ASPECTS OF POPULATION BIOLOGY AND BEHAVIOUR OF MOBULID RAYS

By

Michelle Marie Carpenter

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Principal investigator:
Michelle Carpenter
Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
crpmic001@myuct.ac.za

Primary supervisor:
Emer. Prof. Charles Griffiths
Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
Charles.Griffiths@uct.ac.za

Co-supervisor:
Dr. Andrea Marshall
Marine Megafauna Foundation, West Palm Beach, Florida, USA
andrea@marinemegafauna.org

DECLARATION

I, Michelle Marie Carpenter, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been referenced in the thesis.

Michelle Marie Carpenter

ETHICS STATEMENT

Research in Závora, Mozambique, was conducted under permission of the Memorandum of Understanding between the Marine Megafauna Foundation and Instituto Oceanográfico de Moçambique. Individual permission to study in Mozambique was granted under study permit 331/GED/PI.

Research in the iSimangaliso Wetland Park was conducted under the SharkLife Research Agreement dated 10/06/2020 and WildOceans Agreement dated 01/10/2020. The iSimangaliso Wetland Park authorities are specifically acknowledged for supporting this work.

The KwaZulu-Natal Sharks Board is granted permission to catch animals as part of its operational activities as per its permit conditions listed in OCS/TOPS/STANDINGPERMIT/2022/02. This permit is issued by the Department of Forestry, Fisheries and the Environment in terms of regulation 7(1) of the Threatened or Protected Marine Species Regulations, 2017 (Government Gazette Notice No. 40876 of 30 May 2017) (the Regulations) read with the National Environmental Management: Biodiversity Act, 2004 (Act no.10 of 2004) (the Act).

All encounters of mobulids for sightings and behaviour research occurred while recreational diving or snorkelling. As these observations were made opportunistically and did not involve the capture, use, care, or manipulation of any alive or dead species, no permit or ethics approvals were required.

Individual permission to study in South Africa was granted under study permit TRR3115910.

Michelle Marie Carpenter

STATEMENT OF CONTRIBUTION BY OTHERS

Chapter 1
The introduction was my own work. My supervisor, Emer. Prof. Charles Griffiths, as well as Mr Geremy Cliff, provided feedback on earlier versions of the chapter.

Chapter 2
The general methods chapter was my own work. My supervisor, Emer. Prof. Charles Griffiths provided feedback on earlier versions of the chapter.

Chapter 3
This chapter was published in the Journal of Fish Biology in 2022. I am the primary author and Nakia Cullain, Stephanie Venables, Yara Tibiriçá, Prof. Charles Griffiths, and Andrea Marshall are co-authors. I was responsible for conceiving the central idea of the study, some data collection, analysis, interpretation, and writing. Nakia Cullain and Yara Tibiriçá collected the data with some contributed by all authors. Stephanie Venables provided assistance with statistics and interpretation. All authors contributed to writing the manuscript. Two reviewers from the Journal of Fish Biology gave helpful feedback that improved the manuscript.

Chapter 4
This chapter is in preparation for submission to a journal in 2023. I collected data in South Africa, collated citizen science data, and managed the database. Matt Dicken contributed data from Protea Banks. Ryan Daly, Jennifer Olbers, Grant Smith, and Nakia Cullain supported fieldwork in the iSimangaliso Wetland Park. Chad Minaar donated citizen science data from Ballito. I wrote the chapter with feedback from Emer. Prof. Charles Griffiths.

Chapter 5
This chapter was published in Frontiers in Marine Science in 2023. I am the primary author and Denham Parker, Matthew Dicken, and Emer. Prof. Charles Griffiths are co-authors. Two reviewers from Frontiers in Marine Science helped improve the manuscript.

Chapter 6
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Chapter 7
This chapter was published as a short communication in the African Journal of Marine Science. I was responsible for data collection and writing the manuscript. Emer. Prof. Charles Griffiths contributed editorial feedback. Two anonymous reviewers helped improve upon the manuscript.

Chapter 8
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Aspects of population and behaviour of mobulid rays: MICHELLE M CARPENTER

ABSTRACT

Manta and devil rays (*Mobula* spp.) are charismatic yet threatened species that remain poorly understood. The Inhambane Province in Mozambique is known to be home to the largest populations of manta rays in Africa, including the reef manta ray (*M. alfredi*) and the oceanic manta ray (*M. birostris*). However, research on other mobulid species, and in the region south of Tofo, including Závora in Mozambique and South Africa, has been limited. This thesis identifies critical sites and describes their characteristics, including habitat use, population abundance, and behaviour, for three species of mobulids: *M. alfredi*, *M. birostris*, and the shortfin devil ray, *M. kuhlii*. Chapter 1 comprises a literature review on the status and knowledge on mobulids in a global context, and within the southern African region, with focus on *M. alfredi* and *M. birostris*, due to the increased amount of information available on these species. Chapter 2 provides detailed information on the methodologies used throughout the thesis, some of which overlap.

Chapter 3 utilises an 11-year photographic mark-recapture dataset spanning from 2010-2021 to investigate the size and structure of the *M. alfredi* population that aggregate at 'Red Sands' (RS), a reef cleaning station located at Závora, Mozambique. A total of 583 individual *M. alfredi* were identified using photographic mark recapture (photo-ID) of the unique ventral spot patterning. Pollock's Robust Design models were used to estimate annual abundances, emigration, annual apparent survival, and capture probability at RS during peak season, July-November, between 2016-2021. The resulting abundance estimates varied each year, with a range of 35 individuals (95% CI 30-45) up to 233 individuals (95% CI 224-249). Lagged Identification Rates were additionally used to describe the residency patterns of *M. alfredi* at Red Sands, revealing that despite site affinity to RS, individuals were overall transient within a given season, with an average residency of four days.

Chapter 4 applies the methods used in Chapter 3 to report on South Africa's first photo-ID dataset for manta rays, including both *M. alfredi* and *M. birostris*, spanning from 2020-2022. Through the use of citizen science photographic donations and opportunistic snorkelling and

diving, were used to document manta ray aggregation sites in the KwaZulu-Natal (KZN) Province. These same data were used to further document connectivity of *M. alfredi* between Mozambique and South Africa, revealing that at least 20% of all photographed individuals in South Africa had made international migrations to or from Mozambique. The distances travelled ranged from 435 km and 1,000 km, between the Aliwal Shoal Marine Protected Area (MPA), KZN and Závora, the second longest known migration for this species. Additionally, previously undocumented aggregations were found, including *M. alfredi* at the iSimangaliso Wetland Park and possibly *M. birostris* at Ballito. Aggregations in KZN were primarily related to surface feeding, with cleaning and courtship behaviours also documented.

Chapter 5 analyses a 41-year catch dataset (1981-2021) provided by the KZN Sharks Board bather protection programme to investigate broad spatial-temporal trends in catch for manta rays (*M. alfredi*, *M. birostris*). Using Generalised Additive Models and the probability of encounter, the effects of annual and seasonal factors, moon phase, and location on catch rates are assessed. The size composition and demographics of the caught manta rays and the catch per unit effort at different beaches are also examined. The results revealed a significant decline in overall catches around 2000 ($p<0.0001$). Catches increased in summer, indicating seasonal visitation to South African waters. Moon phase had an effect on manta ray presence, with more catches during new and full moon phases. Over half of the catches included juveniles, whereby a total of 841 individuals (52% of total catch) of confirmed juvenile size class (1400-2500 mm disc width) were caught, particularly in the southernmost sampled area, from Hibberdene in the north to Mzamba in the south.

Chapter 6 investigates a previously undocumented aggregation of *M. kuhlii* in the Aliwal Shoal MPA, and their cleaning behaviour by the bluestreak cleaner wrasse, *Labroides dimidiatus*. The study documented *M. kuhlii* sightings, behaviours, and associated environmental conditions during recreational snorkelling/diving. Generalised additive models (GAMs) were used to assess predictors of *M. kuhlii* presence and cleaning behaviour duration. The results showed that *M. kuhlii* were present in 56% of the survey days, with up to 150 individuals observed. The study recorded a total of 4.04 hours of *M. kuhlii* cleaning behaviour which was processed using frame by frame analysis. Sea surface temperature was found to be a significant predictor for *M. kuhlii*

presence, while the absence of current and estimated horizontal visibility were significant predictors for longer cleaning durations.

During recreational diving in the Aliwal Shoal MPA, first-time observations of *M. kuhlii* courtship were unexpectedly encountered and videoed in November 2020 and January 2021, which are detailed in Chapter 7. The common behaviours associated with mobulid courtship were documented: multiple males following a single female, rapid speed bursts, avoidance of the female, and swerving, which confirmed that *M. kuhlii* mating occurs in KZN waters.

The collective results are concluded in Chapter 8, which indicate that both Závora and KZN waters provide important seasonal habitat for mobulid species along the southern African coastline. The findings of this work support other studies that show manta rays to use an expansive area, with seasonal affinities to certain locations, and transience during periods when conditions are not favourable for visitation. This demonstrates the importance of understanding site use within the larger home range of a given mobulid population. Therefore, this thesis has filled critical knowledge gaps and improved the overall understanding of mobulids in southern Africa, providing valuable information for conservation management efforts.

Keywords: Mobulidae, spatial ecology, behaviour, fisheries, population demography, marine conservation, photo-ID

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CHAPTER 1

INTRODUCTION

"You have to deeply deny the evidence to conclude that humans alone are conscious, feeling beings"
-Carl Safina, *Beyond Words*

The increasing global human population and associated rapid transformations across all types of ecosystems threaten biodiversity and are restricting the natural spaces needed for conservation (Crist *et al.* 2017). Recognising important or 'critical' habitats that support keystone species, high levels of biodiversity, or numbers of endemic species, is one successful method of combating this biodiversity decline (Keitt *et al.* 1997, Martin *et al.* 2015). Terrestrial examples include the recognition of Sumatran orangutans, *Pongo tapanuliensis* (Sloan *et al.* 2018), and mountain gorillas *Gorilla beringei* (Robbins *et al.* 2011) as flagship/umbrella species for the forests they inhabit, with conservation of their habitat subsequently protecting many other species. In the ocean, research, monitoring, and conservation are logistically more difficult, but similar strategies can be applied. For example, Martin *et al.* (2015) identify 'Critical Habitat' and 'Key Biodiversity Areas' to include cold- and warm-water coral reefs, seamounts, mangroves, seagrass beds, saltmarshes, sea turtle nesting sites, hydrothermal vents and cold seeps, based on the increased number of species and/or iconic species found in these sites. As in the terrestrial examples cited above, 'flagship' species, or groups, can be used in marine habitats to help promote the conservation of critical areas. Sharks and rays (Elasmobranchii) are one such group that include a number of flagship species (Osgood *et al.* 2020).

1.1. Defining critical habitat for elasmobranchs

Sharks and rays, collectively termed elasmobranchs, are among the most threatened groups of fish (Pacoureau *et al.* 2021). They are generally slow-growing with low reproductive outputs, rendering them vulnerable to overfishing (Musick *et al.* 2000). Indeed, global abundance of oceanic elasmobranchs has declined 71% since 1970 due primarily to overfishing, making this group of international conservation concern (Pacoureau *et al.* 2021). Initiatives to conserve elasmobranchs and their marine habitats include spatial protection through the establishment of Shark Sanctuaries, first established in 2009, and more recently, Important Shark and Ray Areas, initiated in 2022 by the International Union for the Conservation of Nature (IUCN). In under a decade, Shark Sanctuaries have resulted in reduced directed fishing, which in turn has reduced market sales and arrested the rate of population decline, as well as increased optimism about the conservation of sharks in adjoining local waters (Ward-Paige and Worm 2017). This has a positive impact on both the environment and the economy, as many elasmobranchs are valuable to ecotourism (Vianna *et al.* 2012, O'Malley *et al.* 2013, Zimmerhackel *et al.* 2019, Mustika *et* *al.* 2020). Of the rays, the most popular ecotourism group of species, and therefore the most charismatic (Ducarme *et al.* 2013), are members of the family Mobulidae (*Mobula* spp.) (O'Malley *et al.* 2013, Murray *et al*. 2020).

1.2. Family Mobulidae

The Mobulidae family, comprising manta rays and devil rays, is a poorly understood and threatened group of species (Couturier *et al.* 2012, Croll *et al.* 2016). Mobulid rays were once considered 'devil-like', as they possess cephalic lobes that resemble 'horns', and were once thought to be solitary, non-intelligent, even dangerous animals. Extensive research over the last few decades has, however, shown that they are complex, social animals with derived characteristics and diverse distributions.

1.2.1. Taxonomy

Mobulidae includes nine extant zooplanktivorous species (White *et al.* 2017, Notarbartolo di Sciara *et al.* 2019) (Table 1.1). Although not used in feeding, mobulids possess a tooth band of miniscule teeth, located on the edge of the jaw, which varies in size and tooth number across species (Adnet *et al.* 2012). Until recently, the manta rays were regarded as comprising a separate genus *Manta,* but genetic analysis has demonstrated that the family comprises just one genus, *Mobula* (Marshall *et al.* 2009, White *et al.* 2017). Morphological evidence shows that devil rays and manta rays are distinct from one another; two species of manta rays having evolved a terminal mouth, large body size, and paddle-like cephalic lobes, compared to the seven species that have a ventral mouth, reduced body size, and small cephalic lobes (Notarbartolo di Sciara 1987, Marshall *et al.* 2009, Couturier *et al.* 2012).

The two recognised species of manta rays are the reef manta ray (*M. alfredi*) (Kreft 1868), and the oceanic manta ray (*M. birostris*) (Walbaum 1792), and it was only in 2009 that they were described as distinct species (Marshall *et al.* 2009) (Table 1.1). These are the two largest of all ray species (*M. alfredi*; 5500 mm maximum disc width (DW); *M. birostris*; 8000 mm maximum DW) (Marshall *et al.* 2009). A putative third manta species, *M. cf. birostris* has been proposed, but not yet formally described, and occurs in the Atlantic Ocean (Hinojosa-Alvarez *et al.* 2016). Several morphological differences distinguish the two manta species, including distinct colouration patterns. *Mobula birostris* has a sharp, T-shaped white shoulder patch on the dorsal surface, charcoal shading on the ventral edge of the pectoral fins, and dark mouth and cephalic lobe regions, whereas *M. alfredi* may have shoulder patches that are a blurry shape, with possible shading on the edge of the pectoral fins, and may have light or dark faces (Marshall *et al.* 2009). Colouration patterns in other mobulids also vary; shortfin devil rays, *Mobula kuhlii* (Müller and Henle 1841) possess grey to black colouring on the ventral surface of the pectoral fin apex which fades into white on the remainder of the pectoral fin and on the abdomen (Notarbartolo di Sciara *et al.* 2017, White *et al.* 2017).

1.2.2. Evolution

The taxonomy of Mobulidae is often questioned and adapted due to the difficulty of encountering and identifying these wide-ranging species (White *et al.* 2017, Notarbartolo di Sciara *et al.* 2019). Mobulid rays evolved after the Cretaceous-Paleogene boundary, approximately 66 mya, which is comparatively recent, given that the first elasmobranchs evolved over 400 mya (Snyder *et al.* 1983, Cappetta and Stringer 2002). Despite the great overlap in some geographic ranges of some species, differences in habitat use and behaviour have likely sustained the speciation of mobulids (Kashiwagi *et al.* 2011, 2012). The periods in cladogenesis in Mobulidae were simultaneous with phases of global warming, with speciation occurring as recently as the last million years, likely as a result of fragmented upwelling or reduced food availability (Kashiwagi *et al.* 2012, Poortvliet *et al.* 2015). Additionally, the periods of ice ages likely caused physical barriers in population dispersal and connectivity (Poortvliet *et al.* 2015, Stewart *et al.* 2017). Further, the different foraging strategies may have driven speciation and allowed for sympatry of these closely-related species in several locations (Andersen *et al.* 1993, Kashiwagi *et al.* 2012, Braun *et al.* 2014, Stewart *et al.* 2016a, Burgess *et al.* 2016, Stewart *et al.* 2019).

Manta rays (*M. alfredi*, *M. birostris*) are the most derived species in this group, dating back approximately 4.8 mya (Snyder *et al.* 1983, Cappetta and Stringer 2002). White *et al.* (2017) found the family Mobulidae to be paraphyletic, with *M. alfredi* and *M. birostris* being closely related, and their speciation having occurred 0.5-1.0 mya (Kashiwagi *et al.* 2012). *Mobula birostris* possesses a calcified mass with a small spine on the base of the tail, a remnant of when the group split from myliobatids, and is thus inferred to be the older of the two species (Marshall *et al*. 2009).

1.2.3. Distribution

Mobulid species are found circumglobally in tropical, subtropical, and temperate seas (Notarbartolo di Sciara 1987) (Table 1.1). Presently, four species (*M. birostris*, *M. thurstoni*, *M. tarapacana*, *M. mobular*) are known to exhibit circumglobal distribution (between 40S and 40N), the other species being restricted to certain ocean basins (Notarbartolo di Sciara 1987, White *et al*. 2017) (Table 1.1). Distributions of the oceanic manta ray (*M. birostris*), the giant devil ray (*M. mobular*), and the bentfin devil ray (*M. thurstoni*) extend into temperate waters (Duffy and Abbott 2003, Duffy and Tindale 2018), while the other species are restricted to subtropical/tropical waters (Table 1.1). Within the manta rays, *M. birostris* generally occurs more offshore than the more coastal *M. alfredi* (Marshall *et al*. 2009, Armstrong *et al*. 2020a). For several mobulid species their home ranges remain unknown (e.g. *M. kuhlii*), but present migration records include 1150 km for *M. alfredi* (Armstrong *et al*. 2019), >1400 km for *M. birsotris* (Hearn *et al*. 2014), and up to >3800 km for *M. tarapacana* (Thorrold *et al*. 2014). Mobulids are known to dive deep, the maximum recorded diving depths being 672 m for *M. alfredi* (Lassauce *et al*. 2020), 700 m for *M. mobular* (Canese *et al*. 2011), and up to 1800 m for *M. tarapacana* (Thorrold *et al*. 2014). Diving behaviour in mobulids is linked to the exploitation of the demersal layer, where an abundance of zooplankton occurs, especially at night (Burgess *et al*. 2016, Peel *et al*. 2019a). Despite their ability to inhabit large horizontal and vertical spaces, mobulids exhibit site affinity, or ‘fidelity’ (Chapman *et al*. 2015) to certain sites, often related to foraging (Couturier *et al*. 2012).

1.2.4. Feeding ecology

Mobulids are the only planktivorous rays (Notarbartolo di Sciara 1987, Notarbartolo di Sciara *et al*. 2019). The availability of zooplankton, as a temporal and scarce resource in a vast ocean, is a primary driver of mobulid movement patterns, since these planktivores mostly inhabit subtropical/tropical waters (Behrenfeld and Boss 2014, Harris *et al*. 2020). Climate patterns indeed affect mobulid distribution, due to the relationship between temperature and nutrient presence with chlorophyll-a and subsequent zooplankton blooms (Folt and Burns 1999, Jaine *et* *al*. 2012, Weeks *et al*. 2015). Research has shown a significant correlation between the El Niño Southern Oscillation and the presence of *M. birostris* at both the Revillagigedo National Park, Mexico (Cabral *et al*. 2023) and Raja Ampat, Indonesia (Beale *et al*. 2019). This indicates that these events can affect the distribution of *M. birostris*, most likely due to changes in zooplankton availability. It is plausible that this correlation extends to all species of Mobulidae.

The large size (+2000 mm DW) of several mobulids (*M. alfredi*; *M. birostris*, *M. mobular, M. tarapacana;* Table 1.1) may be an advantage for increasing the geographic range of zooplankton exploitation; the dorsoventrally compressed body shape being beneficial for gliding, hence enabling greater distances to be covered in search of prey (Braun *et al*. 2014). By foraging in multiple water layers and also having an affinity to certain shallow sites, mobulids help supply nutrients to shallow systems, such as oligotrophic coral reefs through excretion (Williams *et al*. 2018). Large dead mobulid species also provide substantial "food falls" to deep water scavengers (Higgs *et al*. 2014). These rays thus uniquely influence both shallow and deep-water ecosystems as secondary consumers and as a transport mechanism between depths (Braun *et al*. 2014, Thorrold *et al*. 2014).

Table 1.1. Extant mobulid species, including their scientific and common names, size ranges (disc width), broad distribution, temperature ranges, International Union on the Conservation of Nature (IUCN) Red-List statuses, southern African distribution, and associated references.

Species name	**Common name**	**Disc width (mm)**	**Broad distribution**	**Temperature range**	**IUCN Red-List Status**	**Southern Africa distribution**	**References**
Mobula alfredi	Reef manta ray	1300-5500	Indian and Pacific Oceans	Tropical-subtropical	Vulnerable	Southern Mozambique; KwaZulu-Natal, Eastern Cape, South Africa	Kreft 1868, Marshall *et al*. 2009, Marshall *et al*. 2022a
Mobula birostris	Oceanic manta ray	2000-8000	Circumglobal	Temperate, tropical-subtropical	Endangered	Southern Mozambique; KwaZulu-Natal, Eastern Cape, Western Cape, South Africa	Walbaum 1792, Marshall *et al*. 2009, Marshall *et al*. 2022b
Mobula eregoodoo	Long-horned pygmy devil ray	347-1300	Indian and West Pacific Oceans	Tropical-subtropical	Endangered	KwaZulu-Natal, South Africa	Bleeker 1859, Lebepe and Dippenaar 2013, Stewart *et al*. 2018a, Broadhurst *et al*. 2018, Notarbartolo di Sciara *et al*. 2019, Rigby et al. 2022a

Mobula hypostoma	West Atlantic pygmy devil ray	550-1290	Atlantic Ocean	Tropical-subtropical	Endangered	NA	Bancroft 1831, McEachran and Carvalho 2002, Patella and Bullard 2013, Marshall *et al*. 2022c
Mobula kuhlii	Shortfin devil ray	310-1220	Indian and West Pacific Oceans	Tropical-subtropical	Endangered	Southern Mozambique; KwaZulu-Natal, Eastern Cape, South Africa	Müller and Henle 1841, Compagno and Last 1999, Rohner *et al*. 2013, Notarbartolo di Sciara *et al*. 2017, Rigby et al. 2020b
Mobula mobular	Spinetail devil ray	920-3100	Circumglobal	Temperate, tropical-subtropical	Endangered	Southern Mozambique; KwaZulu-Natal, South Africa	Müller and Henle 1841, Notarbartolo di Sciara 1987, White *et al*. 2006, Marshall *et al*. 2022d, Cliff pers. comms
Mobula munkiana	Munk's devil ray	350-1300	East Pacific Ocean	Tropical-subtropical	Vulnerable	NA	Notarbartolo di Sciara 1987, Stewart *et al*. 2018a, Marshall *et al*. 2022e
Mobula tarapacana	Sicklefin devil ray	1052-3400	Circumglobal	Temperate, tropical-subtropical	Endangered	KwaZulu-Natal, Eastern Cape, Western Cape, South Africa	Philippi 1892, Notarbartolo di Sciara 1987, Stewart *et al*. 2018a, Marshall *et al*. 2022f, Cliff pers. comms
Mobula thurstoni	Bentfin devil ray	650-1830	Circumglobal	Temperate, tropical-subtropical	Endangered	KwaZulu-Natal, South Africa	Lloyd 1908, Notarbartolo di Sciara 1987, Stewart *et al*. 2018a, Marshall *et al*. 2022g, Cliff pers. comms

1.2.5. Cleaning symbiosis

Mobulid ray aggregations can be directly related to foraging opportunities, but are also associated with cleaning stations located in close proximity to feeding grounds, where they solicit cleaning services by symbiotic fish (Limbaugh 1961, Couturier *et al.* 2012, Stevens 2016). Cleaning symbiosis is crucial in maintaining healthy fish communities, as one organism (the 'cleaner') benefits by obtaining food through removing ectoparasites, mucus, and wounded tissues, thus providing health benefits to another (the 'client') (Losey 1987). Cleaner-client symbiotic relationships occur globally on coral reefs in all ocean basins (Grutter 1999, Baliga and Law 2016, Quimbayo *et al*. 2018). Cleaning behaviour has evolved across five clades of fishes; persisting for over 20 million years (Feder 1966, Gasparini and Floeter 2001, Whitney and Motta 2008, Quimbayo *et al*. 2017a, b, Baliga and Mehta 2019). The areas where this behaviour occurs, 'cleaning stations', are characterised by protuberant structure on a reef or seamount, providing three-dimensional habitat for the cleaner fish in the form of corals, sponges, and/or large rocks (Losey 1972, Oliver *et al*. 2011).

The relationship between cleaner and client is not arbitrary; individual cleaners and clients recognise one another over time, cleaner individuals repeatedly prefer cleaning familiar clients, and clients visit the same small area of cleaning station up to 144 times in a single day (Losey 1972, Grutter 1995, Tebbich *et al*. 2002). However, this relationship can be exploited, whereby a cleaner fish may ‘cheat’ by removing the protective mucus coating from the client, which has higher nutritional value (Grutter and Bshary 2003). This causes the client to ‘jolt’, suddenly twitching away, acting aggressively towards the culprit, or leaving the cleaning station (Soares *et al*. 2008). Nonetheless, the risks of cheating cleaners are outweighed by the health benefits (Ros *et al*. 2011), as parasite loads significantly increase without access to cleaning (Grutter *et al*. 2018).

‘Facultative cleaners’ are ephemeral cleaners which have other sources of nutrition, whilst ‘dedicated cleaners’ depend on cleaning as their food source for survival (Gasparini and Floeter 2001). Dedicated cleaners are associated with a hierarchical, structured framework that results in more effective cleaning, compared to unstructured facultative cleaner networks (Quimbayo *et al*. 2018). The family Labridae (wrasses, parrotfishes, and hogfishes) contains the greatest number of cleaner species (58) within any one fish family. One of the best known of these species is the blue streaked cleaner wrasse, *Labroides dimidiatus*, a dedicated cleaner found to service an average 2,297 clients each day, consume over 1,000 parasites from clients per day, and remove up to four parasites per minute of interaction (Grutter 1996, Bshary and Côté 2008). Cleaning behaviour is therefore a complex and intricate relationship that not only impacts the behaviour and ecology of the local reef, but also affects the visitation by pelagic species.

Four mobulid species (*M. alfredi*, *M. birostris*, *M. thurstoni*, *M. kuhlii*) are currently known to repeatedly return to aggregate at specific cleaning stations (O’Shea *et al*. 2010, Murie and Marshall 2016, Mukharror *et al*. 2018, Armstrong *et al*. 2020a). The preferences for these selected sites can remain unchanged for up to 30 y in *M. alfredi* (Couturier *et al*. 2014), however, there have been reports of abandonment of certain cleaning stations in southern Mozambique (Cullain, unpublished data). As a result of their health benefits, the home ranges of mobulid species must include an abundance of both zooplankton and cleaning stations within their habitat

(Couturier *et al*. 2012). Apart from manta rays (*M. alfredi, M. birostris*), there is limited information on the cleaning behaviour of other mobulid species.

1.2.6. Reproduction

Mobulids are aplacental, viviparous, matrotrophs that reproduce via internal fertilisation (Wourms 1977, Dulvy and Reynolds 1997, Conrath and Musick 2012). They are slow-growing, with late maturation and low fecundity (Marshall and Bennett 2010a, Stevens *et al*. 2018). Males possess a pair of claspers (external reproductive organs anatomically associated with each of the pectoral fins), while the female reproductive organs are internal (Notarbartolo di Sciara 1987, Marshall and Bennett 2010a). Males are classified as immature if the claspers are small and un-calcified (not rigid), and mature if the claspers are easily visible, calcified (rigid), and extended beyond the edge of the pelvic fins (Notarbartolo di Sciara 1987, Marshall and Bennett 2010a). Females are classified as mature when reproductive scars are present, or if they have been sighted as pregnant, but otherwise it is difficult to assess female maturity (Marshall and Bennett 2010a). *Mobula kuhlii* size at maturity is estimated to be 1030-1120 mm disc width (DW) for males and at least 1160 mm for females (Compagno and Last 1999, White *et al*. 2006; Notarbartolo di Sciara *et al*. 2016, Rigby *et al*. 2020). Male and female manta ray size at maturity ranges from 2700 and 3200 for *M. alfredi* and 3189 mm and 4130 mm for *M. birostris*, (White *et al*. 2006, Deakos 2010, Stevens 2016, Rambahiniarison *et al*. 2018). The age of maturity for most mobulid species remains unknown, but for *M. alfredi* ranges from 3-13 for males (Marshall et al. 2011, Stevens 2016) and 8-17 for females (Kashiwagi 2014, Stevens 2016). Nevertheless, the age and size at maturity may vary between populations.

Elasmobranch mating behaviour in the wild is seldom documented due to the innate challenges of studying these highly mobile marine species. Thus far, only 14 reports on reproductive behaviour of free-living batoids exist, including five for mobulids: *M. thurstoni* (McCallister *et al*. 2020), *M. alfredi* (Marshall and Bennett 2010a, Stevens *et al*. 2018), *M. birostris* (Yano *et al*. 1999, Stevens *et al*. 2018), *M. tarapacana* (Sobral 2013, Mendonça *et al*. 2020), *M. mobular* (Duffy and Tindale 2018). Reproductive activity is well-studied in *M. alfredi* and *M. birostris* which can occur at aggregation sites, such as cleaning stations, or within surface or deep feeding grounds, places where individuals most likely encounter potential mates (Stevens *et al*. 2018, Stewart *et al*. 2019).

Mating behaviour appears to be consistent across batoids with the general sequence of events being: 1) prolonged following of a female by one or more males; 2) pre-copulatory biting by the male onto one of the female's pectoral fins; 3) copulation/insertion of one of the male's claspers into the female's cloaca, flapping his pectorals to stay in place; 4) resting; and, 5) separation (Chapman *et al*. 2003). In mobulids, courtship of oceanic manta rays (*M. birostris*) was first described by Yano *et al*. (1999), who noted the 20-30 min pursuit of one female by multiple males (a 'mating train'), whereas the 'prolonged following' by male southern stingrays (*Hypanus americanus*) described by Chapman *et al*. (2003) included additional behaviours within the 'following' stage. Mating trains have variable numbers of up to 40 males and observed durations up to 48 hr for reef manta rays (*M. alfredi*) (Clark 2010) and up to 147 min for the spinetail devil ray (*M. mobular*) (Duffy and Tindale 2018). This behaviour appears to be similar across mobulid species, but there is a lack of observations of mating in *M. kuhlii*, *M. eregoodoo*, *M. hypostoma* and *M. munkiana*.

