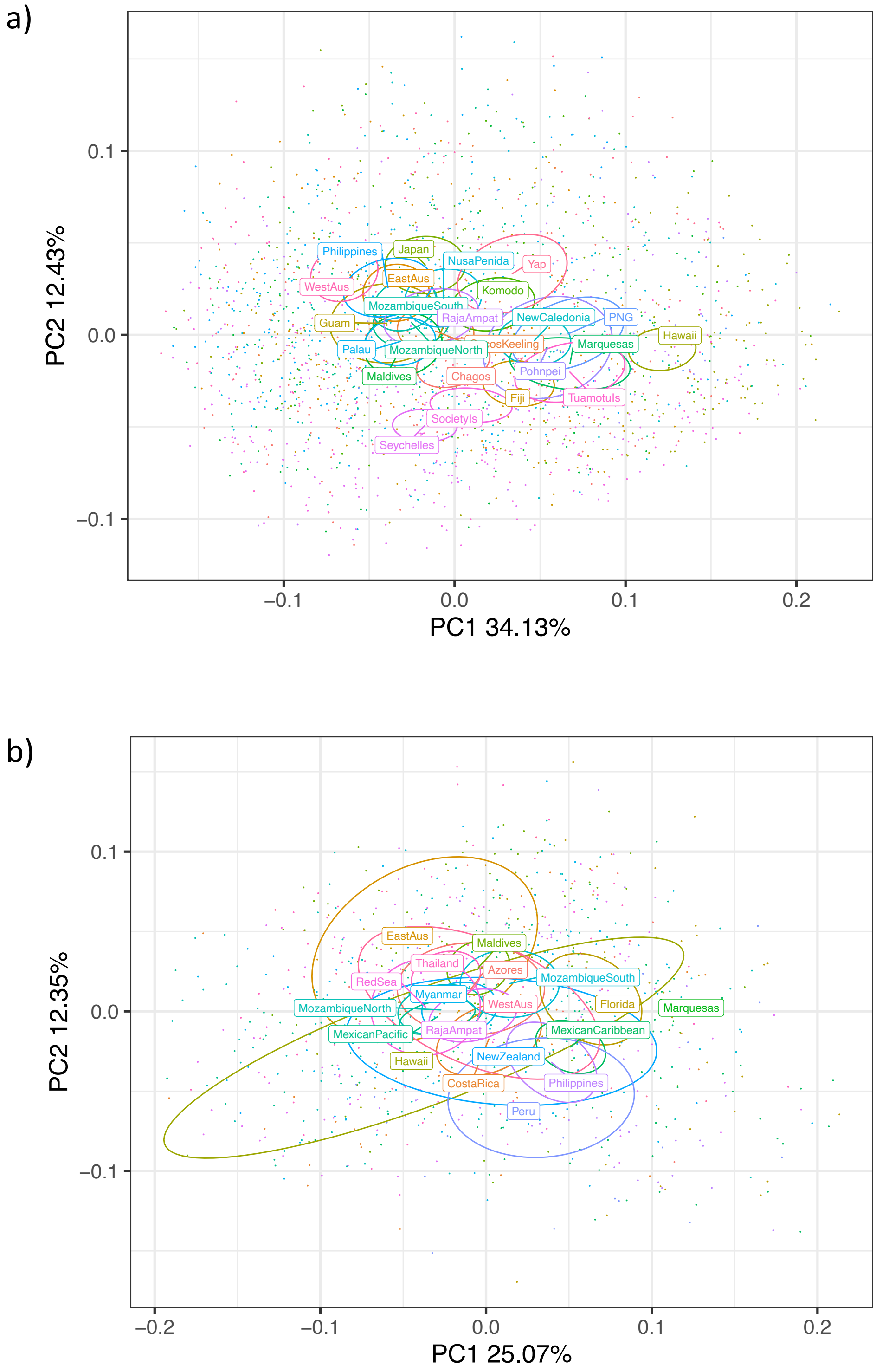

Figure A7. Example of principle components analysis output when sample size of manta ray populations are decreased to n < 100. a) *Mobula alfredi* and b) *M. birostris*. Points are individuals and labels represent the centroid for each population. Ellipses are standard error around the mean.

Appendix B: Publications

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ORIGINAL RESEARCH

Ecology and Evolution Open Access WILEY

Mutualism promotes site selection in a large marine planktivore

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Abstract

1. Mutualism is a form of symbiosis whereby both parties benefit from the relationship. An example is cleaning symbiosis, which has been observed in terrestrial and marine environments. The most recognized form of marine cleaning symbiosis is that of cleaner fishes and their clients.
2. Cleaner species set up cleaning stations on the reef, and other species seek out their services. However, it is not well understood how the presence of cleaning stations influence movements of large highly mobile species. We examined the role of cleaning stations as a driver of movement and habitat use in a mobile client species.
3. Here, we used a combination of passive acoustic telemetry and in-water surveys to investigate cleaning station attendance by the reef manta ray *Mobula alfredi*. We employed a novel approach in the form of a fine-scale acoustic receiver array set up around a known cleaning area and tagged 42 rays. Within the array, we mapped structural features, surveyed the distribution of cleaner wrasse, and observed the habitat use of the rays.
4. We found manta ray space use was significantly associated with blue-streak cleaner wrasse *Labroides dimidiatus* distribution and hard coral substrate. Cleaning interactions dominated their habitat use at this site, taking precedence over other life history traits such as feeding and courtship.
5. This study has demonstrated that cleaning symbiosis is a driver for highly mobile, and otherwise pelagic, species to visit inshore reef environments. We suggest that targeted and long-term use of specific cleaning stations reflects manta rays having a long-term memory and cognitive map of some shallow reef environments where quality cleaning is provided. We hypothesize that animals prefer cleaning sites in proximity to productive foraging regions.