Copulation involves the male grabbing a pectoral fin of the female and simultaneously inserting one clasper into the cloaca (Yano *et al*. 1999). Being filter-feeders, manta ray teeth may function solely for mating (Motta and Wilga 2001, Marshall and Bennett 2010a). Following copulation, the individuals separate, and no further interaction occurs (Yano *et al*. 1999). Yolk sac viviparity is the initial source of nourishment for the single embryo, with supplementary nutrition provided by the secretion of lipids from cells in the uterine wall (Snelson *et al*. 2008). The ovum size at fertilisation is unknown. The gestation periods are unknown for most mobulids, but ranges from 10 months for *M. eregoodoo* (Broadhurst *et al*. 2018) to 12-13 months for *M. alfredi* (Marshall and Bennett 2010a). All mobulid species thus far documented typically carry only one pup, with a maximum of two rarely reported (Bigelow and Schroeder 1953, Notarbartolo di Sciara 1987, Compagno and Last 1999, White *et al*. 2006). Birth has never been observed for any mobulid species in the wild, but *M. alfredi* births have been documented in the Churaumi Aquarium in Japan, whereby a mother of 3000 mm DW gave birth to a ~1500 mm pup (Anon 2007) (Table

1.1). Mobulids have resting periods between pupping ranging from 1-3 y (Rambahiniarison *et al*. 2018).

Parental care is absent in mobulid rays, as in all elasmobranchs. Juveniles segregate by age class, as documented in several coastal shoreline and lagoon habitats (McCauley *et al*. 2014, Stevens 2016) and also sometimes takes place offshore (Stewart *et al*. 2018b). Currently confirmed nursery habitats include Flower Garden Banks, USA for *M. birostris* and *M. cf. birostris* (Childs 2001, Stewart *et al*. 2018b); Raja Ampat, Indonesia for *M. alfredi* (Setyawan *et al*. 2022); and the Gulf of California for *M. munkiana* (Palacios *et al*. 2021). Several other areas have been suggested to be possible nursery grounds: Florida, USA for *M. cf. birostris* (Pate and Marshall 2020); Sharm el-Sheikh, Egpyt, for *M. alfredi* (Knochel *et al*. 2022); Nusa Penida, Indonesia for *M. alfredi* (Germanov *et al*. 2019); Fernando de Noronha Archipelago, Brazil for *M. cf. birostris* (Bucair *et al*. 2019); the Gulf of Mexico for *M. hypostoma* (Moral-Flores *et al*. 2020) and *M. mobular* (Gaskins 2019); Peru for *M. mobular* (González-Pestana 2022); Palmyra Atoll for *M. alfredi* (McCauley *et al*. 2014); and the Eastern Cape, South Africa for *M. alfredi* (Marshall *et al*. 2022). Globally, most mobulid nursery habitats have not yet been discovered.

1.2.7. Brain anatomy and sensory systems

Mobulid rays are considered to be intelligent, which has prompted researchers to explore this subject comprehensively (Ari and D'Agostino 2016, Stewart *et al*. 2019, Perryman *et al*. 2019, Perryman *et al*. 2021). Mobulid brains have countercurrent brain mechanisms (Alexander 1996, Northcutt 1977, Ari 2011), which reduces metabolic heat loss, resulting in more efficient information processing and swimming activity and increasing ability to use higher latitudes or deeper water. This mechanism occurs via the *rete mirabile cranica*, found in *M. birostris*, *M. tarapacana*, *M. thurstoni* and *M. mobular* (Schweitzer and Notarbartolo di Sciara 1986, Alexander 1996). Mobulids include the species with the largest known encephalization quotient of all fish, *M. birostris* (Ari 2011). Yopak *et al.* (2007) examined the encephalization quotients of several shark families and found the largest brains to be associated with benthopelagic or pelagic species in reef, coastal, or oceanic sub-habitats, with the highest values seen in sphyrnids, most notably the scalloped hammerhead, *Sphyrna lewini*. Applying this correlation to mobulids suggests pressure for encephalization due to their reliance on reef or coastal habitats for cleaning stations, and the fact that they often aggregate near these habitats during cleaning, foraging and mating (Marshall *et al.* 2009, Couturier *et al.* 2012). Scalloped hammerheads, though solitary feeders, school on seamounts for social aggregation (Klimley and Nelson 1984). Likewise, teleost species found in complex reef habitats developed advanced brains with cognitive abilities, such as tool use (Jones *et al.* 2011, Brown 2012).

Mobulid rays evolved a large telencephalon, considered the coordinating centre for elasmobranchs, where olfactory projections, mechanoreceptive and electroreceptive modalities, and visual modalities converge, influencing motor movement, memory, and cognition (Ari 2011). In *M. birostris*, 61% of total brain mass is composed of the telencephalon and 19% forms the cerebellum, which is responsible for balance, coordination, error correction, and smooth muscular activity (Ari 2011). These enlargements are most likely associated with other complex behaviours, rather than foraging strategies, since filter-feeding is generally passive. Moreover, Ari (2011) observed enlarged anterior lobes in mobulid cerebellums, the region where visual responses are processed, indicating highly-developed vision. Mobulids are sensitive to visual stimuli (McComb and Kajiura 2008). Ari (2011) reported a large optic tectum and enlarged cerebellum anterior lobe, where visual responses predominate, in *M. birostris*. Mobulids lack stingers/serrated caudal spines (Notarbartolo di Sciara 1987), making speed and large size their only real defence mechanism against predation. This escape mechanism in turn may rely on effective eyesight to detect predators at a distance and to react quickly. Vision is thus essential for mobulids to avoid predators and possibly also for recognising and responding to social cues.

1.2.8. Social behaviour

Sociality is considered a significant outcome of cognitive evolution (Kamil 2004) and has been documented in several elasmobranch species (Guttridge *et al.* 2011, Mourier *et al.* 2012). *Mobula alfredi* exhibit fusion-fission societies in Raja Ampat, Indonesia, in which separate communities were discovered and mature females were more likely to form stable social bonds with one another (Perryman *et al.* 2019). Cleaning stations are sites for regular social interactions; evidence for this includes the fact that individuals are repeatedly sighted being cleaned together, specific individuals are regularly seen together on certain cleaning stations, and that courting and copulation events take place at these sites (Marshall and Bennett 2010a, Stevens *et al.* 2018, Perryman *et al.* 2019).

As social elasmobranchs, *M. alfredi* exhibit numerous intraspecific interactions while cleaning. The presence of *M. alfredi* at a cleaning station may attract other individuals to join, who will then imitate their behaviour, while other studies propose that there are agonistic displays, in which individuals compete to control access to cleaning (Kitchen-Wheeler 2013). Specific ‘following’ and ‘leading’ behaviours have been identified in *M. alfredi*, which may reflect a learned ritual (Perryman *et al.* 2019, Perryman *et al.* 2021). Individuals that showed higher use of the cleaning station, as tracked by acoustic telemetry, also had stronger social affiliations (Perryman *et al.* 2022). The substantial energentic investment required for increased brain size highlights the potential importance of social behaviour in the reproductive success of mobulids, as supported by the existing evidence.

1.2.9. Conservation and ecotourism

Mobulid rays have k-selected life history traits and long lives of at least 27-30 y, and potentially up to 40 y for manta rays, all characteristics which make them vulnerable to overexploitation (Kashiwagi *et al.* 2008, Couturier *et al.* 2012, Broadhurst *et al.* 2018). Fisheries impacts are the major threat to mobulids, and their populations cannot withstand present fishing mortality, even from small artisanal fisheries (Dulvy *et al.* 2014, Lawson *et al.* 2017). Genus-wide declines are predicted globally, based on the reports of decreasing sightings per unit effort (SPUE) of monitored populations and catch data (Fernando and Stevens 2011, Couturier *et al.* 2012, Hall and Roman 2013, Lewis *et al.* 2015, Croll *et al.* 2016). For instance, global abundance of *M. kuhlii* has declined by 50–79% in the past 38 years (Rigby *et al*. 2022). Population declines are partly attributed to direct harvesting of the gill plates for Asian medicine (Couturier *et al.* 2012, O’Malley *et al.* 2016). The rapidly growing Chinese markets offer USD$130 for a whole *M. kuhlii* and $350 for a *M. birostris* (Zeng *et al.* 2016). Bycatch from unsustainable fishing methods is also a major threat to all mobulids, which are frequently captured both offshore in tuna purse seine nets and in gill nets along the coastline (Couturier *et al.* 2012, Poisson *et al.* 2014). Further, ghost nets, which are lost gill nets, are the most common form of entanglement for elasmobranchs across the globe (Parton *et al.* 2019). Other impacts include coastal development, which in turn leads to increased boat strikes, habitat loss, and pollution (Couturier *et al*. 2012, Croll *et al*. 2016, Fernando and Stewart 2021, Lawson *et al*. 2017, O'Malley *et al*. 2016).

Due to a combination of these major threats and their life history characteristics, all species of Mobulidae are listed on the International Union for Conservation of Nature and Natural Resources (IUCN), as either Vulnerable (two species) or Endangered (seven species) (Table 1.1). In other international agreements all nine mobulid species are listed on Appendices I and II on the Conservation of Migratory Species (CMS), and on Appendix II the Convention on the International Trade in Endangered Species (CITES). Monitoring of mobulid populations is thus crucial for future IUCN Red List assessments and further development of local management actions, such as the designation of new Marine Protected Areas (MPAs) and regulations surrounding fisheries and tourism operations.

As a result of their documented declines and considerable value to the tourism industry, a surge of mobulid research and conservation efforts has occurred during the last two decades, largely focused on manta rays (*M. alfredi*, *M. birostris*) (Lawson *et al*. 2017, Stewart *et al*. 2018a). By 2012, manta ray tourism had an estimated global annual tourism value of $140 million per year (O'Malley *et al*. 2013), compared to a fisheries value of only $11.3 million per year (Heinrichs *et al*. 2011). In Inhambane Province, Mozambique, alone, this value was $10.9 million for manta ray snorkelling/diving (Venables *et al*. 2016). This tourism largely occurs at cleaning stations, which therefore act as focal points for research, monitoring, conservation, ecotourism, and citizen participation in research.

1.2.10. Photoidentification

Manta rays (*M. alfredi*, *M. birostris*) and the sicklefin devil ray (*M. tarapacana*) (Mendonça *et al*. 2020) possess unique ventral spot patterning that is used for photographic mark-recapture ('photo-ID') studies, but has not yet been reported in other mobulids (Kitchen-Wheeler *et al*. 2011, Marshall *et al*. 2011). The unique and stable ventral pattern of individual rays has facilitated photo-identification (photo-ID) studies at these aggregation sites, providing the foundation for manta ray research in many locations across the globe (Kumli and Rubin 2008, Deakos *et al*. 2011, Marshall *et al*. 2011, Couturier *et al*. 2014, Stevens 2016, Germanov *et al*. 2019, Harris *et al*. 2020). This technique has been used to assess home range (Deakos *et al*. 2011, Kashiwagi *et al*. 2011), longevity (Rubin 2002, Clark 2010, Couturier *et al*. 2014, Kashiwagi 2014), migration patterns (Germanov and Marshall 2014, Armstrong *et al*. 2019), site affinity (Couturier *et al*. 2011, Marshall *et al*. 2011, Germanov *et al*. 2019), and reproductive ecology (Marshall and Bennett 2010a, Deakos *et al*. 2011, Stevens 2016). *Mobula alfredi* photo-ID databases vary substantially in the total number of individuals identified over time, ranging from populations in the low hundreds (Deakos *et al*. 2011, Kashiwagi 2014, Axworthy *et al*. 2019, Carpentier *et al*. 2019, Peel 2019) to those in the thousands (Stevens 2016, Armstrong *et al*. 2019, Venables 2020).

With photographs as a main data source, citizen science has amplified the scope and robustness of field studies, coupled with continuously advancing technology, making this powerful tool increasingly useful in research (Dickinson *et al*. 2010). Photo-ID is a primary research technique that has enabled key findings, for instance, documenting the largest known populations of *M. birostris* (Ecuador, Harty *et al*. 2022) and *M. alfredi* (the Maldives, Stevens 2016), and the longest point-to-point migration (1,150 km) for *M. alfredi* (Armstrong *et al*. 2019). Photo-ID records can also be used in statistical analyses for manta rays (*M. alfredi*, *M. birostris*) to estimate population abundances (Marshall *et al*. 2011, Beale *et al*. 2019, Cabral *et al*. 2023), annual apparent survival (Couturier *et al*. 2014), and to document abundance trends over time (Venables 2020). This technique provides researchers with insights into the utilisation of particular habitats, the frequency of visitation by individuals, and facilitates continuous monitoring of these aggregations.

Photo-ID can also be used to estimate the frequency of unique individuals that possess an excess of pigment, melanism, or reduced pigment, leucism, a feature which appears to be unique to manta rays (*M. alfredi*, *M. birostris*) within the family Mobuliae (*Mobula* spp.) and indeed within elasmobranchs as a whole (Marshall *et al*. 2009, Venables *et al*. 2019). As a genetic phenotype, the frequencies of melanism and leucism vary across populations; including over 40% melanism in photo-identified *M. alfredi* in Raja Ampat, Indonesia (Venables *et al*. 2019) and 26% in photo-identified *M. birostris* in the Revillagigedo Archipelago, Mexico (Kumli and

Rubin 2011). Leucism frequencies are difficult to quantify, due to the large fluctuation in the degree of pigmentation between leucistic individuals (Venables *et al*. 2019). It is unknown why these colour morphs exist, since no differences in survivorship have been detected in these individuals, compared to those with 'normal' pigmentation patterns (Venables *et al*. 2019).

1.3. Mobulids in southern Africa (South Africa and Mozambique)

In southern Africa, three mobulid species are frequently encountered: *M. alfredi*, *M. birostris*, and *M. kuhlii*, whereas anecdotal sightings or reports (catches), via the KwaZulu-Natal (KZN) bather protection programme, of other mobulids, are rare (Cliff pers. comms; Table 1.1). Their species distribution is known to extend from the Inhambane Province, Mozambique border to Port St. John's, Eastern Cape, for *M. alfredi* (Marshall *et al*. 2022) in the south, with some occurrences reported for *M. birostris* in the Western Cape (Marshall *et al*. 2022b). The species distribution for *M. kuhlii* is currently unknown in South Africa. Despite contrasting temporal patterns, manta rays (*M. alfredi*, *M. birostris*) in southern Mozambique overlap in their use of cleaning and foraging habitats, which may be a result of resource availability in the area, which may likely also be true for South Africa (Kashiwagi *et al*. 2011). Identifying and describing critical habitats for mobulids which inhabit extensive home ranges poses a significant challenge, and they are likely impacted by numerous anthropogenic imapcts within their overall habitat.

1.3.1. Mobulid conservation in southern Africa

In the Indian Ocean, mobulids are impacted by directed fisheries (Croll *et al*. 2016, O'Malley *et al*. 2016) and as indiscriminate bycatch from the use of gill nets (Lewison *et al*. 2004, Temple *et al*. 2018), purse-seine nets (Guirhem *et al*. 2021), and longlines (Lezama-Ochoa *et al*. 2015). Although directly fished and caught as bycatch in Mozambique (Couturier *et al*. 2012, Croll *et al*. 2016), one of the sources of fishing mortality for *M. alfredi* and *M. birostris* in South Africa is the bather protection programme in KZN (Dudley and Cliff 1993, Cliff and Dudley 2011, Young 2001). Although not a fishery in the conventional sense, this is the only shark fishing operation in South Africa documented to catch mobulids (Dudley and Cliff 1993, Croll *et al*. 2016, Marshall *et al*. 2008).

A 20-year study in Mozambique documented the largest photo-identified population of *M. alfredi* in Africa, with the number of identified individuals currently reported to be 1209 (Marshall *et al*. 2011, Venables 2020). With increased annual sampling effort, *M. alfredi* continues to exhibit long-term affinity to monitored cleaning stations in Mozambique; some individuals returning to the same sites for more than 15 years (Venables 2020). Estimations of annual abundances in Tofo peaked at 836 individuals in 2004-2005 (Venables 2020). However, *M. alfredi* sightings declines of up to 88% between 2003-2011 (Rohner *et al*. 2013) and estimates of only 100 individuals sighted in Tofo after 2013 (Venables 2020), has raised concern about the health of this population, and it has been declared the most threatened in the world (Stevens 2016, Carpentier *et al*. 2019, Peel 2019, Venables 2020). Further, *M. kuhlii* was also reported to decline 99% in sighitngs in Tofo (Rohner *et al*. 2017). The substantial research and conservation efforts in Tofo, Mozambique over the last two decades (Marshall *et al*. 2011, Venables *et al*. 2016) resulted in *M. alfredi*, *M. birostris* and *M. kuhlii* being declared nationally protected (Boletim da Republica 2021). In South Africa, manta rays (*M. alfredi*, *M. birostris*) are nationally protected (Notice No. 40875 under No. 476 of the Biodiversity Act, 10 of 2004,2017), but *M. kuhlii*, as well as all other mobulids, remain unprotected.

1.3.2. Knowledge gaps

While mobulids (mostly *M. alfredi*, *M. birostris*) have been studied for two decades in Tofo, Mozambique, they remain relatively understudied in other locations in the region (e.g., Závora, Mozambique), with little research to date in South Africa, despite sightings from KZN and the Eastern Cape (Marshall *et al*. 2022). Further, few studies have been conducted on *M. kuhlii*. At monitored locations in southern Mozambique, manta ray habitat use is seasonal; *M. alfredi* sightings increase in Tofo during austral summer (November to February) (Marshall *et al*. 2011) and *M. birostris* sightings peak in April (Rohner *et al*. 2013), but the identification of other sites and the respective spatio-temporal trends of mobulids along the coast remains unknown.

Genetic analysis suggests that there is a single breeding population of *M. alfredi* common to both Mozambique and South Africa (Venables *et al*. 2021, Marshall *et al*. 2022), and it is likely that KZN coastal waters serve as critical habitat for southern African manta ray populations. Preliminary research has identified *M. alfredi* sightings in KZN, with the same individuals migrating to or from Mozambique, with return distances of up to 505 km (Marshall *et al*. 2022). Further, acoustic telemetry revealed a *M. alfredi* individual that travelled up to 90 km in a single day in Mozambique (Venables *et al*. 2020).

Mobulid movement patterns in southern Africa may be driven by temporal and spatial patterns of zooplankton abundance (Sims *et al*. 2006, Rohner *et al*. 2013, Stewart *et al*. 2019). The waters off KZN are well-known to be important habitat for many elasmobranch species (Dudley and Cliff 2010, Daly *et al*. 2014, Dicken *et al*. 2018, Daly *et al*. 2021). On the east coast of South Africa, the narrow continental shelf (Martin and Flemming 1988) and shifting seasonal water temperatures and currents (Walker 1990, Roberts *et al*. 2010) allow numerous elasmobranch species, such as the tiger shark (*Galeocerdo cuvier*) and diamond ray (*Gymnura natalensis*) to exploit a wide range of habitat and area (Connell 2001, Wetherbee and Cortés 2004, Dicken *et al*. 2006, Daly *et al*. 2018, Daly *et al*. 2022). However, most of the South African coastline has yet to be surveyed for mobulid ray habitat use.

1.3.3. Recognising critical habitats for mobulids in southern Africa

Stewart *et al*. (2018a) emphasised the necessity to find and recognise critical habitats for mobulids to facilitate the recovery and preservation of their populations. These habitats or 'hotspots' need to be identified to determine which, if any, are in existing MPAs and which fall outside formal conservation areas and therefore are in need of protection to safeguard the species. Understanding population trends of large, migratory, marine species can be challenging due to the difficulty in assessing habitat use within their estimated home ranges. Knowledge of fine-scale visitation patterns at specific locations through site-specific studies can be beneficial to understanding population dynamics, identifying priority areas for protection, and developing local management regimes.

1.4. Aims and thesis structure

This thesis aims to improve understanding of three southern African mobulid rays (*M. alfredi, M. birostris, M. kuhlii*) and how they utilise specific areas along the coastline from southern Mozambique in the north to KZN, South Africa in the south. To avoid duplication, the text is presented in the form of single overarching Introduction, Methods, and Conclusions sections, paired with five Results and Discussion sections, which each representing the findings of a specific research paper.

Chapter 1 provides an introduction and comprehensive literature review. Chapter 2 describes the methodologies used to provide information about mobulid distributions, habitat use, behaviour, abundances, and spatiotemporal trends. Chapters 3-7 then detail the major contributions of the thesis, which include:

Chapter 3 aims to assess population demographics and abundance of the *M. alfredi* aggregation in Závora, Mozambique, which has not yet been evaluated, compared to 90 km north in Tofo, which has been under continuous observation since 2003. To assess the importance of this aggregation within the broader *M. alfredi* population in southern Mozambique, I use an 11-year photo-ID database of individuals to describe population demographics, site affinity, and resightings data. Using PRD mark-recapture modelling, I estimate annual abundance and population parameters including apparent survival, emigration and recapture probability at RS between 2016 and 2021. The findings can be used to inform the development of local conservation strategies and guide the design and implementation of spatial management approaches, such as Marine Protected Areas, in the Závora Bay region of the Inhambane coastline.

Chapter 4 applies the manta ray (*M. alfredi*, *M. birostris*) photo-ID techniques used in Tofo, Mozambique for over two decades, and in Závora for 11 years, to South Africa, where little research had been done to date. I construct the first photo-ID database of manta rays in South Africa, and combine two years of in-water baseline data, historical and current citizen science data, and anecdoteal reports, to identify hotspots and their functions along the KZN coastline. I cross-reference *M. alfredi* photo-identified in South Africa with the Tofo and Závora databases to build upon Marshall *et al*. (2022)'s initial findings that allude to a single population movement between the two countries. Given the challenging study of wide-ranging marine species, this chapter aims to establish a foundation for future research recommendations and management insights.

Chapter 5 aims to determine temporal and spatial patterns in catches of manta rays (*M. alfredi*, *M. birostris*) in KwaZulu-Natal (KZN), South Africa. Due to the number of sightings and the behavioural patterns documented in KZN in Chapter 4, these coastal waters likely serve as a vital habitat for populations of southern African manta rays. I use 41 years of catch data from the KZN bather protection program to investigate long-term trends in manta ray occurrence, body size, and demographic composition. Covering a geographical expanse spanning several hundred kilometres and encompassing a duration of over four decades, this dataset represents the most extensive and longterm assessment of trends in manta ray sighitngs on Africa to date. The results can serve as a foundation for understanding baseline trends used for future assessments and pinpointing key areas where targeted research and management strategies can be applied.

Chapter 6 aims to describe the first known shortfin devil ray (*M. kuhlii*) cleaning station in the Aliwal Shoal MPA, South Africa, and the trends in sightings and behaviours associated with this critical area. I use Generalised Additive Models to assess the 1) environmental effects (e.g., sea surface temperature, current) on the presence of *M. kuhlii* on the Aliwal Shoal MPA and 2) cleaning durations on Angels Ledge (AL) cleaning station. Using Remote Underwater Video (RUV) I describe intraspecific cleaning behavior and interactions with cleaner fish, which has been evaluated in manta rays but not yet in *M. kuhlii*. The results are informative for understanding fine-scale habitat use, and to establish a sustainable ecotourism model centred around this understudied, unprotected species.

Chapter 7 aims to describe shortfin devil ray (*M. kuhlii*) courtship behaviour for the first time. I record the first scientifically documented videos of *M. kuhlii* courtship, a description of the environmental conditions that prevailed during these events, and a description of pre-mating behaviour.

This thesis advances the understanding of the biology of mobulids and their critical sites along the southern African coastline, hence facilitating the development of improved management strategies and conservation for these threatened species.

CHAPTER 2

GENERAL METHODS

Citizen science is an effective tool used in research on mobulid species.

Photo credit Zachary Mineo

The study region of this thesis spans approximately 1,000 km, from Závora, Mozambique in the north, to the Protea Banks Marine Protected Area, South Africa in the south. Most of the research efforts were concentrated in South Africa due to the knowledge gaps of manta ray spatio-temporal habitat use in this country. Where focused research was conducted, specific study maps are provided, otherwise, short descriptions are given. The study site for Chapter 3 was Závora, Mozambique. The study area for Chapters 4-7 was the KwaZulu-Natal Province, South Africa, with a citizen science photo contributed from the Eastern Cape Province included in Chapter 4.

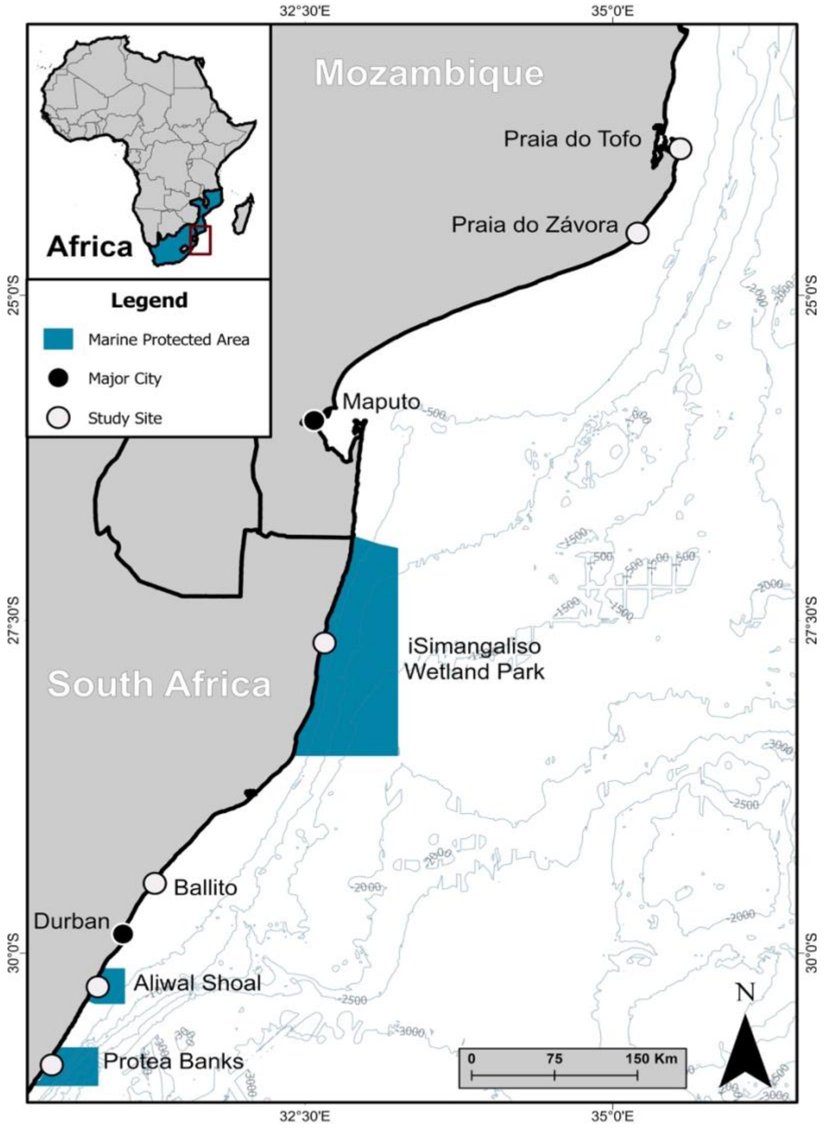


Figure 2.1. Study region for this thesis in South Africa and Mozambique, including sites where sightings data was gathered (Protea Banks, Aliwal Shoal, Ballito); and photographic data (Závora, iSimangaliso), including opportunistic citizen science images (Tofo, Aliwal Shoal).

2.1. Study site: Závora, Inhambane Province, southern Mozambique

In Mozambique, mobulids are most commonly encountered in the coastal waters of the Inhambane Province, particularly from the Bazaruto Archipelago in the north to Závora in the south (Figure 2.2). This 350 km stretch of coastline joins a narrow continental shelf that experiences regular upwelling events, resulting in productive waters that attract reef manta rays (*M. alfredi*), oceanic manta rays (*M. birostris*) and shortfin devil rays (*M. kuhlii*) (Rohner *et al*. 2013, Quartly and Srokosz 2004). The most visited site in Závora is a cleaning station called Red Sands (RS), a rocky reef with scattered corals and sponges, at 12-18 m depth, approximately 3 km offshore (Figure 2.2). The site is characterised by variability in environmental conditions; with horizontal visibility ranging 1-20 m, various levels of current and surge, and sea temperatures ranging from 16-23 ºC in the winter, and up to 27 ºC in the summer (Cullain unpublished data).

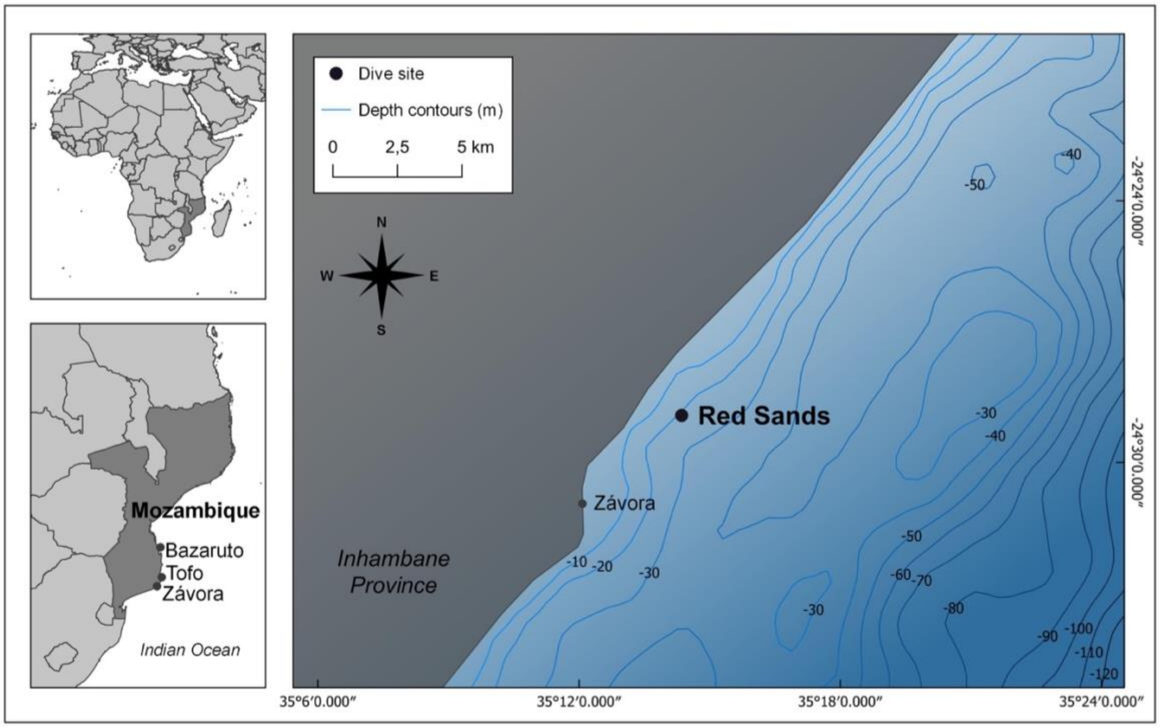


Figure 2.2. Study map of Závora Bay, Inhambane, Mozambique, showing the location of the Red Sands cleaning station and the bathymetry of the bay.

2.2. Study area: KwaZulu-Natal, South Africa

The coastal waters off the KwaZulu-Natal (KZN) Province, on the east coast of South Africa, is subtropical and dominated by the southward-flowing Agulhas Current, which moves between the

Mozambican Channel from Mozambique into South Africa (Lutjeharms 2007). The KZN Province is a wind-driven and high-energy environment (Schumann 1988), and, depending on location, receives little to substantial riverine input (Pretorius *et al*. 2016), near the presence of submarine canyons (Sink *et al*. 2006), and varying distance offshore of continental shelf edge (Green and Garlick 2011). Due to these dynamic ocean systems (Sink *et al*. 2019), the waters are productive and vary temporally and seasonally (Lamont and Barlow 2015, Govender *et al*. 2022). Two ecoregions have been described by Sink *et al*. (2019) within KZN borders: 'Maputaland', which extends from the Mozambique border southwards to Cape Vidal, and 'Natal', from south of Cape Vidal to the Eastern Cape border (Sink *et al*. 2005, Griffiths *et al*. 2010). However, within the Natal region there is variation in the flow of the Agulhas Current and how it interacts with the continental shelf (Lutjeharms *et al*. 2000, Roberts *et al*. 2010). This variation is largely due to the presence of the Natal Bight, a 160 km long and 50 km wide coastal offset located between Cape St. Lucia and immediately south of Durban (160 km), which interrupts the strong, stable flow of the Agulhas Current evident along most of the coast (Fennessy *et al*. 2016). South of the Natal Bight, the continental shelf break becomes narrower and closer to shore, extending southwards to the Eastern Cape border (Fennessy *et al*. 2016).

2.2.1. iSimangaliso Wetland Park

The iSimangaliso Wetland Park (ISWP) is located in northern KZN (Figure 2.1), within the biogeographic zone 'Maputaland' (Sink *et al*. 2005). It is a multipurpose Marine Protected Area (MPA) including both inshore and offshore sectors (Notice No. 10950 under Volume 647 of the Protected Areas Act, 57 of 2003). Sampling was conducted from 9-Mile in the north (-27.4129, 32.7282), to roughly 40 km south to Raggie Reef (-27.9138, 32.5974). This encompassed three management zones: the Sodwana Bay Dive Restricted Zone, the iSimangaliso Offshore Pelagic Line Fishing Zone, and the iSimangaliso Offshore Wilderness Zone (IOWZ). The ISWP is characterised by prolific shallow coral reefs along the coastline (Schleyer and Celliers 2003) adjacent to several submarine canyons which drop to 100-500 m depths (Green and Uken 2008).

2.2.2. Ballito

Ballito is a coastal town approximately 54 km north of Durban (-29.4376, 31.2226) (Figure 2.1). The marine environment is popular for recreational fishing, including shore and boat angling, and spearfishing. The area does not have any designated MPAs, the closest existing MPA being at Tugela Mouth, roughly 50 km north. This location was opportunistically visited after two years of communications with local fishermen suggested a potential periodic manta ray (*M. alfredi, M. birostris*) aggregation. A study map was not made for this site because sightings were collated opportunistically and infrequently.

2.2.3. Aliwal Shoal Marine Protected Area

The Aliwal Shoal MPA (-30.2672, 30.8203) (Figure 2.1, 2.3) is a rocky reef composed of fossilised sandstone that formed approximately 80,000 years ago and submerged 6,500 years ago (Bosman *et al*. 2007). Aliwal Shoal is located at a biogeographical transition zone between Natal, which also includes the southern warm-temperate Pondoland reefs, characterised by a narrow continental shelf between <5-50 km and Maputaland, the northern tropical/subtropical reefs (Schleyer 2000, Sink 2001, Mann *et al*. 2006, Celliers *et al*. 2007, Olbers *et al*. 2009). This marine environment is subjected to substantial input of freshwater discharge and sediments/pollutants from rivers along the coast, and with summer rains, increased phytoplankton blooms and turbidity (Boucher 1975, Schleyer *et al*. 2006). Aliwal Shoal was proclaimed an MPA in 2004 to prevent anchoring and mooring of vessels, and extraction of marine resources (Marine Living Resources Act No. 18 of 1998, Government Gazette No. 26433, South Africa 2004). It covers 18.3 km of coastline and protects 126 km² of ocean (Marine Living Resources Act No. 18 of 1998, Government Gazette No. 26433, South Africa 2004). The study area of this research was in the Crown Area Restricted Zone (Figure 2.3) which is approximately one kilometre in length, encompassing an area of 2.1 km² (Bosman *et al*. 2005). The width varies from 280-890 m, with an average depth of 12.5 m below mean sea level, reaching as shallow as 6 m (Bosman *et al*. 2007).

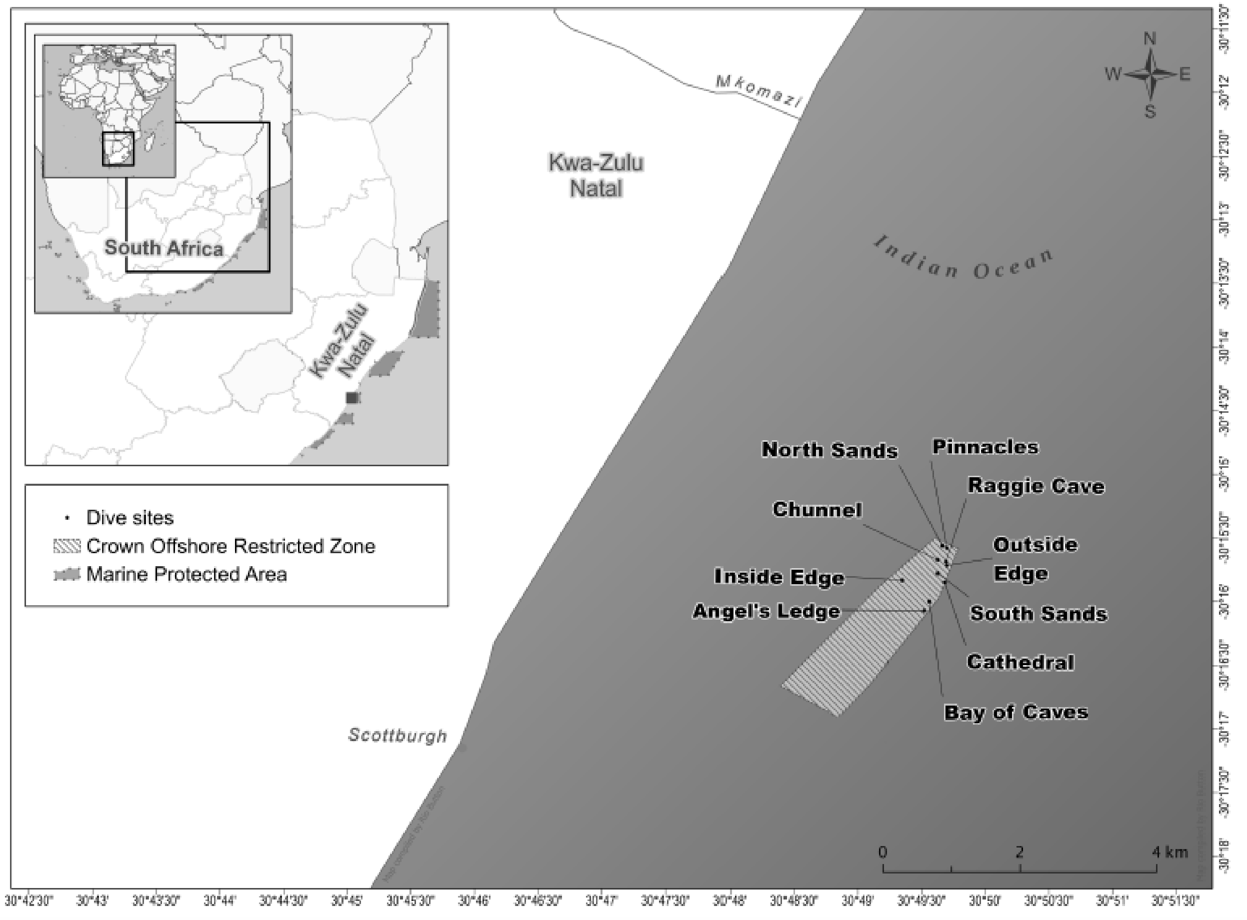


Figure 2.3. Map showing dive sites where *Mobula kuhlii* were encountered during recreational snorkelling or SCUBA diving at the Crown Restricted Zone of Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa between 2020-2022.

2.2.4. Protea Banks Marine Protected Area

The Protea Banks MPA (-30.8656, 30.4853), is approximately 85 km south of the Aliwal Shoal MPA (Figure 2.1). It is a fossilised dune reef that formed during the last glacial events (Jackson 2000) and is a popular shark dive destination. The reef has minimum depths of 27 m and drops to 60 m on the perimeters (Jackson 2000). It is roughly 6 km long and 800 m wide, with several pinnacles, caves, and deep reefs providing extensive three-dimensional structure (Sink *et al.* 2011). The area is characterised by frequent strong currents and year-round pelagic shark sightings (Sink *et al.* 2011). It was proclaimed an MPA in 2019 (National Environmental Management 2019). A study map was not constructed because data from this area were from a donated citizen science database, and not sampled since 2018.

2.3. Photoidentification

The use of photoidentification (photo-ID) has rapidly co-evolved in parallel with both technological advancements in camera equipment, open-source websites, algorithm development, and growth in citizen science initiatives (for example, public data submissions and increased internet access), making it a powerful tool for long-term population monitoring of wide-ranging species across many taxa and habitat types (Karanth 1995, Dala-Corte *et al.* 2016, Marshall and Pierce 2012, McConkey 1999, Schofield *et al.* 2008, Towner *et al.* 2013, Wiirsig 1990). Citizen science participation in manta ray research occurs on online platforms such as ‘Manta Matcher’ (Global; Town *et al.* 2013), ‘ID the Manta’ (Global; Stevens 2016), and ‘Project Manta’ (Australia; Couturier *et al.* 2011). Predictable aggregations at certain sites allow snapshots of population sizes, trends, and movement patterns of these elusive species.

Photo-ID, including citizen science-sourced data, was the primary method used to research manta rays (*M. alfredi*, *M. birostris*) in this thesis (Chapter 3, Chapter 4). Photographs were accepted from any year, providing the date and location were accurate. Individual profiles were completed for each encounter that included the photographer and/or submitter, date, location, and wherever possible, information on sex, maturity, and behaviour. Images were cropped to isolate the standardised spot pattern and oriented head-up (Marshall 2008, Germanov *et al.* 2019). Manta rays were documented in southern Africa at identified locations, to describe newly discovered sites, and to check for connectivity of *M. alfredi* between South Africa and Mozambique. The behaviours of individual manta rays were recorded and compared to assess the function of these sites within the greater southern African region.

2.3.1. Population demographics

Upon encountering a manta ray, the following data were collected when possible and without disturbing the ray: (1) species, (2) photograph of the ventral spot patterning, (3) sex, (4) maturity, (5) pigmentation type, (6) size estimate, and (7) behaviour. Species identification was conducted using the morphological characteristics described by Marshall *et al.* (2009). Sex was determined by the presence of external claspers for males, and absence for females (Marshall and Bennett 2010a). Male maturity was assessed by the size of reproductive organs, individuals being considered adult once the claspers extended past the posterior edge of the pectoral fins (Marshall and Bennett 2010a). Female maturity was determined by the observation of pregnancy (when the abdomen was clearly expanded), or the presence of reproductive scars usually on the left pectoral fin (99% lateralization: Marshall and Bennett 2010a). A female that was not noticeably pregnant, nor had mating scars, was recorded as unknown maturity. If multiple behaviours were observed during a single dive or drift (e.g., surface feeding and cruising), all behaviours were noted. Resightings of an individual were recorded when identified more than 24 h after the last sighting.

In Závora, Mozambique, photo-ID data were collected between 2010-2021 to assess population demographics. Mean counts of individuals or time periods between sightings were calculated to assess the number and resightings of males and females, (± Standard Deviation). The total number of identified and unidentified individuals were pooled for each day of sampling. Photographic sampling by trained researchers was conducted at RS on SCUBA diving. Weather, logistical limitations, and COVID-19 restrictions prohibited consistent, daily sampling effort throughout and between years. During each survey, teams of 2-8 divers swam a transect that covered all monitored cleaning stations that make up RS.

In KZN, South Africa, opportunistic sampling was conducted between 2020-2022 at the iSimangaliso Wetland Park and Aliwal Shoal MPA. The SharkLife Conservation Group also donated citizen science data for the iSimangaliso Wetland Park. At Protea Banks, these citizen science data were collected by a recreational SCUBA diver at African Dive Adventures. In Ballito, recreational boat tours were joined sporadically to search for manta rays on the surface. Volunteer microlight pilots from the Ballito Microlight School also contributed to manta ray searching in Ballito. Unforeseen events, which prevented launching a boat to sea, included COVID-19 between 2020-2021, the KZN Riots in July 2021, and the KZN Floods which affected March-September 2022.

2.3.2. Mark-recapture modelling of M. alfredi in Závora, Mozambique

Mark-recapture population modelling provides a tool through which photo-ID data could be analysed to provide abundance estimates. Predictable patterns in the use of critical habitats, such as cleaning stations and feeding locations, make reef manta rays (*M. alfredi*) a suitable candidate for this technique (Couturier *et al*. 2011, Venables 2020). With an initial photo of the ventral spot patterning signifying an individual's 'mark' and subsequent photos representing their 'recaptures', these data were further analysed through models to estimate population parameters (Couturier *et al*. 2014, Grusd *et al*. 2019). Previous mark-recapture studies of *M. alfredi* have used various model types (e.g., Cormack-Jolly-Seber (CJS); Petersen's method; Pollock's Robust Design (PRD)) (Deakos *et al*. 2011, Marshall *et al*. 2011, Kitchen-Wheeler *et al*. 2012, Couturier *et al*. 2014).

2.3.3. Pollock's Robust Design

The Pollock's Robust Design is regarded as a useful modelling system to account for temporary emigration and capture heterogeneity, which are inherent in mobile marine species (Couturier *et al.* 2014, Venables 2020). PRD models are characterised by marginal dependence between abundance and survival estimators, as well as estimation of temporary emigration, all of which improve the precision of population estimates and interpretations of the relationship between abundance and survival (Pollock 1981, Pollock *et al*. 1990, Kendall *et al*. 1995, Grusd *et al*. 2019).

Mobulids have been monitored in Závora, Mozambique, since 2010, however, due to limited resources, the remoteness of this location, and minimal tourism/recreational diving, a comprehensive sampling design was not initiated until 2016. Mark-recapture models require consistent survey effort and due to the nature of the current dataset, only the most recent six years fitted these criteria (82% of total identifications). This is because very little sampling effort, including many months with zero surveys, and little no data collection was recorded at RS in the database prior to 2016. Data collected at RS during the five-month peak season (July-November) of 2016-2021 were included in the Pollock's Robust Design (PRD) modelling, resulting in six primary periods (years) and 29 secondary periods (months), selected based on higher *M. alfredi* sightings. Each winter season had five monthly secondary periods (July-November), except 2016, which had four (July-October), due to no survey effort in November 2016. Reef manta ray (*M. alfredi*) sightings were lower in December-June; therefore, these months were excluded to allow adequate time between primary periods to detect fluctuations in the population (Kendall 1999, Silva *et al*. 2009).

Pollock’s Robust Design models have the following assumptions: all ventral markings on *M. alfredi* individuals were unique and remained stable over time, the population was open to immigration, emigration, natality, and mortality between years, full closure within the aggregation months, and equal survival probability on all individuals (Kendall *et al.* 1995, Williams *et al*. 2002, Cooch and White 2006, Smith *et al*. 2013). Closure was not assumed at RS specifically, rather it was assumed that the individuals encountered at RS remained in the Závora Bay region during these time periods and were thus recaptured at RS.

A PRD with Huggins’ estimator was used to analyse six-year photographic mark-recapture data of *M. alfredi* at RS, Závora (Huggins 1989, Pollock *et al.* 1990). The annual abundances and population parameters, including apparent survival, emigration, and recapture probability were estimated at RS between 2016-2021. Models were assembled using package ‘RMark’ (Laake 2013), in R Version 4.1.2 (R Core Team 2021) the R interface to program MARK (White and Burnham 1999, Cooch and White 2006).

Apparent survival between primary periods was evaluated as time-constant φ (·), time varying φ(t), and with a group effect for sex φ(sex). Models including time-varying survival consistently yielded inestimable parameters. It was appropriate to exclude time-varying survival from the final model set due to the longevity of *M. alfredi* once mature; previous studies on *M. alfredi* populations found survival estimates close to 1.0 between years (Kitchen-Wheeler *et al*. 2012, Couturier *et al*. 2014). The temporary emigration parameter represents the probability of present individuals in the population being absent for capture in a specific period (Kendall *et al.* 1997). This was assessed as Markovian (γ' and γ''), random γ ($\gamma' = \gamma''$), or none ($\gamma', \gamma''=0$). Capture p, and recapture c, probabilities were modelled as time-constant p (·), time-varying per year p(y), and with effects of sampling effort p(s). Equal capture and recapture probability (p=c) was excluded from the final candidate model set due to inestimable parameters resulting from the variability of encounters per secondary period. Parameter estimates were model averaged based on the model weight. The confidence interval (CI) and standard error (SE) of each estimated parameter were evaluated. The PRD analysis was subsequently conducted on the same data with pooled sexes to yield numbers for total population abundance across the primary periods. Akaike’s information criterion (AICc) was used to evaluate the best model that fitted the data, determined by the smallest AICc value (Burnham and Anderson 2004). A Mann-Whitney U test was conducted using the ‘exactRankTests’ R package to analyse the effect of sex on the total number of recaptures during the study period, with individuals of undetermined sex excluded from the analysis (Hothorn and Hornik 2021). Significance was accepted at $p < 0.05$.

2.3.4. Lagged Identification Rates

Lagged Identification Rates (LIR), the probability of resighting an individual after a given time lag, were used to estimate site use of *M. alfredi* at RS, Závora, Mozambique (Whitehead 2001). The SOCPROG 2.9 program (Whitehead 2009), specifically the ‘movement analysis’ module, was used. Observed individual sighting data from 2016-2021, when there was consistent survey effort, was compared to several exponential mathematical models that represented various habitat use scenarios including permanent residency, emigration and mortality, emigration and re-immigration, emigration and re-immigration with mortality, and a cyclical pattern of appearance. The quasi-Akaike information criterion (QAIC) values were used to select the best supported model due to the overdispersion of the data (Whitehead 2007). Data were bootstrapped 100 times, with 1,000 maximum evaluations, to estimate the standard error and parameter precision (Buckland and Garthwaite 1991, Whitehead 2001).

2.3.5. Cross checking for migrations between South Africa and Mozambique

Photographs of the ventral surfaces of individual *M. alfredi* were collected opportunistically by dive operators and recreational divers at tourist dive locations: the IWSP and Aliwal Shoal MPA in KZN, and Tofo and Závora in the Inhambane Province, Mozambique. A linear continuous display of each photograph collected in South Africa was compared with the Závora and Tofo databases from Mozambique. The ventral spot patterning was manually compared against every positively identified individual in the respective databases at the time (Tofo: 875, Závora: 583, South Africa: 87).

2.4. Catch analysis of the KwaZulu-Natal bather protection programme

In Chapter 3, a total of 41 years of catch data from the KZN bather protection programme was used to investigate long-term trends in manta ray occurrence (*M. alfredi*, *M. birostris*), body size and demographic composition. The influence of environmental variables on manta ray occurrence was determined using Generalised Additive Models (GAMs), and to describe patterns of temporal and spatial habitat use.

2.4.1. KwaZulu-Natal bather protection programme

The KZN bather protection nets are large-mesh gill nets installed year-round at public recreational beaches since 1952 to mitigate shark-human interaction (Cliff and Dudley 1992). The nets are 214 m long, 6.3 m deep, and set parallel to, and 300-500 m from the shore, in a water depth of 10-14 m (Cliff and Dudley 1992, Daly *et al*. 2022). The nets were deployed at a maximum of 46 fixed locations throughout the study (Figure 2.4), and are currently installed at 37 locations along the KZN coastline (Table 2.1). The deployed nets are regularly inspected, whereby trained field staff visit each net by boat, a process called ‘meshing’. Meshing usually occurs at first light, between 17-19 times per month (Dudley and Cliff 2010). The monthly average number of nets per day per location multiplied by the average net length was used as a measure of the unit effort. Statistically reliable bycatch data (in this case mobulids) from the bather protection nets began in 1981, therefore data prior to that were excluded. Observers were trained to distinguish between devil ray and manta ray species; but individuals were excluded with a Disc Width (DW) less than 1.4 m from the analysis, as these could be misidentified *Mobula kuhlii* or *M. eregoodoo* specimens (Cliff, pers. comm.). The DW is measured ray by measuring the distance from one edge of the ray's flattened body to the other edge across its widest point. Due to the relatively recent speciation of manta rays (Marshall *et al*. 2009) and limited access to training, observers could not distinguish between manta ray species, as well as between all mobulid species. Therefore, the two manta ray species were pooled together. All individuals used in the study were measured in the field. When an individual manta ray was caught, the individual was sexed using the presence or absence of claspers, and the DW was measured to the nearest mm as the straight-line distance between pectoral fin tips.

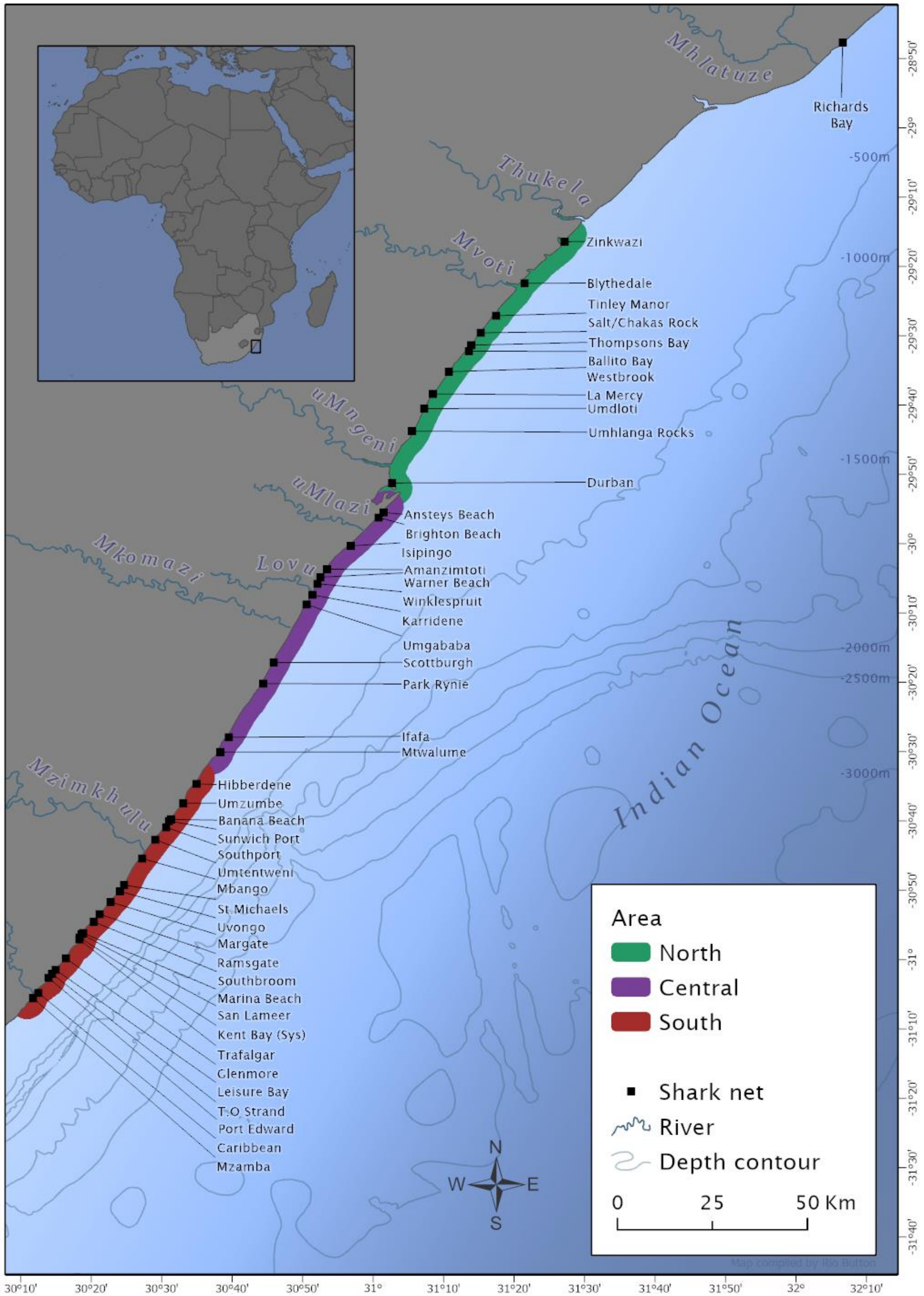


Figure 2.4. Map of KwaZulu-Natal showing sites where bather protection nets were deployed and defining the three designated coastal areas that were used in the study between 1981-2021. Also shown are depth contour (500 m intervals) and defined major river systems. Map constructed by Rio Button.

Table 2.1. List of each beach in KwaZulu-Natal with bather protection nets between 1981-2021, the designated areas for the study, the respective latitude and longitude (in decimal degrees), the minimum, maximum, and average number of nets deployed (± SD), the minimum, maximum, and average length of nets (in meters (m)) deployed (± SD), and the time intervals of installed nets.

Beach	**Code**	**Area**	**Latitude**	**Longitude**	**Min. nets**	**Max. nets**	**Avg. nets**	**Months and years deployed**	**Average net length (m)**	**Min net length**	**Max net length**
Richard's Bay	R.B	R.B	-28,79513056	32,11033333	7	26	14±4	1981-Mar 2020; Oct 2020-Dec 2021	1,488.0±474.5	374.0	2,455.0
Zinkwazi	ZIN	North	-29,27413611	31,45256111	4	10	9±2	1981-Aug 2019; Nov 2019-Mar 2020; Nov 2020-Jun 2021; Nov-Dec 2021	913.8±257.4	178.	1,068.0
Blythedale	BLY	North	-29,37410833	31,35793889	2	6	5±1	1981-Jul 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	482.4±162.3	89.0	641.0
Tinley Manor	TIN	North	-29,45210000	31,29044167	6	6	6±0	1981-Feb 1994; Feb-May 1996	576.9±170.3	107.0	641.0
Salt/Chakas Rock	SAL	North	-29,49321111	31,25428056	2	11	7±2	1981-Aug 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	742.4±288.6	89.0	1,174.0
Thompson's Bay	T.B	North	-29,52288611	31,23198889	2	6	5±1	1981-Jul 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	475.0±159.6	89.0	641.0
Ballito	BAL	North	-29,53737778	31,22623056	4	14	10±2	1981-Aug 2019; Nov-2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	991.5±277.1	178.0	1,495.0
Westbrook	TON	North	-29,58674167	31,17899722	2	6	5±1	1981-Jul 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	503.2±147.1	89.0	641.0
La Mercy	L.M	North	-29,64015000	31,14098056	6	6	6±0	1981-1993; Feb-Mar 1996	573.3±178.9	107.0	641.0
Umdloti	UMD	North	-29,67531111	31,12037222	2	6	5±1	1981-Jul 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	503.2±147.1	89.0	641.0
Umhlanga Rocks	UMH	North	-29,72961389	31,09171944	4	18	14±3	1981-Aug 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	1,450.8±435.1	178.0	1,922.0
Durban	DUR	North	-29,85436667	31,04450000	27	63	56±7	1981-Mar 2020; Sep 2020-Dec 2021	5,557.3±1,073.2	1,448.0	6,401.0

Anstey's Beach	ANS	Central	-29,92516389	31,02438333	2	6	6±1	1981-Jun 2019; Dec 2019-Mar 2020; Dec 2020-May 2021; Nov-Dec 2021	535.9±153.5	71.0	610.0
Brighton Beach	BRI	Central	-29,93743056	31,01278056	2	6	4±1	1981-Jun 2019; Dec 2019-Mar 2020; Dec 2020-May 2021; Nov-Dec 2021	401.2±165.0	71.0	610.0
Isipingo	ISP	Central	-30,00553611	30,94677500	2	8	5±2	Oct 1995-Jun 2019; Dec 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	458.0±204.5	89.0	854.0
Amanzimtoti	AMA	Central	-30,06164722	30,89058333	8	34	24±7	1981-Jun 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	2444.8±874.0	356.0	3,630.0
Warner Beach	WAR	Central	-30,08084722	30,87538056	2	8	7±1	1981-Jun 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	706.3±190.8	89.0	854.0
Winklespruit	WIN	Central	-30,09649722	30,86816389	2	6	5±1	1981-Jun 2019; Nov 2019-Mar 2020; Nov 2020-May 2021; Nov-Dec 2021	507.5±148.9	89.0	641.0

In an effort to reduce bycatch of non-target species, there was substantial removal of nets at 34 of the 37 beaches in the early 2000's, which were replaced by drumlines (Cliff and Dudley 2011, Dicken *et al*. 2016, Dicken *et al*. 2018). Each drumline is anchored adjacent to the nets and consists of a single Mustad 4480DT 14/0 J hook (Gjøvik, Norway) suspended 4 m beneath a large float (Dudley *et al*. 1998, Cliff and Dudley 2011). The hooks were baited and checked every weekday (weather permitting) and re-baited as necessary. In 2007, a total of 79 drumlines replaced almost half (4 km) of the nets at 17 of the 18 protected beaches along the Hibiscus Coast (Hibberdene, beach 25, to Port Edward, beach 44; Figure 1). An additional 28 drumlines were installed between Zinkwazi and Ballito in 2015, and an additional 70 between Tongaat and Umgababa in 2019. The 177 drumlines currently in operation were deployed at a replacement ratio of four drumlines to one net. Specifics of the drumline deployments are given in Dicken *et al*. (2016).