KEYWORDS

acoustic tracking, animal navigation, coral reef, elasmobranch, location accuracy, megafauna, movement ecology, VEMCO Positioning System

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Marine Biodiversity Records

MARINE RECORD Open Access

Photographic identification and citizen science combine to reveal long distance movements of individual reef manta rays *Mobula alfredi* along Australia's east coast

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Abstract

Research into the movement ecology of terrestrial and marine animals is growing globally, especially for threatened species. Understanding how far an animal can move and the extent of its range can inform conservation planning and management. On the east coast of Australia, reef manta rays *Mobula alfredi* are the subject of a photographic identification study, *Project Manta*. In June 2018, videos of reef manta rays from the SS Yongala (19.31° S, 147.62° E), were submitted to the *Project Manta* east coast sightings database. The videos were of two individuals previously identified from North Stradbroke Island (27.42° S, 153.55° E), about 1150 km to the south of the SS Yongala. This represents the greatest point-to-point distance travelled by individual *M. afredi* and extends the latitudinal range for this sub-population on the east coast. This study highlights that citizen science input can provide valuable data to address knowledge gaps in the distribution and population range of marine species. Knowledge of the 1000+ km range movement potential of individual *M. alfredi,* highlights the possibility that regional sub-populations may span jurisdictional zones of more countries than previously considered likely, complicating conservation management of this species.

Keywords: Home range, Animal movement, Population connectivity, Photo-ID, Great barrier reef, Elasmobranch, Dispersal capacity

Introduction

Knowledge of the movement ecology of species, and how far individuals move is important for understanding population structure and for conservation planning and management (Hays et al. 2016). The movements and distributions of non-commercial fish species are generally poorly-understood when compared to commercially important fishes, and other marine megafauna such as sea turtles (Fossette et al. 2010; Houghton et al. 2006; Schofield et al. 2013), cetaceans (Christal and Whitehead 1997; Williams et al. 2009; Cheney et al. 2013) and seabirds (Hennicke and Weimerskirch 2014; Ludynia et al. 2012; Péron et al. 2013).

The reef manta ray *Mobula alfredi,* is a large, pelagic elasmobranch (disc width up to 5 m) found in tropical and subtropical waters of the Indo-Pacific Oceans, often associated with coastlines and coral or rocky reef habitats. This species displays aggregative behaviours at predictable locations that have provided good opportunities to investigate their biology and ecology (Marshall et al. 2011; Dewar et al. 2008; Kitchen-Wheeler et al. 2012). The application of photographic identification (photo-ID) and electronic tagging has shown a relatively high degree of site fidelity to meso-scale geographical regions (Couturier et al. 2018; Braun et al. 2015; Setyawan et al. 2018). One outcome of such site fidelity, could be the formation of local sub-populations, particularly if there are barriers to interchange of individuals with adjacent populations. Currently, there is little evidence of

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BRIEF COMMUNICATION

JOURNAL OF FISH BIOLOGY

The geographic distribution of reef and oceanic manta rays (*Mobula alfredi* and *Mobula birostris*) in Australian coastal waters

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Abstract

The known distribution of manta rays in Australian waters is patchy, with records primarily centred around tourism hotspots. We collated 11,614 records of *Mobula alfredi* from photo-ID databases (n = 10,715), aerial surveys (n = 378) and online reports (n = 521). The study confirms an uninterrupted coastal distribution from north of 26°S and 31°S on the west and east coasts, respectively. More southerly *M. alfredi* records relate to warm-water events with a southernmost extent at 34°S. Coastal sightings of *Mobula birostris* were rare (n = 32), likely reflecting a preference for offshore waters, but encompass a wider latitudinal extent than *M. alfredi* of 10–40°S.

KEYWORDS

citizen science, connectivity, photo-ID, reef manta ray, species distribution range

There are currently two recognised species of manta ray (order Myliobatiformes), which are large, pelagic, planktivorous rays with broadly overlapping, near-global distributions in tropical and warm temperate oceanic waters. The reef manta ray, *Mobula alfredi* (Krefft, 1868), has a geographical distribution throughout much of the tropical and subtropical Indo-Pacific region, with the majority of records from relatively shallow waters associated with mainland coastlines, offshore islands and reefs (Couturier *et al*., 2012; Marshall *et al*., 2009) (Figure 1). However, the known distribution of *M. alfredi* is extremely patchy, with most records from dive ecotourism hotspots in Mozambique, South Africa, Maldives, Japan, Guam, the Red Sea, Philippines, New Caledonia, Indonesia and Australia (Couturier *et al*., 2012; O'Malley *et al*., 2013) (Figure 1). This could be indicative of the environmental preferences of this species, or a consequence of effort-mediated sampling bias.

In Australian waters, *M. alfredi* is reported to occur to at least ~26°S on the west coast and to ~30°S on the east coast (Couturier *et al*., 2012; Last & Stevens, 2009). The majority of sightings are from subtropical locations where the species aggregates seasonally coinciding with regular ocean-based tourism activities. The occurrence of the species elsewhere remains mainly anecdotal. Large *M. alfredi* aggregations are typically associated with feeding behaviours and higher localised zooplankton concentrations, although the species also shows high site fidelity to patches of reef used for cleaning (Armstrong *et al*., 2016; Couturier *et al*., 2018). On the east coast, seasonal, southward movements coincide with a strengthening of warm southerly flowing

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