2.4.2. Sampling design

For the occurrence analysis in this study, the Natal region was further divided into three areas to allow for the possibility of the heterogeneity of ocean processes along the coastline (Figure 2.4). The study area extended approximately 350 km from Richard's Bay in the North, to Mzamba Beach in the South (Figure 2.4, Table 2.1). The three areas (North, Central, and South) from North to South measure 84.9, 84.6, and 86.1 km, respectively and are broadly consistent with designated regions defined by previous local studies (Dicken *et al*. 2006, Dudley and Cliff 2010).

2.4.3. Catch per unit effort

Catch per unit effort (CPUE) was measured at each beach by calculating the total catch divided by the total of the monthly average number of nets multiplied by the average net length used at each location between 1981-2021. This is because the number of nets and net length varied at each beach throughout the study period (Table 2.1). The CPUE can be represented as

$$\frac{\text{Number of rays caught}}{\text{Average net length*Average number of nets per month}}$$

Means (± Standard deviation, or, 'SD') were calculated to assess the following: the average annual number killed as a result of catch, the average number of nets and net length at each location, and the average DW of each individual caught.

2.4.4. Standardising Probability of Encounter

Generalised Additive Models (GAMs) were used to examine the relationships between the Probability of Encounter (PE) (0 = absent, 1 = present) of manta rays caught in the bather protection nets and predictor variables assuming a binomial error distribution. All analyses were conducted in R software (R Core Team 2021). Probability of encounter is preferred over count distributions when a species is rarely captured, as overdispersion is accounted for. Furthermore, simulation testing has shown that if PE decreases below a certain threshold, the information provided by non-zero observations is minimal and the relationship between PE and abundance becomes approximately linear (Parker *et al*. 2016, Kerwath *et al*. 2019). Moon phase has been repeatedly documented to predict manta ray occurrence (Dewar *et al*. 2008, Rohner *et al*. 2013) therefore this was intergrated into the model set. Daily moon phase data were extracted from the ‘suncalc’ package (Thieurmel and Elmarhraoui 2019). Effort was treated as an offset in natural logarithmic scale which included the average number of nets and net length and each location. The full GAM included the smoothing functions for the variables month and moon phase as follows:

$$\text{logit}(p) = \alpha + \text{offset}(\log(\text{effort})) + \text{Year} + \text{Area} + s_1(\text{Month}) + s_2(\text{Moon phase})$$

where logit denotes the binomial link function, p is the probability of catching at least one individual per net deployment, α is the intercept, s1-2 denotes cyclic cubic smoothing functions for Month and Moon phase (Wood 2006). Year and Area were treated as categorical variables. Sequential F-tests were used to determine the covariates that contributed significantly ($p < 0.001$) to the deviance explained and GAMs were fitted in R statistical software using the ‘mgcv’ and ‘nlme’ (Wood 2006). For PE analysis, significance was accepted at $p < 0.001$ based on past studies that analysed similar covariates (e.g., year, month, moon phase) in the region (Parker and Kerwath 2020, Daly *et al*. 2021). The annual value of PE was standardised by fixing all covariates other than Year in the prediction dataset. Drumline data were excluded from GAM analysis because of the short time-frame of their deployment and low catches of manta rays compared to the nets.

2.4.5. Size and sex composition

All caught manta rays were sexed based on the presence or absence of external claspers, and measured, using DW (Marshall and Bennett 2010). While a threshold of ≥6 m DW was used to identify *M. birostris*, the majority of catch data included unknown manta species. Nevertheless, detection of juveniles versus adults was possible using known sizes of maturity for both *M. alfredi* and *M. birostris* (Table 2.2). Juvenile and adult maturity status for an individual was determined by a DW between 1400-2500 mm and 3801-8000 mm, respectively (Table 2.2). Individuals that had a DW between 2501-3800 mm were recorded as being of unknown maturity. Sex ratios were calculated using an exact binomial test in the ‘stats’ package in R (R Core Team 2021) with a significant difference in sex ratio accepted at $p < 0.05$.

Table 2.2. Presently known juvenile and mature size classes for *Mobula alfredi* and *M. birostris*, and the respective sources from which these were derived.

Species	Juvenile size (mm)	Adult size (mm)	References
M. alfredi female	1300-3000	3000-3200	Marshall and Bennett 2010, Stevens 2016, Stewart *et al*. 2018a
M. alfredi male	1300-3200	2700-3000	Marshall and Bennett 2010, Stevens 2016, Stewart *et al*. 2018a
M. birostris female	≤ 3800	4130	Marshall *et al.* 2009, White *et al.* 2006
M. birostris male	≤ 3750	3750-4000	Marshall *et al*. 2009, White *et al.* 2006
Size classes used presently	1400-2500	3880-8000	Current study

2.5. Mobulid sightings and behaviour

To gain an understanding of where mobulid (*M. alfredi*, *M. birostris*, *M. kuhlii*) hotspots occur in South Africa, and the behaviours that transpire at these locations, baseline information was collected opportunistically during recreational snorkelling/freediving and SCUBA diving between 2020-2022 (Chapter 4, Chapter 6, Chapter 7). Underwater Visual Census (UVC) surveys were conducted at the iSimangaliso Wetland Park by the SharkLife Conservation Group, and opportunistic sightings collated from Ballito, Aliwal Shoal MPA, and Protea Banks MPA.

During each recreational dive or ‘drift’ (snorkelling over several sites, depending on the current, for a given amount of time), several parameters were recorded including the dive site name, depth, time, current (N, S, E, W, or NONE), water colour, sea surface temperature (SST; 1°C intervals), bottom sea temperature (BST; 1°C intervals), and estimated cloud cover (%). Estimated horizontal visibility (m) was binned into six 5 m categories to account for human error. Plankton composition and abundance was categorised and recorded according to Rohner *et al*. (2013) as either: (i) ‘suspended sediment’, representing mostly detritus; (ii) ‘zooplankton’, representing zooplankton scattered throughout the water column; (iii) ‘phytoplankton’, representing thick phytoplankton clouds; (iv) ‘surface slick’, representing dense zooplankton communities concentrated on the water surface; or (v) ‘no visible plankton’. All parameters were assessed visually *in situ*, while depth, time, and SST were recorded using a Mares Puck Pro underwater dive computer.

Sightings per unit effort (SPUE) was used as a rudimentary comparison tool for manta ray species composition and mobulid habitat use at the different areas. To calculate sightings per unit effort (SPUE) at the recreational dive locations for manta rays, a single dive, defined as a recreational dive from start to finish, or a recreational snorkel ‘drift’ was assigned as the unit of survey effort (since the number of minutes visiting a certain place was not always recorded by citizen scientists on a regular basis at each diving location). Yet, the effort remained overall consistent, given the standardisation of dive routes and the similarity of dive profiles. Because citizen science was excluded for data on *M. kuhlii*, during each dive/drift, the number of rays sighted was recorded, including maximum number of individuals (Max N), and their location(s). The names of the sites and the times spent at each were recorded *in situ* using an underwater slate and underwater dive computer. The SPUE was then calculated for each site.

When encountered, photographs and videos of mobulid (*M. alfredi*, *M. birostris*, *M. kuhlii*) behaviours were recorded with an underwater camera. Cruising behaviour was identified when one or more mobulid individuals swim in a given direction, with the cephalic lobes furled. Ram feeding, which included surface ram feeding (<2 m depth) were identified when a mobulid had unfurled cephalic lobes, simultaneously pushing zooplankton into the open mouth (Paig-Tran *et al*. 2013). Courtship behaviour, or ‘mating trains’ were recognised to include some or all of the following behaviour patterns: (i) males closely chasing a female; (ii) fast swimming; (iii) males performing similar movements after the female and one another; and (iv) many instances of swerving and lunging (Yano *et al*. 1999, Marshall and Bennett 2010, Stevens *et al*. 2018). The total number of mobulid individuals in each group was recorded *in situ*, as well as the number of individuals involved in a mating train. Cleaning behaviour was recognised when one or more mobulid would slow their swimming speed over an area near reef engage in interactions with symbiotic cleaner fish. Further descriptions of behaviour of *M. kuhlii* are extensively described in Chapters 6 and 7.

2.5.1. Investigating Mobula kuhlii cleaning behaviour

‘Cleaning’ behaviour was specifically defined as observation of a cleaner fish making obvious contact with the body of one or more *M. kuhlii*. The observation of cleaning behaviour at a specific location in at least two survey days resulted in the location being designated a cleaning station. A surface marker buoy signalled the skipper on the surface to mark the precise coordinates of such sites on a Lowrance Elite 5 GPS system. Due to frequent observations of cleaning behaviour during preliminary surveys, one cleaning station, Angels Ledge, at a depth of 22 m, was selected for more detailed observation of *M. kuhlii* cleaning behaviour. Remote Underwater video (RUV) was obtained by placing a GoPro Hero 5 or Hero 9 attached to two 1 kg dive weights in the sand facing the ledge by freediving or during recreational SCUBA dives. Two RUVs were placed for one hour at a time. These then gathered video data of *M. kuhlii* cleaning behaviour in the absence of humans.

Videos were processed using frame by frame analysis in BORIS Software (Friard and Gamba 2016), whereby behaviour could be permanently logged, as point or continuous observations, and their frequencies and durations saved in Excel spreadsheets. When *M. kuhlii* were present in the video data the maximum number (Max N) of individuals was always recorded, due to the fact that *M. kuhlii* individuals cannot be photo-identified. The sex of each individual, maturity status, and any injuries were additionally recorded if possible. Sex was determined by presence or absence of claspers, with fully extended claspers signifying a mature male, and the presence of mating scars or pregnancy indicating mature females (Notarbartolo di Sciara 1987, Marshall and Bennett 2010a, White *et al*. 2006). Injuries were identified by crescent-shaped scars attributed to predation (Marshall and Bennett 2010b), or triangular scars and/or truncated tail injuries attributed to either predation or entanglement in monofilament (Deakos *et al*. 2011, Germanov *et al*. 2019). Sex ratio and injury prevalence were calculated based only on individuals which were close enough to the camera during video recording to describe these.

Cleaning interactions were defined as any cleaner fish making contact with (’striking’) a *M. kuhlii*, indicating parasite removal (Oliver *et al*. 2011, Murie *et al*. 2020), however cleaning time was defined as periods of time where cleaner fish were surrounding *M. kuhlii* individuals, or within 1 m of them, but not necessarily striking the whole time. This allowed for determination of the cleaning effort (average strikes per second of cleaning time). Several aspects of cleaning behaviour were recorded, including swimming speed ‘hovering or swimming slow’ (Figure 2.5a), ‘twitching’ (Figure 2.5b), when a client suddenly jerks part or all of its body, possibly in response to ‘cheating’ cleaner fish (Soares *et al*. 2008) or uncomfortable interactions, ‘posturing’ (Figure 2.5c) which involves terminating pectoral fin beats and opening the mouth and gills (O’Shea *et al*. 2010), and ‘following’ (Figure 2.5d) which involves mobulid individuals following each other around a cleaning station (Perryman *et al.* 2021). 'Cruising' behaviour was when *M. kuhlii* were swimming in one direction, either singly or in a group, with the cephalic lobes furled and clearly not engaged in cleaning. If individuals were observed in the area and exhibiting aspects of cleaning behaviour, but were too far away to detect cleaner fish strikes, it was recorded as 'cleaning out of sight'.

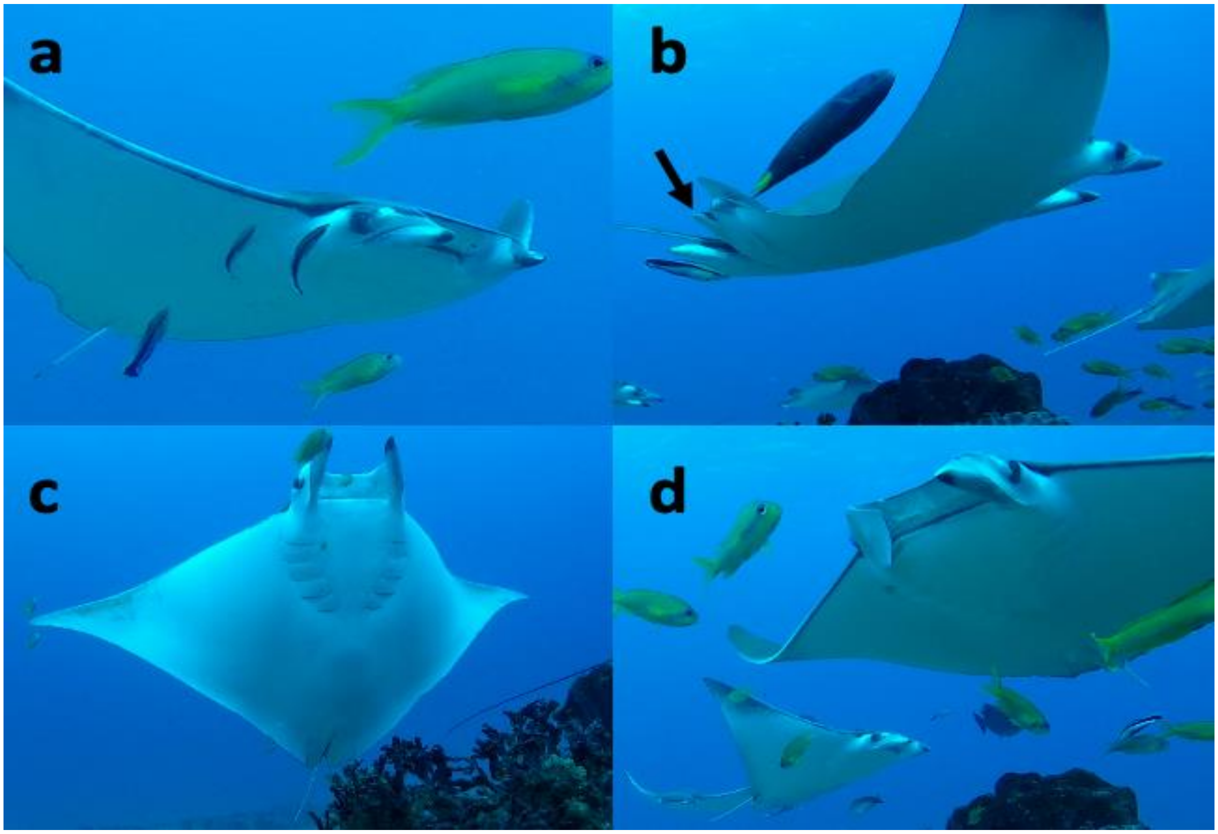


Figure 2.5. Types of *Mobula kuhlii* cleaning behaviour including (a) 'hovering/swimming slow', (b) 'twitching', (c) 'posturing', and (d) 'following' (d) at Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa.

Since different areas of a client's body are known to host different types of parasites (Bshary and Grutter 2002, Caira and Healy 2004, Oliver *et al.* 2011, Murie *et al.* 2020), both the number of bites and the region of the body being cleaned were recorded. The same eight body patches outlined by Murie *et al.* (2020) were used to evaluate the specific areas of *M. kuhlii* cleaned by a cleaner fish: tail, gills, pelvic fins (ventral), ventral body cavity, ventral pectoral fins, ventral head, dorsal head, and dorsal body (Figure 2.6). Cleaner interactions were recorded in BORIS software using point event function named 'Cleaner fish strike' with body part selected as a modifier. Cephalic lobe positions were also recorded when they changed during a cleaning interaction (both unfurled, both furled, one unfurled and one furled) to assess if they were different than manta ray cephalic lobe use at cleaning stations (Perryman *et al*. 2021).

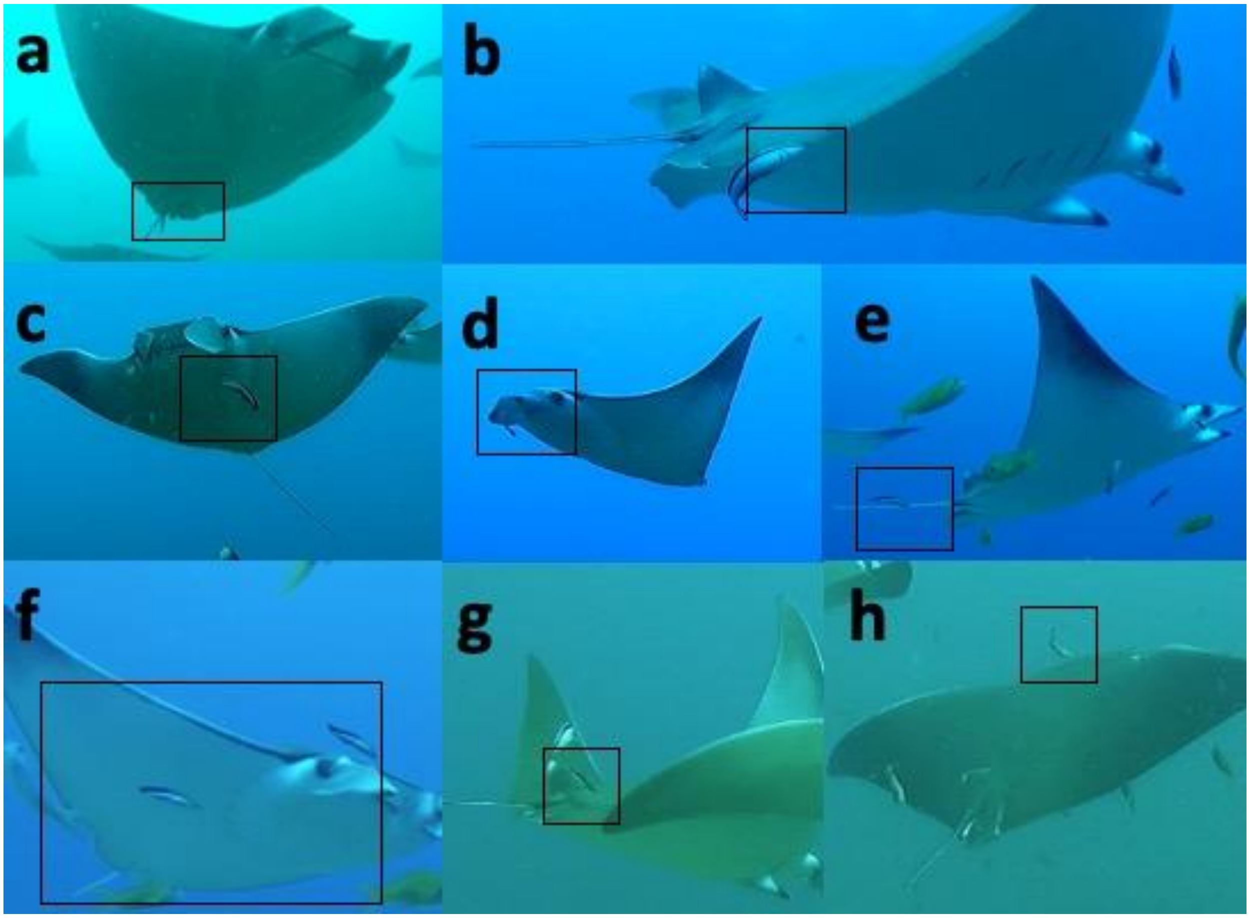


Figure 2.6. Defined *Mobula kuhlii* body patches are as per Murie et al (2020). These include the (a) pelvic fins, (b) ventral pectoral fins, (c) gills, (d) ventral head, (e) tail, (f) ventral body, (g) dorsal body, and (h) dorsal head. Photographs are screenshots from GoPro videos taken on Angels Ledge, Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa between September 2020-March 2022.

Cleaning species have been suggested to be competitors with one another and to prefer larger animals over smaller hosts (Kitchen-Wheeler 2013). Therefore, the presence of other species that had a total length or DW of 1 m or greater simultaneously cleaning on AL, such as spotted eagle rays, *Aetobatus ocellatus*, pickhandle barracuda, *Sphyraena jello*, round ribbontail rays, *Taeniurops meyeni*, potato groupers, *Epinephelus tukula*, bull rays, *Aetomylaeus bovinus*, or giant trevally, *Caranx ignobilis*, were also recorded as observations in BORIS. Total length refers to the measurement of a fish's length from the tip of its snout to the end of its tail, including the caudal fin. This was used to arbitrarily estimate teleosts. Disc width was used for all ray species which is the standard method to describe ray size classes. Interactions with these species were then compared with *M. kuhlii* cleaning durations and cleaner fish bite numbers to determine if there are effects of multiple species using the same cleaning station.

2.5.2. Statistical analysis of M. kuhlii presence/absence on Aliwal Shoal MPA

Generalised additive models (GAMs) fitted with binomial error distributions were used to investigate the effects of environmental conditions on the presence or absence of *M. kuhlii* on the Aliwal Shoal MPA. The time spent at each location on the Aliwal Shoal MPA during a ’dive’ or recreational snorkel ‘drift’ was used as a unit of effort. The maximum number of individuals of *M. kuhlii* (Max N), their location(s) and behaviours, were recorded during each dive. If multiple recreational dives or snorkel drifts were done in a single day, only one was used for the day, to avoid double counting of encountered *M. kuhlii*. Environmental conditions recorded were current direction (N, S, E, W, or NONE), wind speed (km/h), wind direction (°), sea surface temperature (SST; 1°C intervals), bottom sea temperature (BST; 1°C intervals), and estimated cloud cover (%). Estimated horizontal visibility (m) was binned into six 5 m categories to account for human error.

GAMs were fitted using the packages ‘mgcv’ and ‘nlme’ (Wood 2006) in R software (R Core Team 2021). The unit of effort, dive time, was used as an offset in natural logarithmic scale. A stepwise analysis was used to test effects of all variables recorded during a recreational dive or snorkel drift. Daily moon phase data were sourced from the ‘suncalc’ package (Thieurmel and Elmarhraoui 2019), and this variable was included with a cyclical smoother and current direction as a categorical variable (Wood 2006). Sequential F-tests were used to determine the covariates that were significant ($p < 0.001$) to the deviance explained, with non-contributing variables removed from the final model. The model used to predict *M. kuhlii* presence on Aliwal Shoal MPA was as follows:

$$\mathrm{logit}(p) = \alpha + \mathrm{offset}(\log(\mathrm{effort})) + s_1(\mathrm{SST})$$

where logit denotes the binomial link function, p is the probability of encountering at least one individual per minute, α is the intercept, s1 signifies a standard smoothing function for SST (Wood 2006).

2.5.3. Statistical analysis of M. kuhlii cleaning duration on Angels Ledge

GAMs fitted with Gaussian error distributions were used to test if *M. kuhlii* cleaning duration (s) was affected by other megafauna cleaning at the same cleaning station, as well as the same variables tested with *M. kuhlii* presence. Basic statistics on *M. kuhlii* cleaning behaviour were produced and exported using the ‘synthetic time budget’ code in BORIS software (Friard and Gamba 2016). From this, average durations of behaviours were calculated (± Standard Deviation (SD)). All statistics were conducted in R software (R Core Team 2021). To assess for body patches significantly preferred by *Labroides dimidiatus* while cleaning *M. kuhlii,* a Kruskal-Wallis test and subsequently, a post-hoc Dunn test (Kruskal and Wallis 1952, Dunn 1961) was used. The total observation time (s) of each video observation was used as an offset in a natural logarithmic scale. Sequential F-tests were used to determine the covariates that were significant ($p < 0.001$) to the deviance explained, with non-contributing variables removed from the final model. The final model for testing *M. kuhlii* cleaning behaviour duration on AL was as follows:

$$\mathrm{logit}(p) = \alpha + \mathrm{offset}(\log(\mathrm{effort})) + s_1(\mathrm{BST}) + s_2(\mathrm{Visibility}) + \mathrm{Current}$$

where logit denotes the Gaussian link function, p is the number of minutes one or more *M. kuhlii* spends cleaning, α is the intercept, s1-2 denotes standard smoothing functions for BST and Visibility (Wood 2006). Current was treated as a categorical variable.

CHAPTER 3: Population demographics and estimates of abundance of manta rays, *Mobula alfredi*, in Závora Bay, Mozambique

RESULTS AND DISCUSSION

Manta rays can travel over 1,000 km, yet repeatedly return to specific cleaning stations.

3.1. Results

This study advanced the understanding of the population dynamics of *M. alfredi* in remote Závora Bay, Mozambique. Through an 11-year photographic mark-recapture analysis, 583 *M. alfredi* individuals were identified between 2010-2021, revealing an even sex ratio and a seasonal peak sighting period from July to November. Using Pollock's Robust Design population models, I estimated annual abundance, emigration, survival rates, and capture probabilities, demonstrating site affinity of this aggregation to Red Sands. These findings emphasise the importance to consider fine-scale habitat utilisation within the broader range of the Mozambique population for effective local management strategies (e.g., the need for a Marine Protected Area at Závora Bay).

3.1.1. Population demographics

Sampling effort at RS ranged from 0-37 dives per month, with one or two dives of 44-72 min duration conducted per day, resulting in a monthly sampling effort of between 44 and 1252 min (Table 3.1). The number of *M. alfredi* individuals in the photo-ID database increased throughout the study period, with large numbers of new identifications added between 2010-2011, and 2017-2018 (Figure 3.1). Until 2016, the number of newly-identified *M. alfredi* surpassed resights and after 2017, the number of resighted individuals exceeded new IDs (Figure 3.2). An average of three individuals (±4.29), and up to 61 individuals (10% of the photographed population) were identified in a single day visiting RS during peak season (2016-2021; n=274 total identifications in one July – November season).

Table 3.1. Sampling effort (minutes) during primary periods (years) and secondary periods (months) used for Pollock's Robust Design of *M. alfredi* at Red Sands, Závora, Mozambique.

Secondary Period	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
July	68	459	584	833	182	420
August	318	445	570	1252	569	790
September	194	771	725	615	609	828
October	95	481	632	469	44	471
November	0; omitted for PRD	696	456	369	72	333

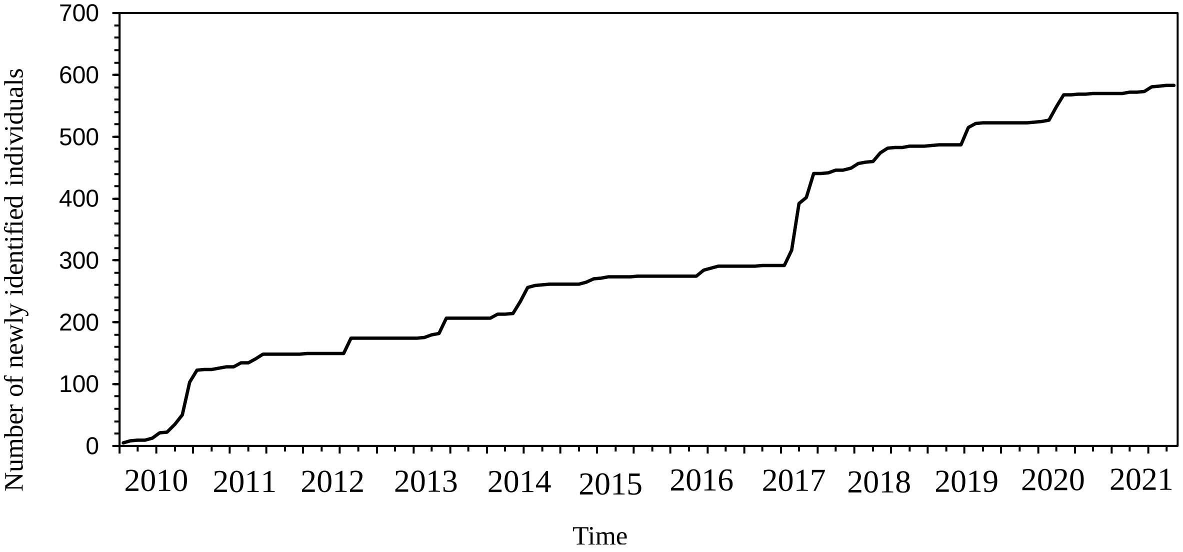


Figure 3.1. Discovery curve of newly identified *M. alfredi* individuals from 2010-2021 in Závora, Mozambique.

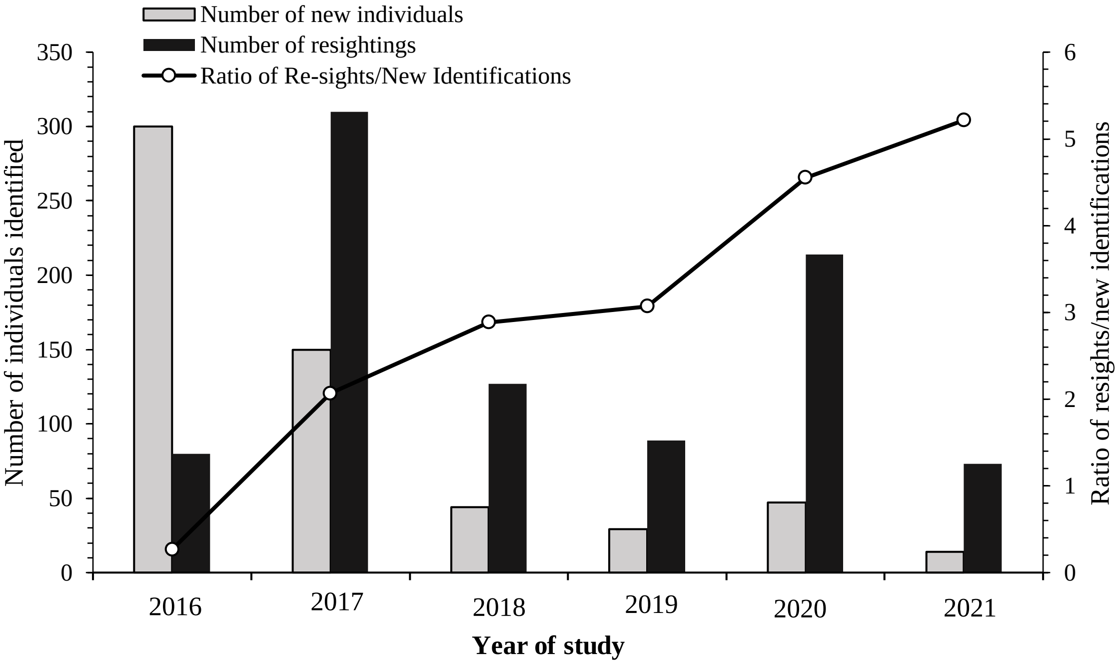


Figure 3.2. Total number of newly identified *M. alfredi* and the total number of resights in each primary period of the study, and the ratio of resights/new at Red Sands, in Závora, Mozambique.

Between 2010-2021, 1509 encounters of 583 individual *M. alfredi* were recorded in Závora Bay. More than half, 54% (n=312) of these were re-sighted at least once. A total of 57% (n=331) of individuals were seen only within a single year and 43% (n=252) across multiple years. The mean time interval between initial and subsequent sightings was 455 days (± 694), with 10 individuals recorded with a re-sighting interval of 1,000 days or more, and a maximum of 10.9 years (3,996 days) between re-sightings. The population exhibited an even sex ratio, whereby 44% (n=255) were females, 50% were males (n=295), and sex could not be determined for 6% (n=33). There was no significant difference in the mean number of sightings between females and males at 2.82 (±2.34) and 2.53 (±2.34), respectively (Mann-Whitney U test; $p = 0.7981$). Though more males than females were re-sighted (males n=171; females, n=139) in Závora, individuals in the database that were sighted six or fewer times consisted of mostly males, whereas individuals sighted seven or more times were almost all females (Figure 3.3). Only mature females had more than 10 sightings during the study period, with the most re-sighted individual identified 18 times. A total of 44 pregnancies across 36 females were noted during the 11-year study period. Five individuals were observed to be pregnant on more than one occasion, with a mean postpartum interval of 33.4 months (± 8.8 months; Figure 3.4). A total of 56% (n=326) of *M. alfredi* were defined as mature, 49% (n=288) of these being males and 7% females (n=38), since for most females, maturity could not be determined (n=250).

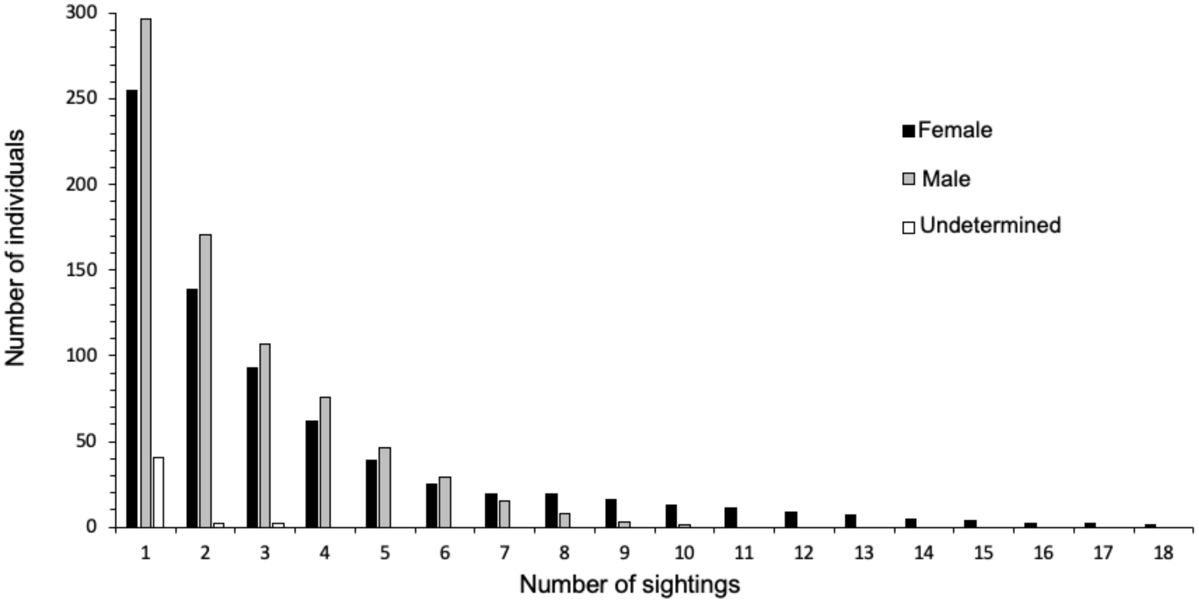


Figure 3.3. Total number of *M. alfredi* identified at Red Sands, in Závora, Mozambique: female (black), male (grey), and undetermined (white).

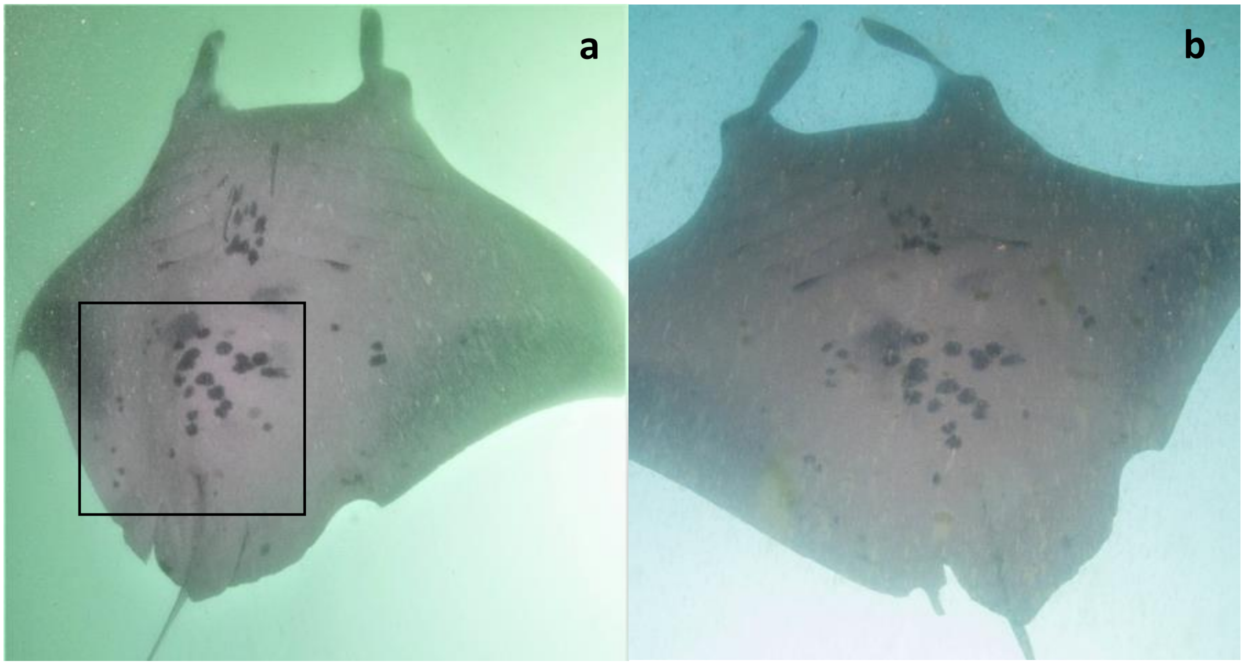
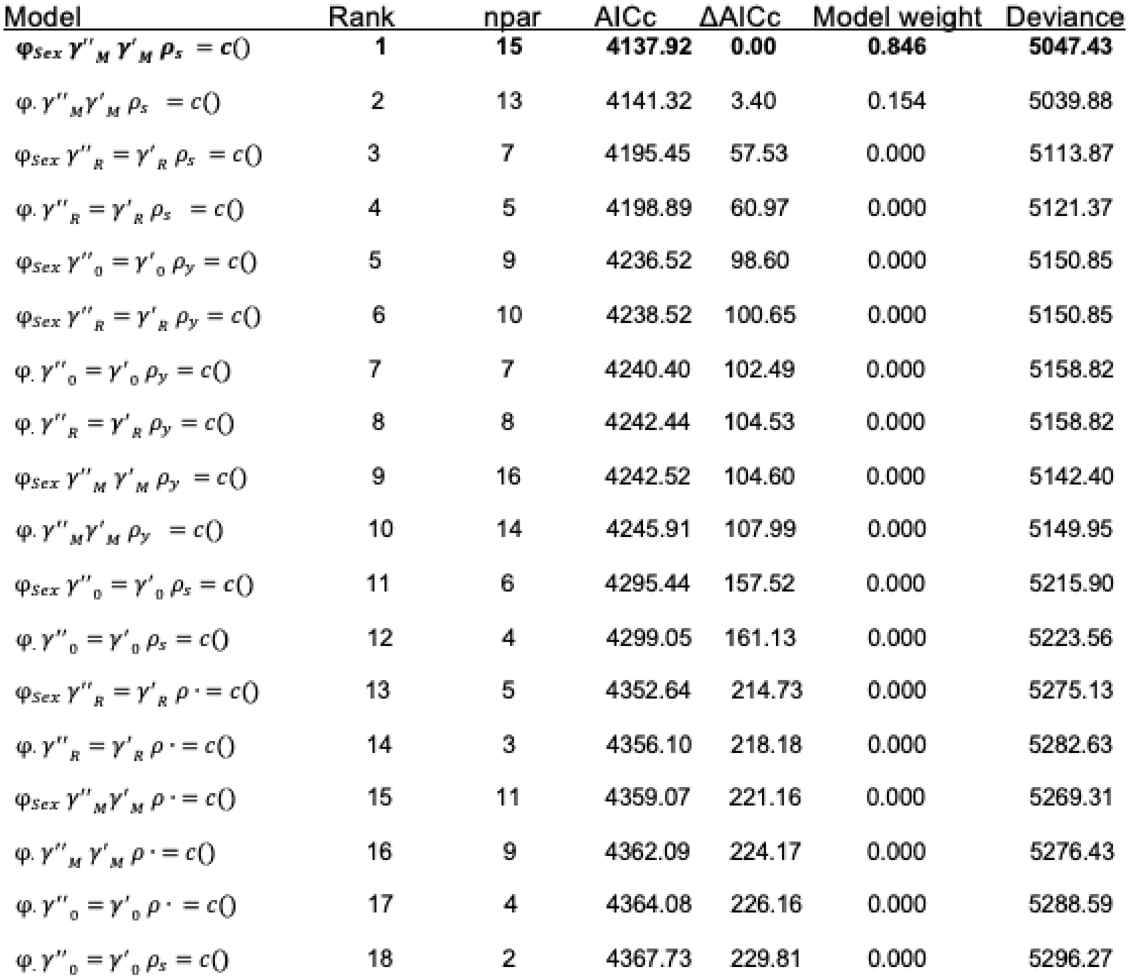


Figure 3.4. Photos of (a) manta Za288 pregnant in 2017 (b) and not visibly pregnant in 2018. Photography credit: MAR Expeditions (top left), Nakia Cullain (top right).

3.1.2. Pollock's Robust Design

Of the total 583 *M. alfredi* individuals catalogued for Závora, 401 were photo-identified at RS between 2016-2021 and were included in the PRD. Of these, individuals of undetermined sex (n=18) were removed for the final PRD analysis that included sex as a covariate. Few *M. alfredi* (n=21) were sighted at other reefs in Závora, but never encountered at RS, therefore these individuals were excluded from the study. Eighteen candidate models were evaluated in the PRD analysis. Models that integrated Markovian emigration, with capture probability varying by sampling effort were best supported (Table 3.2). The best supported PRD model consisted of sex-dependent survival, Markovian temporary emigration, and an effect of sampling effort on capture probability (Table 3.2). Annual apparent survival was estimated to be higher for males than females, at 0.848 (0.09; 95% CI 0.597-0.954) and 0.823 (0.08; 95% CI 0.602-0.935), respectively (Table 3.3). Capture probability dependent on sampling effort fluctuated between primary periods, with the highest in 2020 (0.69; 95% CI 0.60-0.76) and lowest in 2016 (0.16; 95% CI 0.14-0.18) (Table 3.3). Overall annual abundances ranged from 35 (95% CI 30-45) in 2016 to 233 (95% CI 224-249) in 2017 (Table 3.4). Differences in annual abundance estimates were marginal for males and females, at 20-115 and 13-110 respectively (Figure 3.5).

Table 3.2. Selection of Pollock's Robust Design (n=18) candidate models for estimations of population size (N), survival (*φ*; constant or sex varying), temporary emigration (γ'' and γ'; Markovian, random or none), capture (p) and recapture (c) probabilities (constant, with response to capture, varying by year, or varying by sampling effort) of *M. alfredi* individuals that use Red Sands in Závora, Mozambique.



Model	Rank	npar	AICc	ΔAICc	Model weight	Deviance
$\varphi_{Sex}\, \gamma''_M\, \gamma'_M\, \rho_s = c()$	**1**	**15**	**4137.92**	**0.00**	**0.846**	**5047.43**
$\varphi.\, \gamma''_M \gamma'_M\, \rho_s = c()$	2	13	4141.32	3.40	0.154	5039.88
$\varphi_{Sex}\, \gamma''_R = \gamma'_R\, \rho_s = c()$	3	7	4195.45	57.53	0.000	5113.87
$\varphi.\, \gamma''_R = \gamma'_R\, \rho_s = c()$	4	5	4198.89	60.97	0.000	5121.37
$\varphi_{Sex}\, \gamma''_0 = \gamma'_0\, \rho_y = c()$	5	9	4236.52	98.60	0.000	5150.85
$\varphi_{Sex}\, \gamma''_R = \gamma'_R\, \rho_y = c()$	6	10	4238.52	100.65	0.000	5150.85
$\varphi.\, \gamma''_0 = \gamma'_0\, \rho_y = c()$	7	7	4240.40	102.49	0.000	5158.82
$\varphi.\, \gamma''_R = \gamma'_R\, \rho_y = c()$	8	8	4242.44	104.53	0.000	5158.82
$\varphi_{Sex}\, \gamma''_M\, \gamma'_M\, \rho_y = c()$	9	16	4242.52	104.60	0.000	5142.40
$\varphi.\, \gamma''_M \gamma'_M\, \rho_y = c()$	10	14	4245.91	107.99	0.000	5149.95
$\varphi_{Sex}\, \gamma''_0 = \gamma'_0\, \rho_s = c()$	11	6	4295.44	157.52	0.000	5215.90
$\varphi.\, \gamma''_0 = \gamma'_0\, \rho_s = c()$	12	4	4299.05	161.13	0.000	5223.56
$\varphi_{Sex}\, \gamma''_R = \gamma'_R\, \rho\cdot = c()$	13	5	4352.64	214.73	0.000	5275.13
$\varphi.\, \gamma''_R = \gamma'_R\, \rho\cdot = c()$	14	3	4356.10	218.18	0.000	5282.63
$\varphi_{Sex}\, \gamma''_M \gamma'_M\, \rho\cdot = c()$	15	11	4359.07	221.16	0.000	5269.31
$\varphi.\, \gamma''_M\, \gamma'_M\, \rho\cdot = c()$	16	9	4362.09	224.17	0.000	5276.43
$\varphi.\, \gamma''_0 = \gamma'_0\, \rho\cdot = c()$	17	4	4364.08	226.16	0.000	5288.59
$\varphi.\, \gamma''_0 = \gamma'_0\, \rho_s = c()$	18	2	4367.73	229.81	0.000	5296.27

Table 3.3. Parameter estimates, standard errors (SE), and 95% confidence intervals (CI) from the best-fit model: capture probabilities (p) with sampling effort effect between each primary period; Markovian emigration (γ'' γ') between each primary period, and rate of constant apparent survival (φ) across all periods.

Period	*p*	SE	95% CI	γ''	SE	95% CI	Φ^{Sex}	SE	95% CI
2016-17	0.158	0.011	0.137-0.181	0.506	0.123	0.281-0.729	-	-	-
2017-18	0.475	0.030	0.417-0.535	0.714	0.047	0.615-0.796	-	-	-
2018-19	0.293	0.016	0.262-0.326	0.617	0.075	0.464-0.749	-	-	-
2019-20	0.182	0.012	0.160-0.206	0.324	0.090	0.177-0.518	0.848^{Male}	0.087	0.597-0.954
2020-21	0.688	0.041	0.602-0.762	0.852	0.035	0.771-0.909	0.823^{Female}	0.083	0.602-0.935

Table 3.4. Population size (*N*) for males, females, and overall *M. alfredi* at Red Sands in Závora, Mozambique from the weighted average of the best-fit models, and the number of uniquely photo-identified individuals between July 2016 and November 2021.

Sex	Method	Year	Weighted average	SE	95% CI
Male	PRD	2016	20	2.71	17-28
		2017	115	3.79	110-126
		2018	61	2.55	58-69
		2019	59	2.50	56-66
		2020	106	3.60	102-117
		2021	27	1.61	26-33
	Photo ID	2016-2021	215		
Female	PRD	2016	13	2.19	11-21
		2017	110	3.67	105-120
		2018	36	1.88	34-42
		2019	54	2.38	52-62
		2020	74	2.87	70-83
		2021	18	1.31	17-24
	Photo ID	2016-2021	168		
Overall	PRD	2016	35	3.63	30-45
		2017	233	6.16	224-249
		2018	102	3.51	98-112
		2019	114	3.77	109-125
		2020	185	5.22	178-199
		2021	49	2.24	46-56
	Photo ID	2016-2021	401		

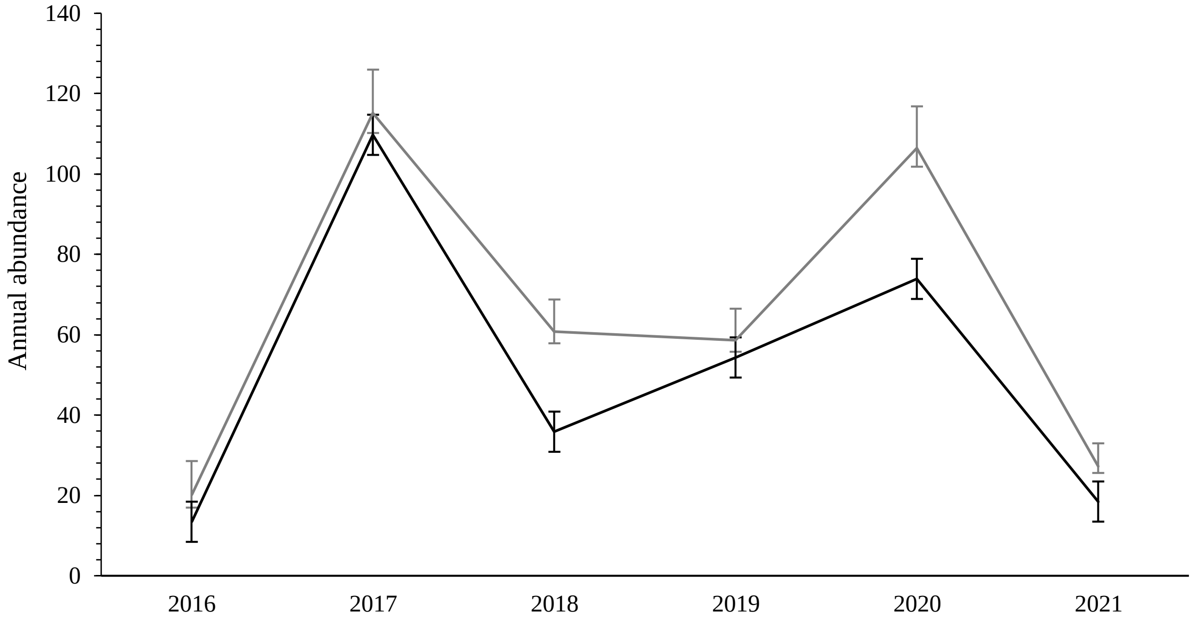


Figure 3.5. Estimates of yearly abundance of *M. alfredi* (*N*) +/- 95% confidence interval at Red Sands in Závora, Mozambique from 2016-2021, estimated from the best-fit model ($\varphi Sex\ \gamma''M\ \gamma'M\ \rho s = c$ ()) with sex as a covariate (a; grey=male, black=female).

3.1.3 Lagged identification rates (LIR)

The best fit LIR models were F and H, which were practically equivalent at <2 based on the ΔQAIC values (Table S3). However, model H made biological sense for the data, which incorporated immigration, reimmigration, and permanent emigration and/or mortality (Table 3.5). Approximately 58 individuals (SE=16.19, 95% CI=35.37-95.71) were estimated to be present in the study area on a given day. *Mobula alfredi* individuals had a mean residence time of four days (SE=27.82, 95% CI=1.53-80.02 days), with 10 days (SE=415.81, 95% CI=5.22-155.49 days) away from the study area. Permanent emigration and/or mortality was estimated at 0.00029 (SE 0.00029, 95% CI = -0.00024-0.00070). The plotted LIR curve decreased rapidly from the date after identification, indicating that most individuals were transient to RS, with temporal annual use of RS (Figure 3.6). The plotted LIR curve then levelled and decreased until the end of the study period suggesting emigration and subsequent return and/or return to the area each season. The declining rate of the LIR displays individual dispersal and the shape of the curve indicated a short residency period at the aggregation site, with re-immigration at a later stage by a proportion of the individuals (Whitehead 2009).

Table 3.5. Model selection for lagged identification rate of reef manta rays in Závora Bay, Mozambique (2016-2021).

Model	Model description	△QAIC
A	Closed (1/*a1* =*N*)	89.60
B	Closed (*a1* =*N*)	89.60
C	Emigration/mortality (*a1* =emigration rate; 1/*a2* =*N*)	46.05
D	Emigration/mortality (*a1* =*N* ; *a2* =Mean residence time)	46.05
E	Emigration + reimmigration (*a1* =emigration rate; *a2* /(*a2* +*a3*)=proportion of population in study area at any time)	28.15
F	Emigration + reimmigration (*a1* =*N* ; a2=Res time in; a3=Res time out	0.95
G	Emigration + reimmigration + mortality	9.93
H	Emigration + reimmigration + mortality a1=N; a2= Res time in; a3=Res time out; a4=Mortality	0.00
I	Cycical a1*cos(a2*td)+a3	93.56

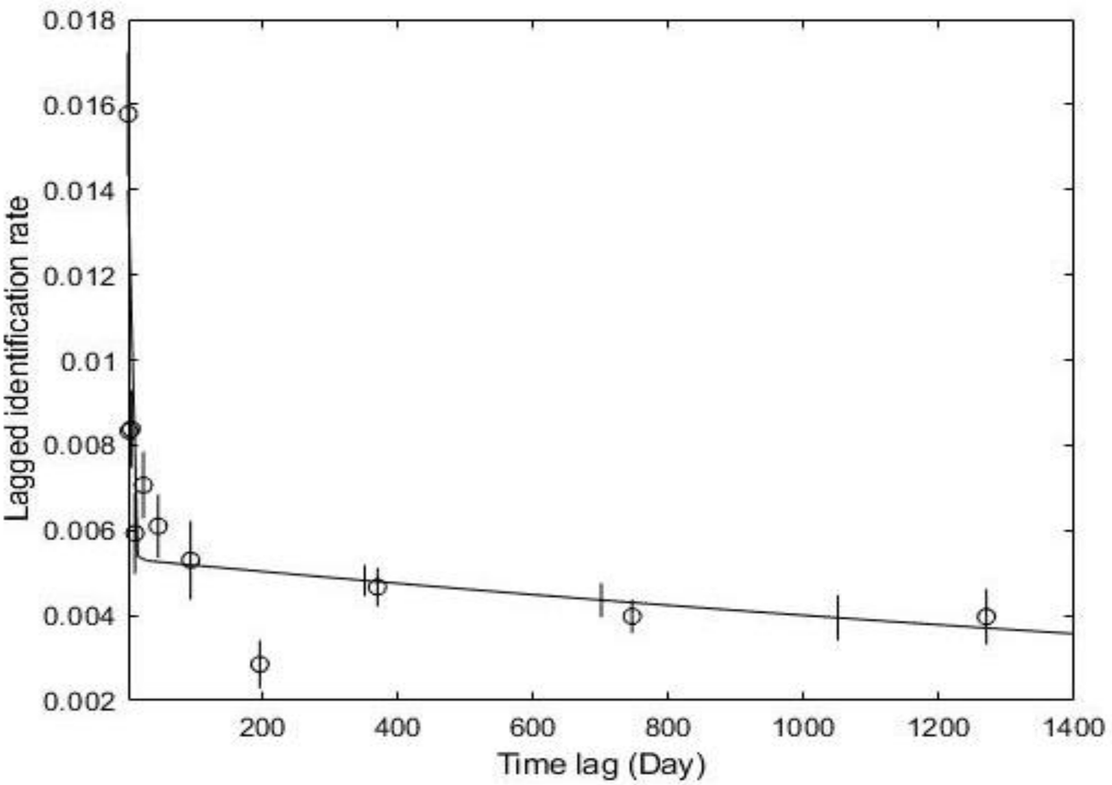


Figure 3.6. Empirical data (mean ±SE) for the lagged identification rate, the probability of re-identifying *M. alfredi* in Závora Bay, Mozambique over increasing time periods, with fitted emigration plus re-immigration plus mortality model.

3.2. Discussion

Site affinity has been globally reported for *M. alfredi*, with individuals consistently returning to cleaning station reefs over long periods of time (Dewar *et al*. 2008, Couturier *et al*. 2014, Venables *et al*. 2020). *Mobula alfredi* habitat use varies spatially and temporally at other well-studied locations (Dewar *et al*. 2008, Armstrong *et al*. 2020a), which is also evident in these findings. In Závora, *M. alfredi* was found to aggregate at one shallow reef, rather than a collection of deeper reefs (25-30m), as documented in Tofo (Marshall *et al*. 2011, Venables 2020). Higher resighting rates were found at RS (54%), compared to cleaning stations in eastern Australia (Couturier *et al*. 2011) and Tofo (Venables 2020), but less than Indonesia, Hawaii, and the Maldives (Couturier *et a*l. 2011, Deakos *et al*. 2011, Germanov *et al*. 2019, Harris and Stevens 2021). Seasonal peaks in sightings were found at Závora, as opposed to year-round sightings at other identified *M. alfredi* aggregations in the Inhambane Province (Venables 2020). Island populations of *M. alfredi* in the Western Indian Ocean also exhibit year-round site use, with seasonally driven peaks related to monsoon winds (Stevens 2016, Peel *et al*. 2019a). While oceanic processes such as monsoonal shifts, seasonal-driven currents, and tides affect *M. alfredi* site use in the Komodo National Park, Indonesia and eastern Australia, ontogenetic patterns were found to influence habitat use in Nusa Penida, Indonesia, the Gulf of Mexico, and Hawaii, and these may be potential drivers of *M. alfredi* use of RS (Dewar *et al*. 2008, Axworthy *et al*. 2019, Germanov *et al*. 2019, Armstrong *et al*. 2020a, Harris *et al*. 2021).

Mozambique and Australia are among few places in the world where *M. alfredi* live along an extended continental coastline, which may explain the observed transience of individuals at these locations outside of peak season (Armstrong *et al*. 2020a, Venables 2020). Long term monitoring of both of these *M. alfredi* populations demonstrate habitat use of an entire coastline where movement patterns may result from temporal shifts in productivity, as opposed to island habitats, which may have more reliable food sources in the area (Rohner *et al*. 2013, Peel *et al*. 2019b, Armstrong *et al*. 2020a, Peel *et al*. 2020, Venables 2020). The Inhambane coastline consists of a narrow continental shelf with mesoscale, eddy-driven upwelling in the Mozambican Channel, which contributes to productivity, thus its' fluctuation may drive *M. alfredi* movements up and down the coast (Quartly and Srokosz 2004, Rohner *et al*. 2013). The support of Markovian emigration in the PRD models further implies that some *M. alfredi* individuals leave for multiple seasons and eventually return. Variations in movement and visitation patterns between the years could be attributed to oceanic processes that affect zooplankton patchiness and distribution, including El Niño Southern Oscillation (ENSO) and/or dipole effects (Folt and Burns 1999, Whitney and Crow 2007, Beale *et al*. 2019).

The annual abundance of *M. alfredi* identified at this single reef in Závora is high when compared to aggregations in Hawaii (Deakos *et al*. 2011, Axworthy *et al*. 2019), the Seychelles (Peel 2019), and Japan (Kashiwagi 2014), and lower when compared to the seasonal, site-specific, aggregation at Lady Elliot Island, Australia (Couturier *et al*. 2014). Previously published abundance estimates (2003-2012) from the Tofo region of the Inhambane Province (Marshall *et al*. 2011, Venables 2020) were larger than the present estimates for Závora, but with fewer overall resightings. However, after 2013, Venables (2020) found <100 *M. alfredi* individuals to be using the reefs around Tofo, whereas in the present study abundances at RS were consistently >100 from 2017-2020. Abundance estimates between 2016-2021 varied noticeably, with 2017 and 2020 having greater capture rates compared to other primary periods. Such variation each year may be attributed to productivity shifts or ontogenetic factors. This seasonal peak in abundance combined with the re-sighting rate of individuals reflects the seasonal importance of the Závora region for a proportion of the larger *M. alfredi* population in southern Mozambique.

Both sexes displayed similar use of RS, in contrast to many monitored locations, where females are more frequently re-sighted (Marshall *et al*. 2011, Setyawan *et al*. 2018). The observed even sex ratio in this study supported preliminary findings by Venables (2020), but contrast to the 61% female-bias found in Tofo (Venables 2020). Often when a greater geographic area is monitored with increased information on the metapopulation, even sex ratios have been reported, including in the Maldives and French Polynesia, or more uncommonly at a single site (Stevens 2016, Carpentier *et al*. 2019, Perryman *et al*. 2019, Venables 2020). Male *M. alfredi* were primarily mature at RS, with several juveniles that later returned as mature over the course of the study. The main difference in site use by the sexes was that specific mature females (n=13) were re-sighted on 10 or more occasions, with some of these individuals encountered at RS over a duration of almost 11 years. Considering that this aggregation of *M. alfredi* returns to this exact reef each year may reflect the importance of this site for sociality and/or courtship ritual (Stevens *et al*. 2018, Thorburn *et al*. 2019, Perryman *et al*. 2021).

An estimated average residence time of four days from the LIR analysis suggests *M. alfredi* individuals visit the study site for a short period in peak season and then leave. The large ranges in standard error and 95% confidence intervals in the LIR analysis are likely due to the individual variability in sightings from the empirical data, and the variation in sightings year to year, which was also apparent in the PRD analysis. The residence time to RS was lower than *M. alfredi* populations around islands in French Polynesia (range 66-130 days), and Coral Bay, Australia (56 days), however residence time out was lower than French Polynesia (range 59-117) and Coral Bay, Australia (92 days) (Carpentier *et al*. 2019, Armstrong *et al.* 2020b). Compared to other monitored *M. alfredi* populations that found constant survival to be ≥0.9 (Deakos *et al*. 2011, Couturier *et al*. 2014), there was a lower apparent survival (males, 0.848; females, 0.823) at RS, suggesting transience during periods when conditions are not favourable for visitation to Závora Bay. The results from LIR and apparent survival found in the PRD analysis suggest that in Závora, *M. alfredi* individuals are more likely to move in and out of the study area even during peak season.

Challenging weather conditions, the logistics of operating in a remote location, and resource availability contributed to uneven sampling effort throughout the study period. We accounted for this in the PRD analysis by modelling capture probability with an effect of sampling effort. Further limitation in sampling for the PRD anlaysis included times when an individual was present at the aggregation but not photographed. Such limitations are characteristic of *M. alfredi* photo-ID studies, including potential violations of model assumptions (i. e., survival probability being the same for all individuals) (Deakos *et al*. 2011, Couturier *et al*. 2014, Venables 2020). Given the level of anthropogenic impact (Venables 2020) and predation pressure (Marshall and Bennett 2010b) affecting southern Mozambique, may result in similar survivorship of this specific aggregation. Considering their longevity, this 6-year analysis is brief relative to the lifespan of *M. alfredi*. Nevertheless, the PRD models in this context provided baseline estimations of the number of *M. alfredi* that use RS in Závora, Mozambique, an area which is currently unprotected.

More than 20 years of research along the Inhambane Province has identified the largest known *M. alfredi* population in Africa, yet with drastic declines in sightings, of up to 88% (Marshall *et al*. 2011, Rohner *et al*. 2013, Venables 2020). This population is now stated to be of immediate conservation concern by local and international scientists (Peel 2019, Rohner *et al*. 2013, Tibiriçá *et al*. 2011, Venables 2020). *Mobula alfredi* is listed in Appendix II (2013) of the Convention for International Trade in Endangered Species (CITES) and in Appendix I and II (2014) of the Conservation of Migratory Species of Wild Animals (CMS). Nationally, manta species were protected under Mozambican law in 2017 (Law 5/2017) which banned fishing of CITES-listed species; however, little was enforced (Boletim da Republica May 2017, Venables 2020). As a Vulnerable (Marshall *et al*. 2018) and economically-important species (Venables *et al*. 2016), *M. alfredi* officially received national protection in 2021 (Boletim da República 2021), however, along the Inhambane coastline, they remain under threat from indiscriminate netting and long-lining, particularly in the south of the province (Marshall *et al*. 2011, Temple *et al*. 2018). In order to increase protection of this mobile species in Mozambique, it is essential to focus on priority habitats where they might be at risk, such as RS, where a seasonal inshore aggregation occurs every year.

At present, the majority of protected critical habitat is concentrated in the north of the province in the Bazaruto Archipelago (Pelegrín *et al*. 2015). Although part of a single breeding population, photo-ID and acoustic telemetry have indicated preferential habitat use to different sites, meaning that *M. alfredi* individuals using the northern regions do not show equal visitation to the southern regions of Tofo and Závora (Venables *et al*. 2020). Anthropogenic pressures from fishing continue to impact the southern *M. alfredi* in most of their home range, including Závora, which is at the southern extent of the area where they are most commonly encountered in Mozambique. Therefore, immediate, site-specific protection of key habitats in the south, such as RS, is recommended as an essential step for conservation management. The design and implementation of a standalone Marine Protected Area in Závora Bay would protect the larger critical habitat for elasmobranchs in this southern region of the Inhambane Province (O'Connor and Cullain 2021). The Inhambane coast was declared a Mission Blue Hope Spot in 2022 in recognition of its diversity of threatened species, and the government of Mozambique has proposed to implement a large seascape-type Environmental Protection Area (EPA) from the

Bazaruto Archipelago southwards towards Závora (Administração Nacional das Áreas de Conservação and Conservation International, 2020). Given the trajectory of the decline of the *M. alfredi* population along this coastline and the seasonal importance of this habitat, the protection of Závora Bay be prioritised during this process.

CHAPTER 4: Descriptions of manta ray (*Mobula alfredi, M. birostris*) aggregations in South Africa

RESULTS AND DISCUSSION

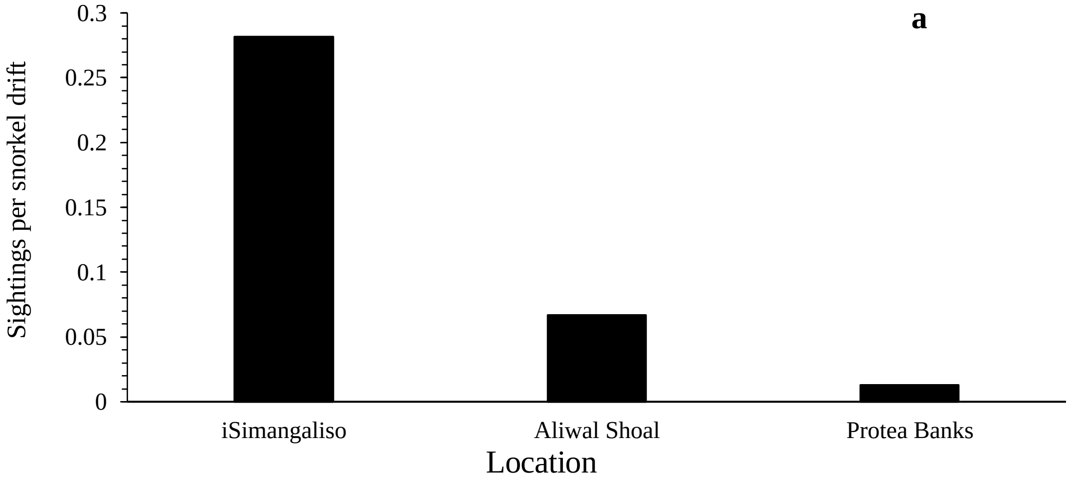
A surprising discovery was the coincidence of identifying the same individulas through photo-identification in South Africa, who I had previously encountered in Závora, Mozambique, just a few years prior.

4.1. Results

This chapter presents South Africa's first photo-ID dataset for manta rays (*M. alfredi* and *M. birostris*) spanning 2020-2022. Through citizen science contributions and opportunistic diving, the research documents novel aggregation sites including *M. alfredi* at the iSimangaliso Wetland Park and possibly *M. birostris* at Ballito, with also revealing substantial connectivity between *M. alfredi* in Mozambique and South Africa (20% of South African individuals showing international migrations), covering distances of 435-1,000 km. These findings provide valuable insights into manta ray behaviour and migration patterns, emphasising the need for transboundary conservation strategies and potential additional Marine Protected Areas.

4.1.1. Manta ray (Mobula alfredi and M. birostris) sightings and habitat use

A total of 558 snorkel drifts were conducted in iSimangaliso Wetland Park, KwaZulu-Natal, between 2020-2022. The highest number of sightings per unit effort throughout the study occurred in the ISWP (Figure 4.1a), most of these being of *M. alfredi*, followed by unknown manta ray species, and *M. birostris* (Figure 4.1b). More than half (59%) of *M. alfredi* sightings occurred at one site, ‘Red Sands’ (RS), in the iSimangliso Offshore Wilderness Zone (IOWZ). This site was discovered to be both a cleaning station and feeding aggregation site, adjacent to a submarine canyon, these being common off the coast in the ISWP.



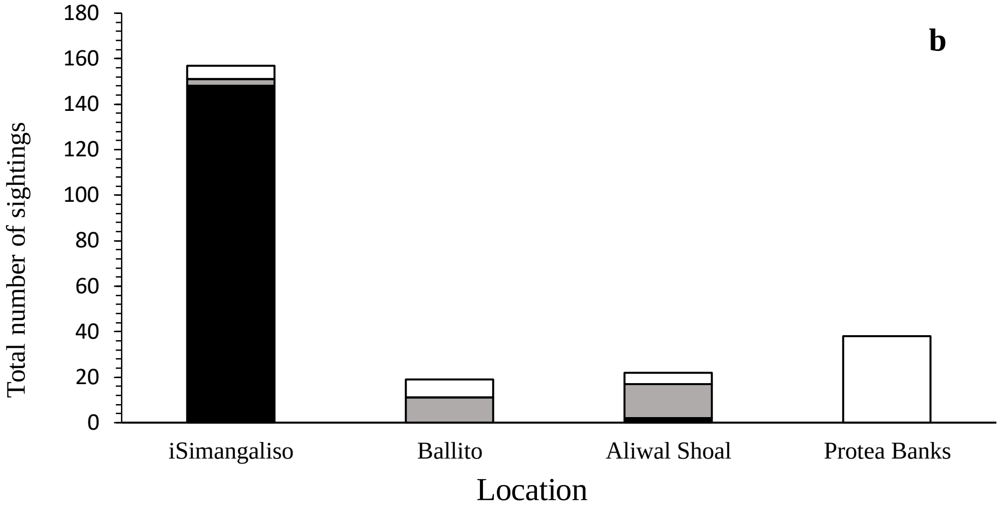


Figure 4.1. The (a) sightings per unit effort (SPUE) of manta ray species combined, and the (b) total number of manta ray sightings, *Mobula alfredi* (black), *M. birostris* (grey), and unknown (white), recorded during recreational diving between September 2020-March 2022.

The maximum number of *M. alfredi* encountered together was eight at Red Sands in the ISWP. The most common behaviours observed were surface feeding (44%), followed by cruising (14%), a combination of cleaning and surface feeding (6%), cleaning (5%), a combination of surface feeding and courtship (3%), and a combination of breaching, cleaning, and cruising (1%), with some recorded as unknown in the database (27%). Individuals were encountered in depths from 8.0-24.8 m, sea surface temperatures (SST) varied between 21-29°C, and estimated horizontal visibility between 15-30 m.

A *M. birostris* feeding aggregation was discovered approximately 300 km south of the ISWP in Ballito, KZN. In 2020, a citizen scientist donated images of a group of 10 *M. birostris* surface-feeding off Ballito. Limited recreational boat trips occurred (n=9) between 2021-2022, therefore SPUE could not be determined at this location. Between 2021-2022, from the boat, a total of 11 *M. birostris* individuals were encountered, with up to seven feeding together at the same time. Citizen scientist microlight (n=11) and drone (n=10) flights information donated by pilots collectively added another 20 *M. birostris* sightings. All *M. birostris* were seen within the top 2 m of the water column, surface feeding (67%) or cruising (33%). They were encountered off Thompson's Bay, Salt Rock, Tinley Manor, Zinkwazi, and Salmon Bay, over depths ranging from 10.0-53.8 m, SST approximately 19-25°C, and estimated horizontal visibility of 1.0-15.0 m, including sightings within brown water.

At Aliwal Shoal MPA, approximately 100 km south of Ballito, 329 recreational snorkel drifts or dives were conducted between 2020-2022. Manta rays were rarely encountered, with more *M. birostris* (n=15) sightings than *M. alfredi* (n=2). The most encountered behaviours in the MPA were surface feeding (27%), followed by cruising (20%), cleaning (13%), and a combination of cleaning, cruising, and surface feeding (13%), with some recorded as unknown (27%). The maximum manta rays seen at the same time in Aliwal Shoal MPA was two *M. birostris*, but this occurred only once, all other encounters being of single individuals. Individuals were encountered in depths ranged from 6-28 m, SST approximately between 20-26°C and estimated horizontal visibility of 10-30 m.

At Protea Banks MPA, approximately 80 km south of the Aliwal Shoal MPA, a total of 2,877 citizen science scuba dives were conducted between 2003-2018. This site had the lowest SPUE of manta rays (*M. alfredi* or *M. birostris*) compared to the ISWP and Aliwal Shoal MPA (Figure 4.1a). Species and behaviour could not be determined. All sightings at Protea Banks MPA were of single individuals and occurred only in November-July, however, seasonality could not be confirmed. Manta rays (*M. alfredi* or *M. birostris*) at Protea Banks MPA were observed in depths of 20-40 m, SST approximately between 21-26°C, and estimated horizontal visibility of 1-35 m.

4.1.2. Photo-ID and reef manta ray connectivity

A total of 110 manta ray individuals were photo-identified in KZN up until 2022, including 87 *M. alfredi* and 23 *M. birostris*. Donated citizen science photographs dated 2005-2022. Most of the identified *M. alfredi* individuals were encountered in the ISWP (92%; n=79) (Figure 4.2), with a sex ratio of males to females being nearly 1:1 (males=34; females=33).

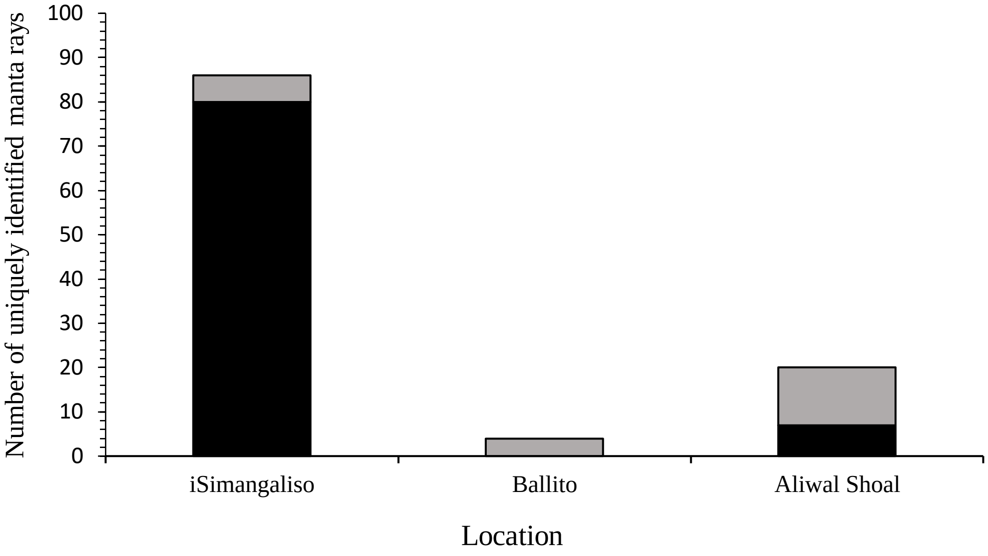


Figure 4.2. Number of unique manta rays, *Mobula alfredi* (black) and *M. birostris* (grey), photo-identified in the Aliwal Shoal Marine Protected Area, Ballito, and the iSimangaliso Wetland Park, KwaZulu-Natal, South Africa.

A total of 14 *M. alfredi* individuals were photo-identified in both South Africa and Mozambique (Figure 4.3, Table 4.1). The greatest distance between such sightings was between the Aliwal Shoal MPA and Závora, Mozambique, a distance of approximately 1,000 km. Other migrations ranged from 435-505 km, between the ISWP and Závora and/or Tofo (Figure 4.3). Four *M. alfredi* were photo-identified in South Africa and Mozambique over a 10-y period, these being SA0057A (Mature Male) SA0091A (Mature Male), SA0062A (Mature Male), and SA0101A (Mature Female). Eight juvenile *M. alfredi* were photo-identified, including a male first identified as a juvenile in 2007, and later identified as a mature male in Závora, Mozambique. Three juvenile *M. birostris* were photo-identified, from the Aliwal Shoal MPA, and donated images from Port St. Johns, Eastern Cape, South Africa. A total of five sightings of melanistic *M. birostris* were encountered, with three of these being uniquely photo-identified individuals. This included a resight of an individual first seen at the Aliwal Shoal MPA in February 2020 and then in Ballito in November 2021.

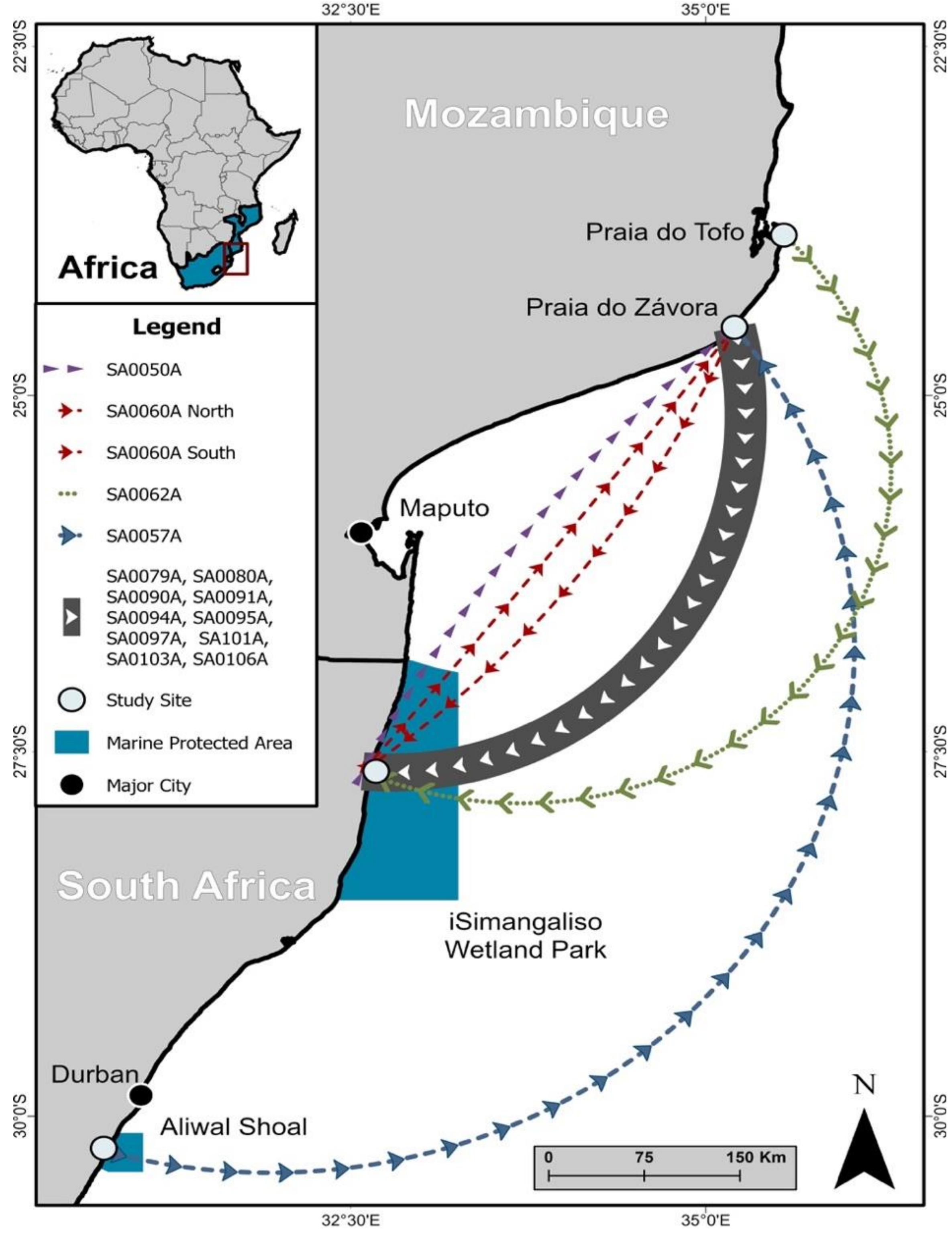


Figure 4.3. *Mobula alfredi* migrations detected between South Africa and Mozambique including the identification name of each individual and their direction of travel. These are additional findings to the initial report by Marshall *et al*. 2022. Map constructed by Mohammed Kajee.

Table 4.1. Encounters of *Mobula alfredi* individuals identified in both southern Mozambique (Tofo=MZ or Závora=Za) and South Africa (SA), including details on the sex, maturity status, sightings records, locations, time between sightings, behaviours, and photographers.

ID SA/MZ	Sex	Sighting records	Time	Behaviour	Photographer
SA0057A / Za540	Juvenile M	2007, Aliwal Shoal MPA, South Africa	Between 3909-4273 days (10.7-11.7 years)	Cruising	Sijmon de Waal
	Mature M	13 September 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	6 August 2020, Závora, Mozambique		Cleaning	Nakia Cullain
SA0091A / Za015	Mature M	5 August 2010, Závora, Mozambique	4304 (11.8 years)	Cleaning	Yara Tibiriçá
	Mature M	6 October 2010, Závora, Mozambique		Cleaning	Yara Tibiriçá
	Mature M	4 September 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	17 September 2020, Závora, Mozambique		Cleaning	Nakia Cullain
	Mature M	14 November 2020, Závora, Mozambique	550 (1.5 years)	Cleaning	Nakia Cullain
	Mature M	18 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0062A / MZ0728A	Juvenile M	10 November 2010, Tofo, Mozambique	3704 (10.1 years)	Unknown	Andrea Marshall
	Mature M	31 December 2020, iSimangaliso Wetland Park, South Africa		Surface feeding, courtship	SharkLife Conservation Group
SA0060A / Za111	Unknown F	11 November 2010, Závora, Mozambique	2422 (6.6 years)	Cleaning	Yara Tibiriçá
	Unknown F	18 November 2010, Závora, Mozambique		Cleaning	Yara Tibiriçá
	Unknown F	29 June 2017, iSimangaliso Wetland Park, South Africa	85 (0.23 years)	Cleaning	Jacques van Jaarsveld
	Unknown F	22 September 2017, Závora, Mozambique		Cleaning	Nakia Cullain
SA0103A / Za212	Subadult M	14 August 2013, Závora, Mozambique	3206 (8.8 years)	Cleaning	Yara Tibiriçá
	Mature M	14 October 2017, Závora, Mozambique		Cleaning	Nakia Cullain
	Mature M	22 August 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	23 August 2018, Závora, Mozambique	1023 (2.8 years)	Cleaning	Michelle Carpenter
	Mature M	13 September 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	6 August 2019, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	25 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	SharkLife Conservation Group
SA0101A / Za147	Unknown F	22 January 2010, Závora, Mozambique	4077 (11.2 years)	Cleaning	Yara Tibiriçá
	Unknown F	9 August 2011, Závora, Mozambique		Cleaning	Yara Tibiriçá
	Mature F	16 September 2013, Závora, Mozambique	2744 (7.5 years)	Cleaning	Yara Tibiriçá
	Mature F	22 March 2021, iSimangaliso Wetland Park, South Africa		Cruising	SharkLife Conservation Group
SA0050A / Za522	Mature M	21 November 2013, iSimangaliso Wetland Park, South Africa	1734 (4.8 years)	Cleaning	Mike Fraser
	Mature M	21 August 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
SA0094A / Za271	Mature M	8 July 2014, Závora, Mozambique	2872 (7.9 years)	Cleaning	Yara Tibiriçá
	Mature M	19 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0080A / Za419	Unknown F	14 September 2017, Závora, Mozambique	1854 (5.1 years)	Cleaning	Nakia Cullain
	Unknown F	17 September 2017, Závora, Mozambique		Cleaning	Nakia Cullain
	Unknown F	14 May 2022, iSimangaliso Wetland Park, South Africa	1700 (4.7 years)	Surface feeding	Michelle Carpenter
	Unknown F	12 October 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0090A / Za364	Mature M	7 September 2017, Závora, Mozambique	1714 (4.7 years)	Cleaning	Nakia Cullain
	Mature M	11 September 2017, Závora, Mozambique		Cleaning	Nakia Cullain
	Mature M	13 August 2019, Závora, Mozambique	1009 (2.8 years)	Cleaning	Michelle Carpenter
	Mature M	18 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter

SA0079A / Za508	Mature F	16 May 2018, Závora, Mozambique	1457 (3.9 years)	Cleaning	Nakia Cullain
	Mature F	22 August 2018, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature F	12 September 2018, Závora, Mozambique	1328 (3.6 years)	Cleaning	Michelle Carpenter
	Mature F	12 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0095A / Za536	Unknown F	4 September 2018, Závora, Mozambique	1354 (3.7 years)	Cleaning	Michelle Carpenter
	Unknown F	17 August 2019, Závora, Mozambique		Cleaning	Michelle Carpenter
	Unknown F	18 September 2020, Závora, Mozambique	609 (1.7 years)	Cleaning	Nakia Cullain
	Unknown F	20 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0097A / Za539	Mature M	13 September 2018, Závora, Mozambique	1345 (3.7 years)	Cleaning	Michelle Carpenter
	Mature M	20 May 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter
SA0106A / Za560	Mature M	13 August 2019, Závora, Mozambique	1533 (4.2 years)	Cleaning	Michelle Carpenter
	Mature M	14 August 2019, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	12 September 2019, Závora, Mozambique		Cleaning	Michelle Carpenter
	Mature M	22 July 2020, Závora, Mozambique		Cleaning	Nakia Cullain
	Mature M	14 November 2020, Závora, Mozambique	709 (1.9 years)	Cleaning	Nakia Cullain
	Mature M	24 October 2022, iSimangaliso Wetland Park, South Africa		Surface feeding	Michelle Carpenter

4.2. Discussion

This study provided first insights into manta ray (*Mobula alfredi*, *M. birostris*) distribution and aggregation sites in South Africa. A combination of opportunistic and citizen science data was used to collate current knowledge on *M. alfredi* and *M. birostris* habitat use. At all locations, surface feeding was the most common behaviour observed for both species, followed by cruising, cleaning, and courtship. Red Sands in the iSimangaliso Wetland Park was shown to be important feeding and cleaning habitat for *M. alfredi*. A total of 110 manta ray individuals were photo-identified (87 *M. alfredi*; 23 *M. birostris*), including an additional 14 *M. alfredi* individuals that migrated between Mozambique and South Africa, with a maximum distance travelled of over 1,000 km. *Mobula birostris* sightings occurred more in Ballito and Aliwal Shoal, but were much less frequently encountered than *M. alfredi*. Among the surveyed regions, Protea Banks MPA exhibited the fewest manta ray SPUE, indicating that it may serve as a corridor for travel rather than a site for aggregations. Three juvenile male *M. birostris* were photo-identified, thereby confirming that juveniles of this species inhabit South African waters. Melanistic *M. birostris* were also encountered, these being previously unknown in southern Africa, with one being resighted. These results support the findings of previous studies suggesting that both *M. alfredi* and *M. birostris* use the South African coastline, but have preference for specific areas, likely driven by plankton availability.

Mobula alfredi and *M. birostris* are known to overlap in mosaic sympatry, with different foraging strategies that may have driven the speciation and that allow these species to share habitat at several locations in southern Africa (Kashiwagi *et al*. 2011). Manta rays were observed in three Marine Protected Areas (Aliwal Shoal, ISWP, and Protea Banks) and Ballito, however there was a difference in overall species composition between the locations. In this study, more *M. alfredi* were documented in the ISWP, and more *M. birostris* documented in Ballito and the Aliwal Shoal MPA, with little sighitngs (38 out of 2,877 dives) of unidentified species in the Protea Banks MPA. Therefore, the waters around KZN may serve as important habitat for both species, with specific areas that may function as critical habitat (e.g., ISWP) related to feeding or cleaning aggregations.

Surface feeding (< 2m water depth from the surface) was the most common behaviour for both species in South Africa. Both *M. alfredi* and *M. birostris* are known to undertake seasonal migrations following zooplankton, which can be related to seasonal or periodic upwellings, seawater temperature, current and tidal patterns, climate events such as El Niño Southern Oscillation, or life stage (Couturier *et al*. 2012, Beale *et a*l. 2019, Farmer *et al*. 2022). The largest numbers of *M. birostris* observed at one time were encountered in Ballito (MaxN=10) and Aliwal Shoal MPA (MaxN=2), however, these were periodic, indicating that inshore habitat use of these locations is conditionally temporal. *M. birostris* is known to spend more time offshore and at depth (Burgess 2017, Stewart *et al*. 2019), while *M. alfredi* associates more with the coast, which may explain the higher number of sightings of this species at one location, the ISWP. Further, between diving at depth to feed on the deep scattering layer, manta ray individuals must increase their body temperatures, which can be facilitated by surface swimming within the top 2 m of the water column, or by visiting cleaning stations, which may explain the behaviours observed in KZN (Couturier *et al*. 2018, Andrzejaczek *et al*. 2021).

At Red Sands, ISWP, surface feeding *M. alfredi* was common compared to the Závora Bay area, where observations of this behaviour are extremely rare in (NRC, pers. Comms). In Závora, the primary food source for *M. alfredi* may be feeding offshore in the deep scattering layer at night, which is also observed in the waters around Saudi Arabia (Braun *et al*. 2014), the Seychelles (Peel *et al*. 2019), and New Caledonia (Lassauce *et al*. 2020). Zooplankton patches must be dense in order for *M. alfredi* to feed, a minimal density of 53.7 mg dry mass^{-3} having been reported to be the threshold in Australia (Armstrong *et al*. 2016). The ISWP encompasses several submarine canyons adjacent to shallow reef, these areas being of high biodiversity, crucial in supporting primary productivity and spawning by pelagic and benthic teleost species (Fernandez-Arcaya *et al*. 2017*,* Sink *et al*. 2006). Despite the fact that the coastal forest and sand dunes in the area are not generally nutrient-rich, some sections of the dunes are red in colour, which suggests the presence of weathered iron. Iron is a scarce but essential nutrient in the marine environment and the runoff of iron into submarine canyons may serve as a fertilizer that supports phytoplankton and subsequent zooplankton blooms (Morel *et al*. 1991). Further, iron-rich water has been observed to run-off from the local river in the ISWP and is a possible reason contributing to the substantial biodiversity of this marine park (Sink *et al*. 2006). The ISWP evidently supports an abundant food source for *M. alfredi,* with several possible environmental factors contributing to suitable plankton densities for feeding.

Cleaning stations for manta rays (*M. alfredi*, *M. birostris*) were confirmed to occur at Aliwal Shoal and the ISWP, but are currently unknown in Ballito. This may be due to the poor visibility characteristic of the area due to adjacent riverine input in the north (Jury 2022) and nearby pollution from Durban in the south (Vetrimurugan *et al*. 2019). In the ISWP, *M. alfredi* display site affinity to Red Sands, IOWZ and are mostly observed cleaning at several stations within the IOWZ and greater iSimanglaiso area (40 km), similar to Tofo, Mozambique (Marshall *et al*. 2011). Yet, the region where *M. alfredi* is consistently encountered in thec ISWP is much larger compared to the selective site affinity to RS in Závora, Mozambique (Chapter 3). This is likely attributed to the extensive stretch of shallow coral reefs that run parallel to the coastline, offering a larger area for cleaning stations in the ISWP as opposed to the rocky reefs found in Závora. The extent of data collected at specific cleaning stations was constrained by the size of the KZN coastline and available funding and logistics for comprehensive exploration; however, this in-water study verified the presence of manta ray hotspots, showing substantial habitat use along the South African coast.

Over 20% of identified individuals reported (6: Marshall *et al*. 2022, 14: current study) in South Africa were also identified in Mozambique, which supports the findings from photo-ID and genetic findings on connectivity between the populations in these countries (Marshall *et al*. 2022, Venables *et al*. 2021). This included the longest *M. alfredi* point-to-point migration in Africa (1,000 km), and the second longest in the world (Armstrong *et al*. 2019), from the Aliwal Shoal Marine Protected Area to Závora, Mozambique. Travelling is energetically expensive, and the observations of manta rays feeding in South Africa suggests that the reward of abundant, nutrient-rich prey outweighs this as well as predation risk (Dudley and Cliff 2010, Marshall and Bennett 2010b, Venables *et al*. 2021). Several elasmobranchs undergo migrations along this coastline, including diamond rays, *Gymnura natalensis* (1,765 km, Daly *et al*. 2022) and bull sharks, *Carcharhias leucas* (709 km, Daly *et al*. 2014). Likewise, manta ray populations inhabiting continental coastlines in southern Africa and Australia are characteristically mobile and more transient (Armstrong *et al*. 2019, Harris *et al*. 2021, Peel *et al*. 2019, Venables *et al*. 2020). The seasonality of *M. alfredi* habitat use in Tofo (Marshall *et al*. 2011) and Závora (Chapter 3) and the connectivity found between these locations and South Africa support this. The cross-border migrations of manta rays underscore the need for international collaboration, particularly with neighboring countries like Mozambique. Joint initiatives such as the Acoustic Tracking Array Platform (Array), have facilitated the resesarch of shared resources and migration corridors. Data sharing, coordinated research efforts, and the establishment of transboundary conservation areas can contribute to the management of manta ray populations across their range. Some of the results from this study have been used in the Maputaland transboundary Important Shark and Ray Area (ISRA) proposal. However, further manta ray acoustic telemetry research is required to develop comprehensive management strategies.

Both juvenile *M. alfredi* and *M. birostris* individuals were identified in South Africa, which further suggests that the coastal waters there may function as nursery habitat, particularly in the Eastern Cape Province, or most southerly part of KZN (Marshall *et al*. 2022). Further, melanistic individuals were identified and resighted in Ballito and Aliwal Shoal MPA, the most ever documented in the Indian Ocean. Melanism is a genetic phenotype with varying frequencies in different populations, for instance, 26% of photo-identified *M. birostris* in the Revillagigedo Archipelago, Mexico were melanistic (Kumli and Rubin 2011). This trait facilitates increased thermoregulation in ectothermic species (Trullas *et al*. 2007), which may be reflective on the horizontal and vertical patterns of the *M. birostris* population in southern Africa. Increased satellite telemetry research is needed to understand *M. birostris* habitat use along the coastline. These additional small findings related to juvenile habita use and melanism allude to the possible differences between the aggregations in South Africa and Mozambique, confirming that additional rsearch is also required at little explored sites such as Ballito, the south part of KZN, and the Eastern Cape.

The scope of this work was limited by studying wide-ranging species along a vast coastline. However, the use of citizen science in various forms has added significant value to this research. Citizen science proved to further engage local communities, tourists, and diving operators in conservation. By conducting this study, previously undocumented habitats of *M. alfredi* and *M. birostris* were identified in South Africa, which provides valuable information around which to design further research (e.g., telemetry and focused habitat research) and to guide future management plans (e.g., increased MPA network) to safeguard these threatened and mobile species in South Africa.

Specific sites identified as critical habitats, such as the ISWP, Aliwal Shoal MPA, and possibly Ballito, call for targeted efforts in habitat protection and restoration. The identification of these sites highlights the importance of effectively managing MPAs. This involves collaborative efforts between government agencies, conservation organisations, and local communities to establish and enforce regulations that restrict impacts (e.g., pollution, human activites, climate change) within these designated areas. In order to effectively develop these ecosysmen-based management practices, more research is needed in the identified areas from this research. For instance, more research to confirm *M. alfredi* prey source in the ISWP, satellite telemetry to confirm the diving patterns of *M. alfredi* and *M. birostris* in KZN, and acoustic telemetry to determine seasonal patterns of *M. alfredi* and *M. birostris* at the ISWP. This study therefore provided a baseline upoin which further research can build upon to develop effective management plans for manta rays and MPAs in KZN.

CHAPTER 5: Long-term trends in manta ray (*Mobula alfredi, M. birostris*) catches in KwaZulu-Natal, South Africa

RESULTS AND DISCUSSION

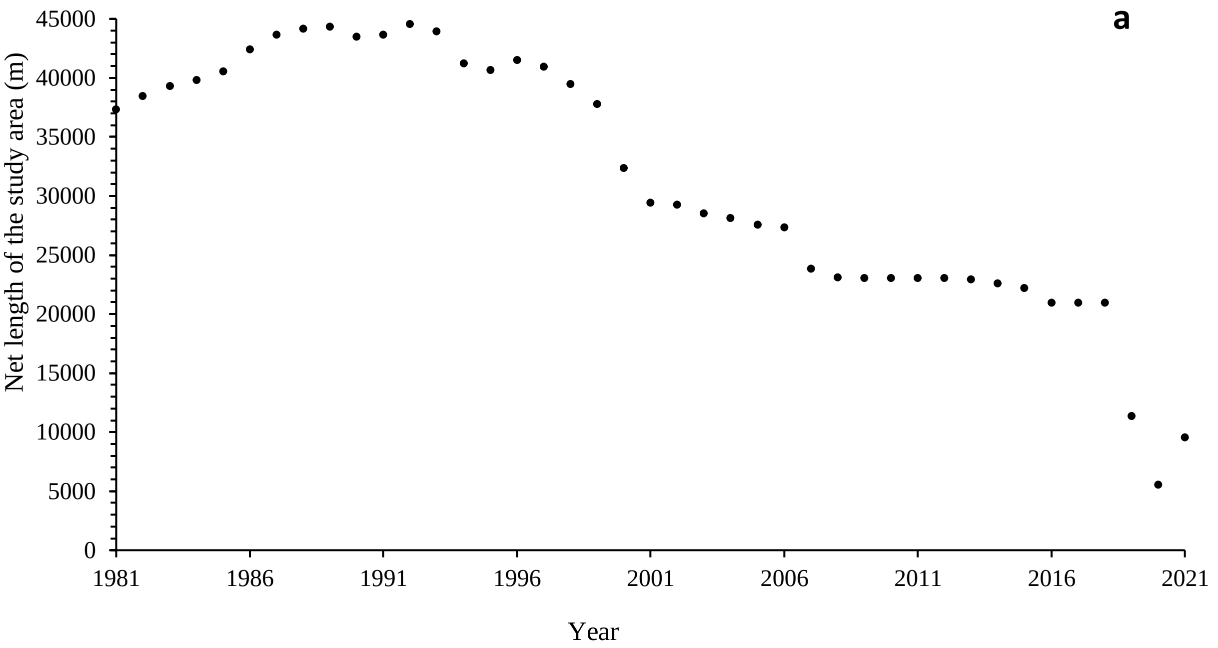
The ocean off KwaZulu-Natal is home to a rich diversity of marine fish species, with many portions of the coastline remaining understudied.

5.1. Results

This chapter utilised a 41-year dataset from the KwaZulu-Natal bather protection program to analyse catch trends between 1981 and 2021. I used Generalised Additive Models (GAMs) and assessed encounter probabilities, which uncovered a noteworthy decline in manta ray catches since the late 1990s. Furhter, the GAM analysis revelealed seasonal visitation patterns and potential key habitat regions along the coastline. The findings emphasise the importance of South African waters as a seasonal habitat for manta rays, necessitating management and conservation actions, while also offering a foundational dataset for future investigations.

5.1.1. Catch and effort

Between 1981-2021, 1,602 manta rays were caught in the KwaZulu-Natal bather protection nets. Between 2007-2021, 10 were caught in the drumlines and therefore excluded from statistical analysis. Manta rays were caught throughout the year, with more caught in austral summer (Dec-Feb; n=534), accounting for 33% of the total catch, compared to the austral winter (Jun-Aug; n=302), which accounted for 19% of the total catch. The size of mantas captured ranged from 1400-8000 mm Disc Width (DW). An average of 40 rays (±29 SD) were caught per year, of which approximately one third (n=527) were found dead, the remaining 70% being released, thus resulting an average of 13 (±11 SD) confirmed mortalities per year. Annual mortality ranged from one (9% of annual catch) in 2017 to 38 (49% of annual catch) in 2001. The majority of catches were single individuals, with a maximum of two manta rays caught in any single deployment. The total net length and manta ray catches exhibited considerable variation across the 41-year period, but there was an overall decrease in net length and overall catches (Figure 5.1).



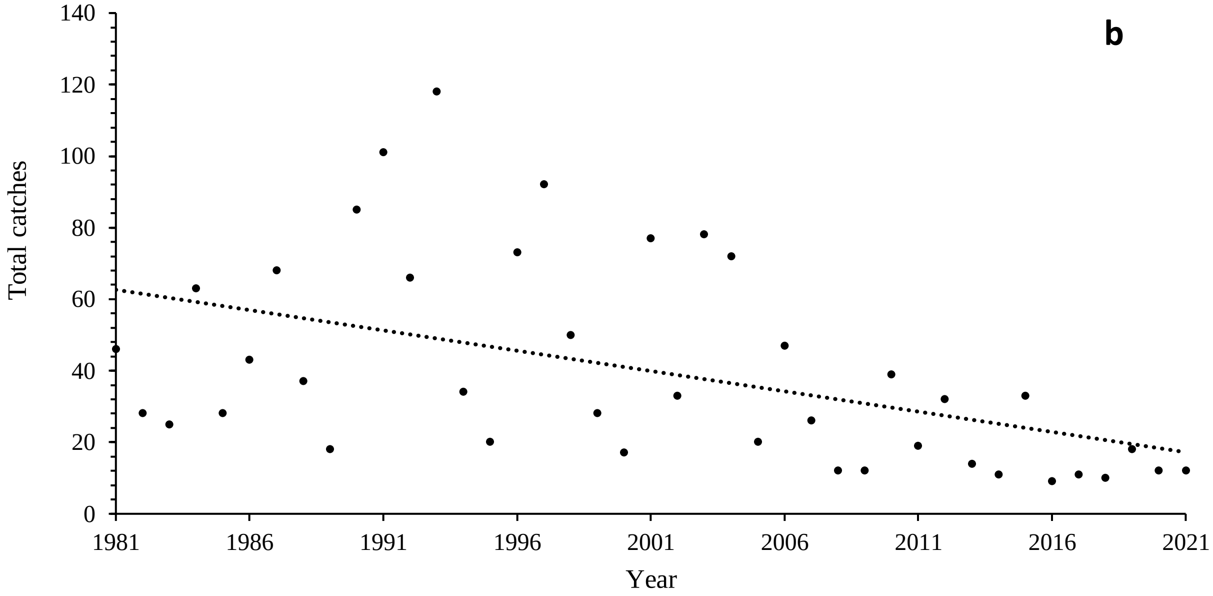


Figure 5.1. The (a) total length of bather protection nets and (b) total annual manta ray catches (b) in these nets in KwaZulu-Natal, South Africa, between 1981-2021. The dashed line (b) represents a linear regression fitted to the data.

Spatially, the Central Area had the highest number of catches throughout the study period (n=649), followed by the South (n=528) and then the North Area (n=414), with 11 additional catches at Richard’s Bay (R.B). Amanzimtoti beach (AMA), within the Central Area (Figure 2.4), had the highest total catch over the entire period (n=120; 7% of total catch). Only two other beaches reported total catches exceeding 100, these being Scottburgh (SCO) and Zinkwazi (ZIN). AMA and SCO are within approximately 35 km of each other in the Central Area, whereas Zinkwazi is the northernmost beach in the North Area (Figure 5.2). When incorporating the unit of effort (net length: calculated as the total of the monthly average number of nets multiplied by the average net length), the highest CPUE occurred at Winklespruit (0.0046) followed by Park Rynie (0.0036), Caribbean Bay (0.0035) and Ifafa (0.0034), three of these beaches being in the Central Area (Figure 5.2).

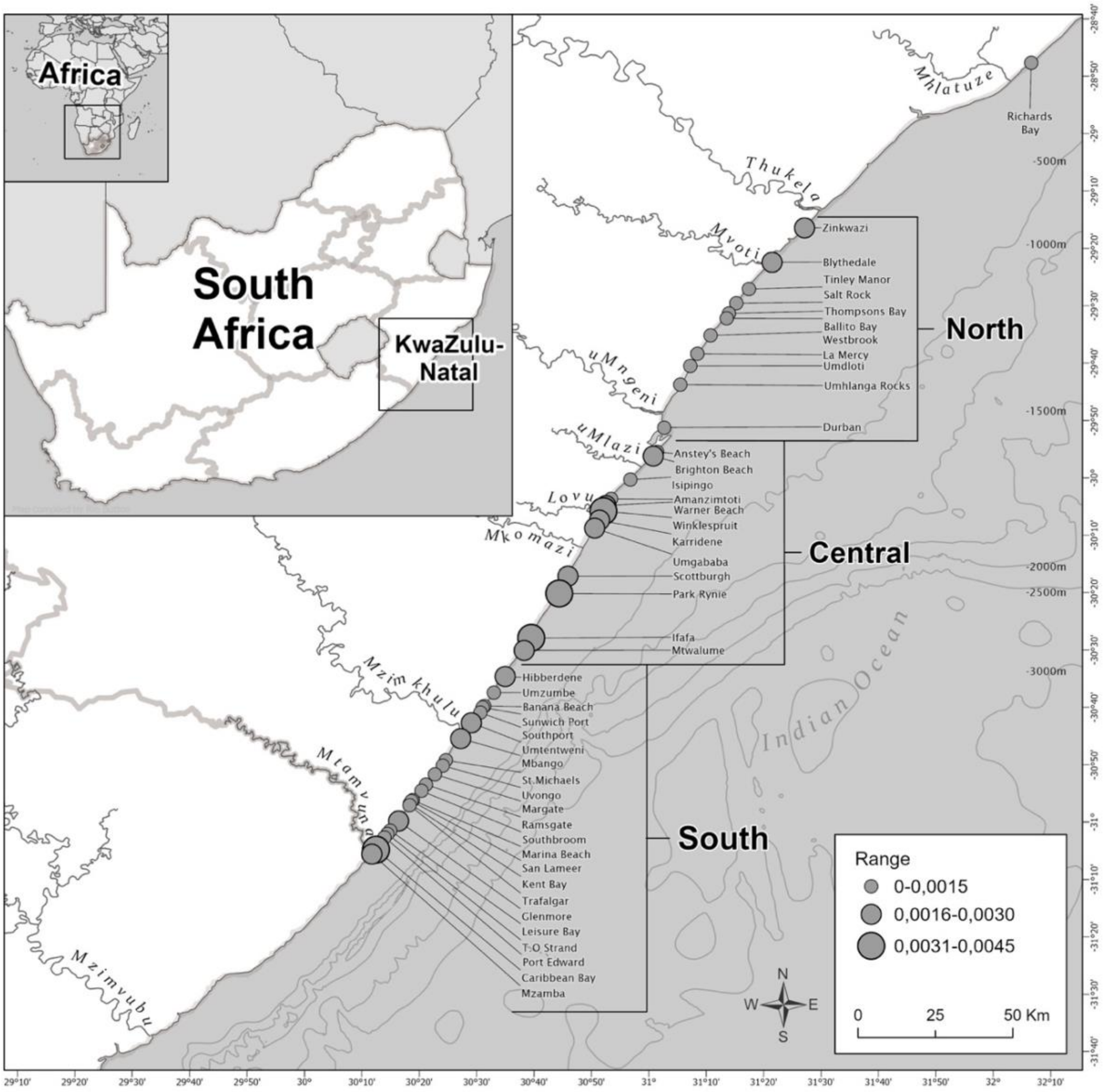


Figure 5.2. Average manta rays caught and standardised by the average net length at the particular beach (catch per unit effort, or CPUE) in the bather protection nets, KwaZulu-Natal, South Africa between 1981-2021. Catch per unit effort was divided into three ranges, the lowest being between 0.000-0.0015, up to the highest being 0.0031-0.0045.

5.1.2. Trends in capture

A total of 1,423 captures were included in the Generalised Additive Models. Month, moon phase, area and year were significant predictors for manta ray capture. This model was offset with the logarithmic of effort. The model explained 3.08% of the total deviance. Year explained 71.4% of total deviation, followed by area (20.5%), month (5.2%), and moon phase (2.8%) (Table 5.1).

Table 5.1. Deviance table documenting the relative importance of the explanatory variables included in the GAM model to assess manta ray catch trends from the KwaZulu-Natal Bather protection net dataset in South Africa between 1981-2021.

Variable	Df	Deviance	% Deviance explained	Pr(>F)	Significance
NULL		17654			
Year	40	17266	71.4	$<2.2e^{-16}$	***
Month	42	17237	5.2	$<6.6e^{-06}$	***
Area	45	17126	20.5	$<2.2e^{-16}$	***
Moon Phase	47	17111	2.8	0.00009	***

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

There was an increase in manta ray catches up until the year 2000 after which there was a significant temporal decline ($p<2.2e^{-16}$) (Figure 5.3). This is especially apparent when viewing year in numerical form, whereby the probability of capture is the lowest in 2015-2021 (Figure 5.4). The probability of capture was highest in the Central Area and lowest in the North Area (Figure 5.3). The probability of catching manta rays peaked in the summer months of December-February and was lowest in winter, between June-August (Figure 5.3). Moon phase had a significant effect on the probability of manta ray capture, with increased catch during new and full moon phases (Figure 5.3).

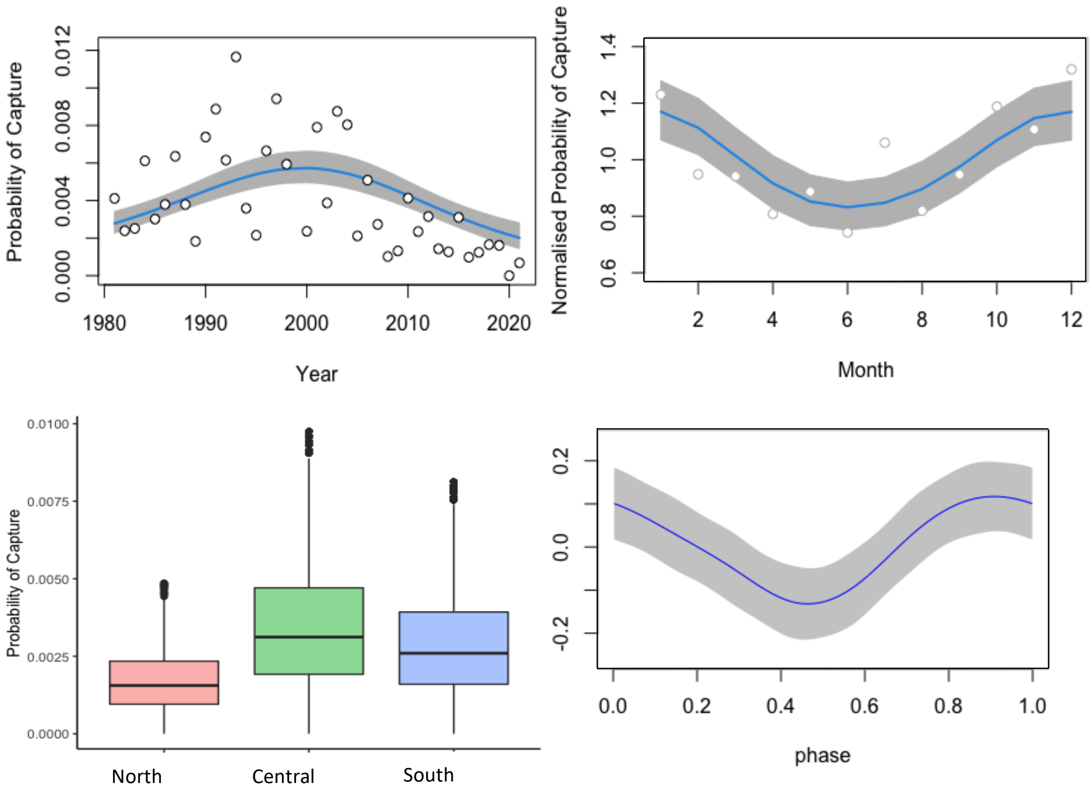


Figure 5.3. Significant predictors for the probability of manta ray capture in the KwaZulu-Natal bather protection nets between 1981-2021 including year (a), month (b), area (c), and moon phase (d). Year and month plots include both numerical and factor models. South Africa austral summer occurs between December-February and winter between June-August.

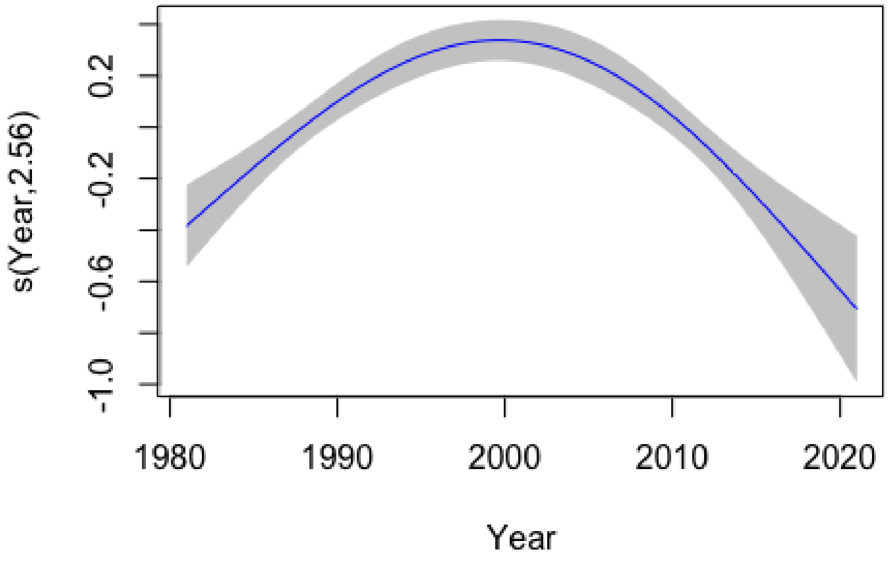


Figure 5.4. Increase in the probability of manta ray capture in the KwaZulu-Natal bather protection nets between 1981-2000 with significant decline (numerical) from then until 2021.

5.1.3. Size and sex composition

Most of the caught rays were sexed (62%, n=997) and of these, 56% were female (n=563) and 44% male (n=434). The remaining 38% were recorded as unknown sex (n=605) and hence were excluded from the analysis of sex ratio. There was no significant difference in sex ratio (p=0.67, exact binomial test). This sex ratio, with slightly more females, remained similar when assessed separately by area (F:M North Area 1.28:1.0; Central Area 1.38:1.0; South Area 1.26:1.0).

Averaged across the entire study period, caught rays had an average DW of 2796 mm (± 1368 SD). More than half (52%, n=841) of caught individuals fell into the juvenile size class, between 1400-2500 mm DW (Figure 5.5). There was an overall ratio of 1:1.8 adults (n=474) to juveniles (n=841) captured, although 18% (n=287) were recorded at unknown maturity due to the overlap in maturity sizes between oceanic and reef manta rays (a size range of 2501-3800 mm) (Figure 5.5).

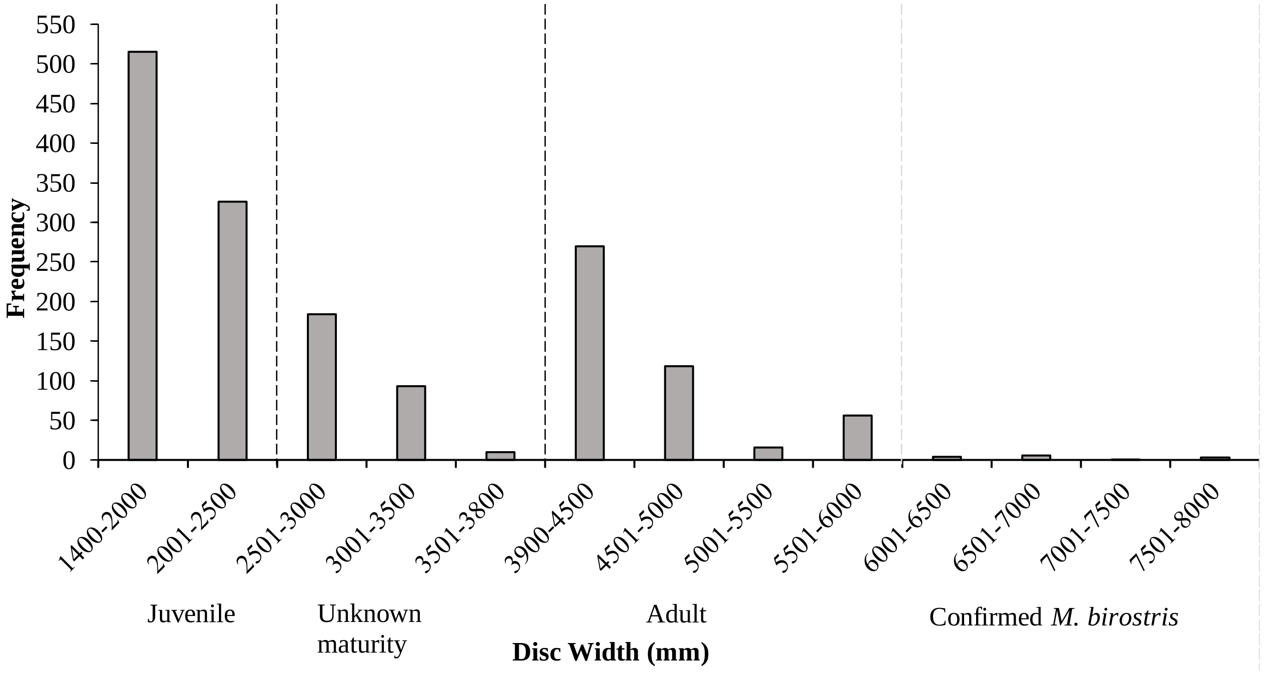


Figure 5.5. Disc width frequency distribution of manta rays caught in the KwaZulu-Natal shark nets between 1981–2021. The (black) dashed lines indicate the division between juvenile, unknown maturity, adult, of unknown species, and (grey) confirmed *Mobula birostris* individuals based on size.

The South Area had the highest proportion of juveniles in the catch (70%) (Figure 5.6), comprising 44% of all juveniles caught throughout the study. Of these, 145 individuals were between 1400-1600 mm in size, the known size range at birth. Both adult and juvenile manta rays were caught throughout the year, with numbers for both peaking in summer, between December-February (Figure 5.7). Confirmed adult manta rays of both species (3800-8000 mm) were caught in their highest numbers in Amanzimtoti (AMA; n=43), followed by Zinkwazi (ZIN; n=39), Scottburgh (SCO; n=38), Park Rynie (PAR; n=36), Winklespruit (WIN; n=36), and Durban (DUR; n=31). Four of these locations (AMA, SCO, PAR, WIN) are within 35 km of one another.

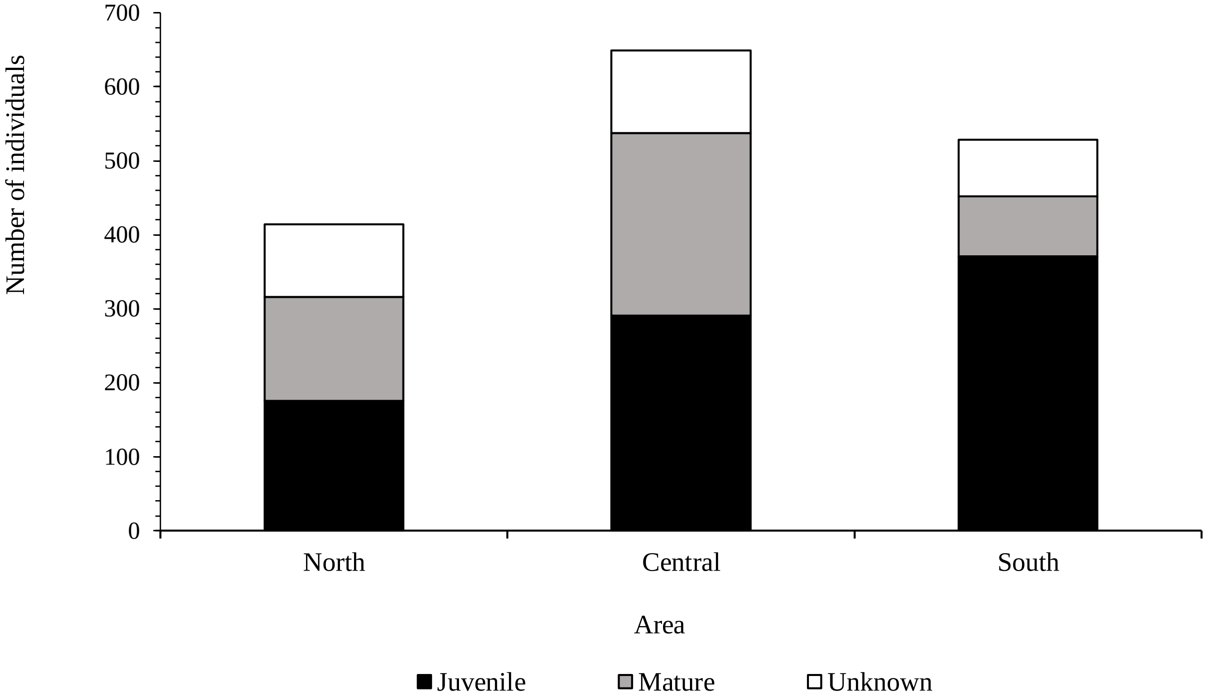


Figure 5.6. Catch and maturity status of manta rays from each area (North Area=Zinkwazi to Durban; Central Area=Anstey's Beach to Mtwalume; South Area=Hibberdene to Mzamba) from bather protection net catch data in KwaZulu-Natal, South Africa 1981-2021.

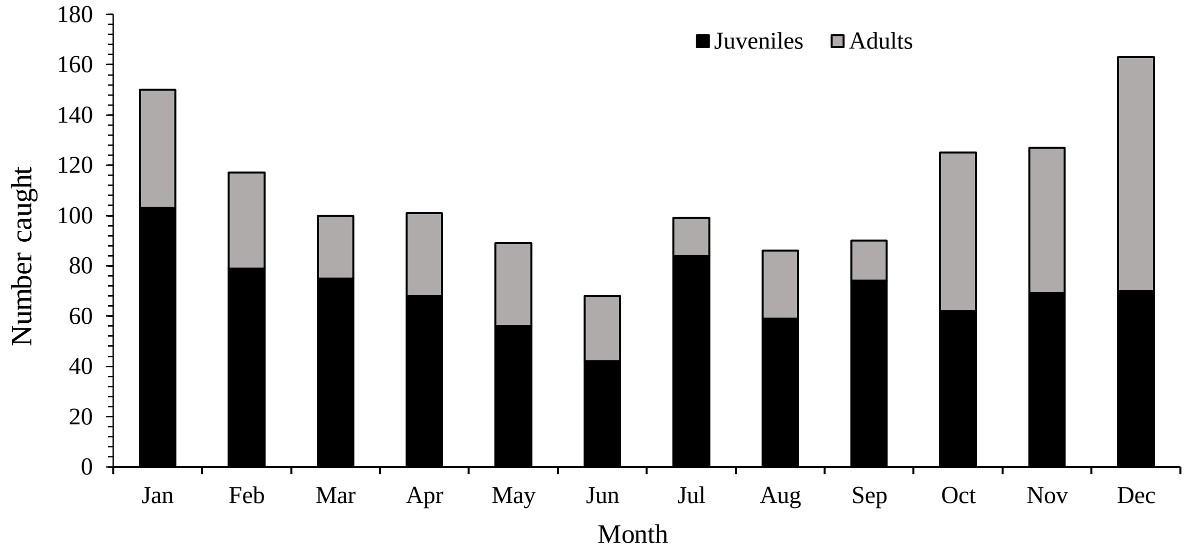


Figure 5.7. Monthly comparison of catch and maturity status of manta rays from bather protection net catch data in KwaZulu-Natal, South Africa over the period 1981-2021.

A total of 70 rays were 5501-8000 mm DW, confirming that they could only have been oceanic manta rays (*M. birostris*), and more than half of these were caught within the Central Area (53%, n=37) (Figure 5.8). The largest *M. birostris* caught in the study were measured to be 8000 mm, two caught in Hibberdene (HIB) and one Brighton (BRI) (Figure 5.8).

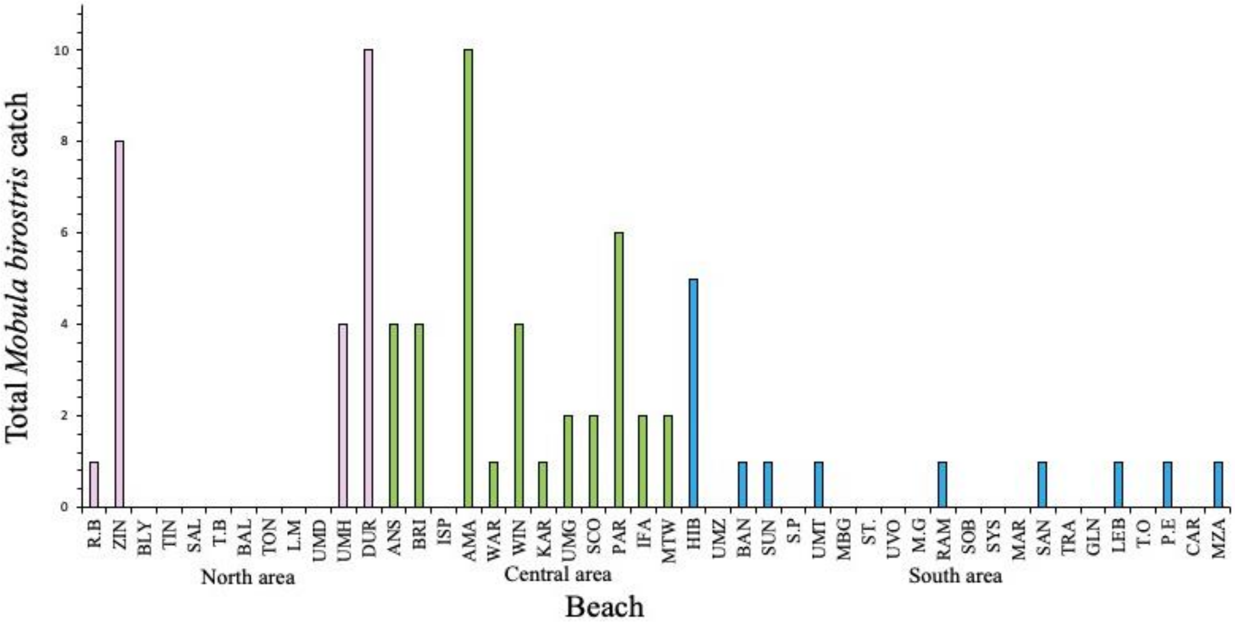


Figure 5.8. Oceanic manta ray (*Mobula birostris*) catches and size composition from each beach from shark net catch data in KwaZulu-Natal, South Africa 1981-2021. Pink represents the North Area, from Zinkwazi to Durban, Green the Central Area, from Anstey's Beach to Mtwalume, and blue the South Area, from Hibberdene to Mzamba.

5.2. Discussion

Manta rays (*Mobula alfredi*, *M. birostris*) were found to have been present in South African waters for at least 41 years. In KZN, manta ray probability of capture showed a peak in the late 1990s, followed by a marked decline thereafter. Further, nominal probability of capture has consistently remained below the annual mean since 2007. This supports findings from the majority of studies from Mozambique, which report that manta ray encounters have generally declined over time (Rohner *et al.* 2013, Venables 2020). Venables (2020) stated that annual landings in an artisanal fishery of 20-50 individuals per year over 16 years could have resulted in the detected abundance decline in Tofo, Mozambique; from 836 in 2004 to less than 100 since 2013 (Marshall *et al.* 2011, Temple *et al.* 2018). Manta ray populations cannot withstand fishing mortality due to their low fecundities (one pup per two years), even from small artisanal fisheries, or as bycatch from destructive fishing practices (Croll *et al.* 2016, Lawson *et al.* 2017, Parton *et al.* 2019). This therefore likely explains the detected abundance decline in the later part of this study.

South Africa was found to encompass important habitat for manta rays, the extent of the visitation to that habitat which differs across seasons. Though manta rays were caught throughout the year, catches were highest in summer (Dec-Feb). Summer is associated with higher sea temperatures, a known predictor for manta ray occurrence (Couturier *et al.* 2018). In KZN, summer is associated with higher rainfall and north-easterly winds that drive the Durban Eddy, both of which increase upwelling and riverine output, and subsequent primary productivity and abundance and diversity of marine taxa (Woodson *et al.* 2012, Guastella and Roberts 2016). Increased copepod and chaetognath abundance occur during summer in KZN, these being known prey of manta rays (Schleyer 1985, Couturier *et al.* 2013, Bennett *et al.* 2016, Peel *et al.* 2019). Therefore, the increased manta ray catches may be due to the increased peaks of phytoplankton and subsequent zooplankton blooms during summer (Pretorius *et al.* 2016). These results indicate that manta rays may be present year-round in the region, but with seasonal peaks, which suggests migration from other parts of the coast driven by life stage, reproduction, food availability, or individual movements.

There were significantly higher catches of manta rays from the area between Anstey's and Mtwalume (Central Area), nearby the Aliwal Shoal MPA. Aliwal Shoal is an important offshore habitat for elasmobranchs (Dicken *et al*. 2006, Dicken and Hosking 2009, Dicken *et al*. 2016). Despite historically high catches, few manta rays (< 15 sightings per year) have been observed at Aliwal Shoal between 2020-2022, during fieldwork for this thesis.

With at least one catch from every beach, this study provides further evidence that manta rays utilise the expansive continental coastline year-round from the Eastern Cape (approximately 175 km south of Mzamba) (Marshall *et al.* 2022) northwards into southern Mozambique. However, the intricacies of habitat use remain unknown in KZN, for instance, the specific hotspots for each species, and how often they move in and out of various areas, and what environmental characteristics in addition to SST affect these patterns. The capture of manta rays along 350 km of continental coastline in this study supported the findings of in-water photo-ID research (Chapters 3 and 4). Additionally, it provided further validation for the cross-border migrations observed in photo-identified *M. alfredi,* as reported by Marshall et al. (2022, Chapter 3). Further, full and new moons were significantly associated with manta ray capture, a known predictor of mobulid sightings (Rohner *et al*. 2013, Fonseca-Ponce *et al*. 2022). This may be due to tidal effects on zooplankton availability (Rohner *et al*. 2013, Barr and Abelson 2019), or the efficacy of nets in capturing manta rays during the full tidal range. The variability in manta ray catches during this study are thus likely consequences of physical processes that drive resource availability and/or net efficacy (Graham *et al*. 2012, Braun *et al*. 2014, Jaine *et al*. 2014, Stewart *et al*. 2016b).

A greater proportion of juvenile manta rays (DW of 1400-2500) were found in the South Area, from Hibberdene to Mzamba. A total of 9% of individuals (n=145) caught were within the estimated birth size (1400-1600 mm) (Stewart *et al*. 2018b), and most were caught at Mzamba (MZA) (n=14), the most southerly location in the present study. Observations in Port St. John's, Eastern Cape, roughly 93 km south of Mzamba, reported six juvenile individuals sighted during winter (Marshall *et al*. 2022). Further, 52% of total catches (n=841) were within juvenile size for either manta ray species, with almost half of these (43%; n=365) from the South Area. Aggregations in Mozambique monitored for 11-20 years have reported small numbers of juveniles (roughly 5% of the photo-identified population in Tofo and Závora and 3% in Bazaruto) (Venables 2020, Chapter 3). These results fit two of three of the criteria outlined by Heupel *et al.* (2007): juveniles were more common in a certain area and the habitat was repeatedly used across multiple years; however, one criterion could not be validated; this being if individuals remain or return to the area for extended periods. In contrast, overall, larger mantas were caught in the North and Central Areas, from Zinkwazi to Mtwalume, where the most confirmed oceanic manta rays (based on size class) were also caught, which may be reflective of a possible oceanic manta ray aggregation. However, it is possible that the nets are incapable of holding large adults.

The primary caveat in this study is that it reports on relatively low sampling coverage over an expansive area. Considering their depth and habitat ranges, both manta ray species are likely to be spending the majority of time outside the limits of bather protection nets or recreational diving in KZN. Further, species could not be distinguished in the catch data, due to the overlap in size and potential confusion with species identification. Both species are known to overlap in habitat use (Marshall *et al.* 2009, Kashiwagi *et al.* 2011) and both have been identified in various locations along the KZN coast (Marshall *et al.* 2022). Therefore, the pooling of species needs to be considered when interpreting the results. In further studies, species identification and data quality could be improved via team training, or the implementation of photographic records for each captured animal, whether dead or alive (and released). Nevertheless, these results are informative for baseline spatial-temporal habitat use, and can be used to inform policymakers on the impacts of bycatch mortality and the need for the development of local conservation management plans.

Identifying sources of mortality of manta rays within the southern African region is key to mitigating impacts. Though the scientific knowledge gained from the bather protection nets has been unprecedented, including pioneering studies on numerous elasmobranch species in KZN (Cliff and Dudley 1991, Dudley and Simpfendorfer 2006, Kock *et al.* 2022), the current design may impact manta ray populations (an average of 13, up to 38 confirmed annual catch fatalities). Manta rays have one of the lowest reported population growth rates (median $r_{\max}$ of 0.116 year^{-1} 95th percentile [0.089–0.139]; Dulvy *et al.* 2014) of 106 assessed elasmobranch species.

Efforts to reduce bycatch have been implemented by the KZN bather protection program, such as reducing the number of nets and drumlines (Guyomard *et al*. 2019), and the removal of gear at three of the four beaches with the highest manta ray CPUE. However, due to the bycatch mortality of many vulnerable species, efforts should continue in seeking solutions to mitigate catches even further, especially at beaches installed within already established species refuges (e.g., Marine Protected Areas). This would help reduce impact to the southern African manta ray populations and facilitate their conservation into the future.

CHAPTER 6: Cleaning behaviour of the shortfin devil ray (*Mobula kuhlii*) in KwaZulu-Natal, South Africa

RESULTS AND DISCUSSION

Mobula kuhlii is an elusive and Endangered species, with only two currently known locations in the world where they use cleaning stations.

6.1. Results

These results feature the Aliwal Shoal MPA's first documented shortfin devil ray (*M. kuhlii*) cleaning station and the associated sighting trends and behaviours. Here, I use Generalised Additive models to examine the influence of environmental factors on *M. kuhlii* presence within the Aliwal Shoal MPA and the duration of cleaning interactions at the cleaning station. Moreover, using Remote Underwater Video (RUV), the research unveils novel insights into intraspecific cleaning behaviours and interactions with cleaner fish. These findings contribute towards establishing a sustainable ecotourism model centered around this understudied and unprotected species.

6.1.1. Shortfin devil ray (Mobula kuhlii) use of Aliwal Shoal

A total of 329 recreational dives/snorkelling drifts by a single diver were made across 144 days between September 2020-March 2022. Among these, *Mobula kuhlii* were observed to be present 81 times. Sea surface temperature was the only significant predictor of the variables tested, with probability of occurrence peaking between 24-25 °C (Figure 6.1). Cleaning stations were observed on all dive sites on Aliwal Shoal, with clients including teleost fish, sea turtles, and shark and ray species. *M. kuhlii* were only observed cleaning in the area of Angels Ledge, Bay of Caves, and Kyles Reef, whereby the only cleaner fish identified to exhibit cleaning *M. kuhlii* on these stations was the blue streak cleaner wrasse, *Labroides dimidiatus*. During cleaning an individual *M. kuhlii* would swim slowly, terminate pectoral fin beats by hovering over the cleaning station, or exhibit posture behaviour, whereby the pelvic fins were angled towards the reef, exposing more of the ventral surface. The number of *M. kuhlii* per minute of diving was highest at Angels Ledge and Kyles Reef (Figure 6.2), which are situated next to one another (roughly a 30 m horizontal distance).

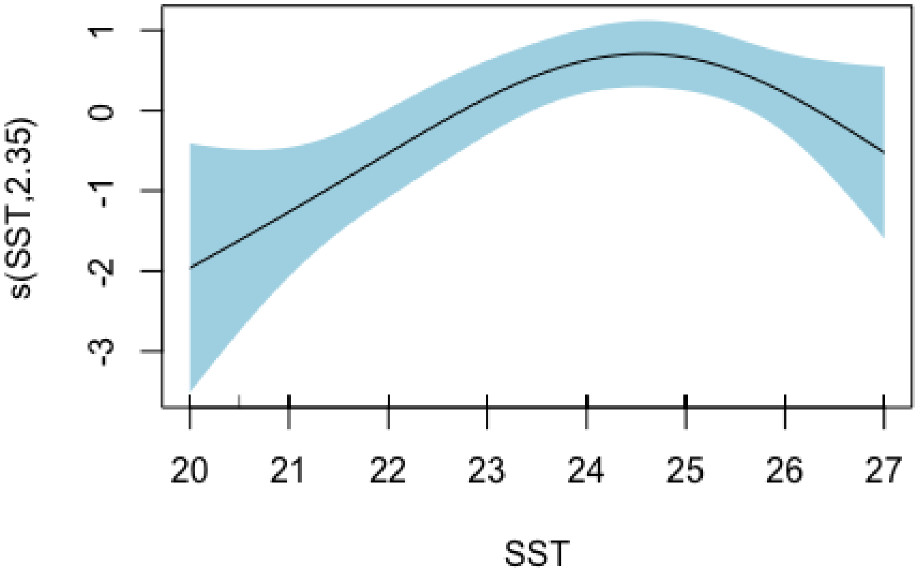


Figure 6.1. Significant predictor, Sea Surface Temperature (SST), of *Mobula kuhlii* visitation to the Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa, recorded on recreational snorkelling or diving, between September 2020-March 2022.

Table 6.1. List of dive sites, including *Mobula kuhlii* cleaning stations*, which were visited opportunistically on recreational dives at the Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa between September 2020 and March 2022. Listed are GPS locations, depth ranges, and total time spent at each place (min).

Dive site	Latitude	Longitude	Maximum Depth (m)	Total dive time (min)
Pinnacles	-30.259917	30.828067	8	821
North Sands	-30.259633	30.827467	14	1338
Chunnel	-30.261467	30.826883	11	973
Inside Edge	-30.264167	30.822222	16	414
Outside Edge	-30.486670	31.002220	18	759
Raggies Cave	-30.261717	30.827950	18	628
South Sands	-30.263250	30.826883	15	896
Cathedral	-30.486670	31.002220	28	570
Bay of Caves*	-30.271110	30.968330	26	769
Angels Ledge*	-30.267833	30.825317	24	1627
Kyles Reef*	-30.306390	30.946390	25	198

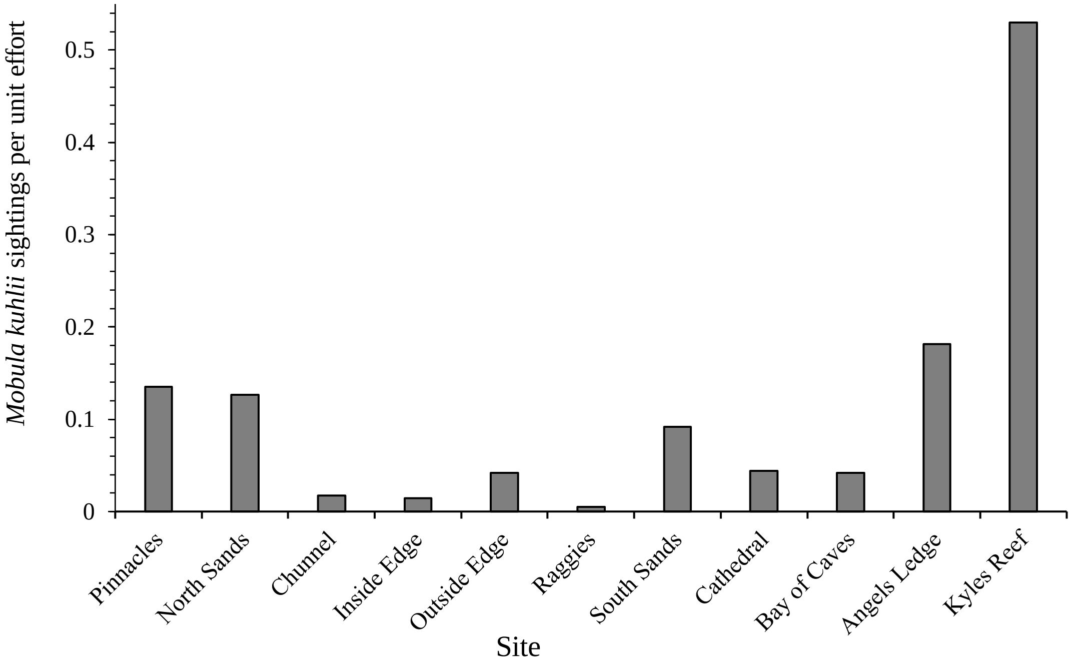


Figure 6.2. Number of *Mobula kuhlii* encounters per minute at dive sites visited during recreational SCUBA dives and snorkel drifts on Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa between September 2020-March 2022.

When encountered, an average number of *Mobula kuhlii* individuals comprising the group was 12 (±10 SD). The largest group observed was >150 individuals, including at least 10-20 individuals RAM feeding, seen on North Sands on 11 December 2021. Other large fevers (between 50-75 *M. kuhlii*) were encountered at Pinnacles, South Sands, Angels Ledge and Kyles Reef. When *M. kuhlii* were encountered during a recreational dive or drift, the most common behaviour was cruising (n=36), followed by cleaning (n=25), courtship (n=6), feeding (n=6), and following spotted eagle rays *Aetobatus ocellatus* (n=2), with unknown behaviour recorded 7% of the time (n=6). Breaching behaviour was also observed (n=5) although reported much more commonly by anecdotal skipper reports. *Mobula kuhlii* were present across all temperatures recorded, ranging from 19-27°C, and in all types of current direction, including the absence of current. However, sightings during East or West currents were uncommon (West, n=1; East n=1). During the study, the estimated horizontal visibility ranged between 6-30 m, cloud cover from 5-100%, wind speed between 0-33 km/hr, and moon phase between 0.005-0.993.

6.1.2. Cleaning behaviour

Remote GoPro mounts were placed in the same spot in the sand within the Angels Ledge cleaning station area opportunistically during recreational diving 41 times between January 2021-March 2022. Of these, 46% (n=19) of days recorded *M. kuhlii* as present. When present, between one and 23 individuals were cleaning on Angels Ledge at the same time, with an average of seven. Up to five *L. dimidiatus* individuals surrounded a single *M. kuhlii* simultaneously (Figure 6.3).

Table 6.2. Ethogram of shortfin devil ray, *Mobula kuhlii*, behaviours opportunistically observed on Angels Ledge, KwaZulu-Natal, South Africa and recorded in BORIS software, between January 2021 and March 2022.

Behaviour	**Type**	**Description**	**Modifiers**
Cleaning	State	One or more *L. dimidiatus* individuals being within 1m of one or more *M. kuhlii*. Sex, cephalic lobe position, and injuries recorded each time, when possible.	Hovering/swimming slow: swimming slowly in and around the cleaning station during cleaning Posturing: Terminating the pectoral fin beats, opening the mouth and gills, and vertical positioning Twitching: body shutters in response to a cleaner fish strike Following: Two or more *M. kuhlii* individuals following one in and around the cleaning station during cleaning
Cleaner fish strike	Point	The mouth of *L. dimidiatus* making contact with a *M. kuhlii* individual	Dorsal head, dorsal body, ventral head, gills, ventral body, pectorals ventral, pelvic ventral, tail
Cleaning out of sight	State	Cleaning behaviour that was observed but too far away to detect *L. dimidiatus* strikes	None
Cruising	State	One or more *M. kuhlii* swimming in a direction, either solitary or in a group, with the cephalic lobes furled and clearly not engaged in cleaning	None
Courtship	State	One or more *M. kuhlii* actively engaged in courtship behaviour as per the description by Stevens *et al*. 2018	Initiation: Male(s) mimic the female's movements, following behind her; both the male and female maintaining cruising speed. A male may attempt to position his body on top of the female's back facing in the same direction. This action may involve the male unfurling cephalic cephalic lobes and positions them so they are in contact with the dorsal head region of the female.
Other species cleaning	State	Other species greater than 1m in total length or disc width cleaning on Angels Ledge	Spotted eagle ray, *Aetobatus ocellatus* Pickhandle barracuda, *Sphyraena jello* Round ribbontail ray, *Taeniurops meyeni* Bull ray, *Aetomylaeus bovinus* Giant trevally, *Caranx ignobilis*

Figure 6.3. Up to five individual bluestreak cleaner wrasse, *Labroides dimidiatu*s, on a *Mobula kuhlii* individual at Angels Ledge cleaning station, Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa.

A total of 4.04 hrs (14,571 secs) of *M. kuhlii* cleaning behaviour (Table 6.2) was recorded, with 1.40 hrs (5,041 secs) being close enough to analyse specific *L. dimidiatus* strikes on specific body parts, types of cleaning behaviours and cephalic lobes positions. A total of 1.18 hrs (4,311 secs) of cruising behaviour were recorded. In a remote video, the observed cleaning behaviour had a mean duration of 53.06 secs (±73.81 sec SD) with *M. kuhlii* receiving direct cleaning strikes from *L. dimidiatus* an average of 31.16 (± 47.71 SD) times, making an overall cleaning rate of 0.59 strikes/s while cleaning. The most common type of cleaning behaviour was 'hovering' at a mean duration of 32.04 s (± 46.54 SD), followed by 'following' (20.46 s ± 40.41 SD), 'posturing' (4.61 s ± 12.61 SD), and 'twitching' (3.15 s ± 5.14 SD). *Mobula kuhlii* was more likely to clean together with other individuals (total = 4,395 s) than alone (total = 645.99 s). There was a significant difference in *M. kuhlii* body patch foraging preference by *L. dimidiatus* ($p<0.001$), with the ventral head, dorsal body, and pelvic fin body patches having the most strikes on average in a given observation (Figure 6.3).

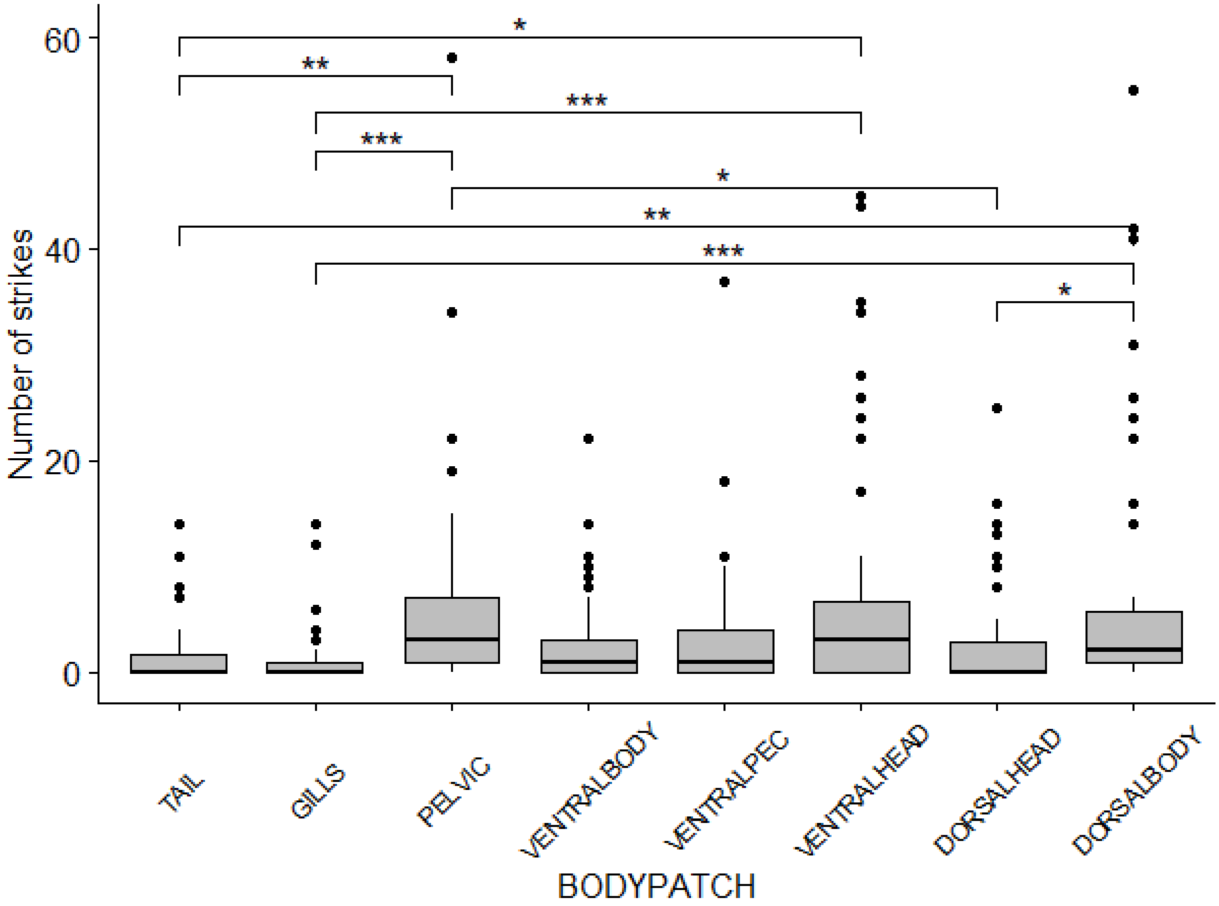


Figure 6.4. *Mobula kuhlii* body patch comparison of foraging preference by *Labroides dimidiatus* on Angels Ledge cleaning station, Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa. Box plots represent non-normalised data. The box encompasses the 25 and 75% confidence intervals with the mean represented by the solid line. Outliers are represented by the solid dots. Asterisks represent significantly different preferences by *L. dimidiatus* between two given body patches using a Kruskal Wallis test and subsequent post-hoc Dunn test.

The final GAM model used to assess *M. kuhlii* cleaning duration included bottom sea temperature (BST), current direction, and estimated horizontal visibility. *Mobula kuhlii* cleaning duration was significantly longer in the absence of a current and in estimated horizontal visibility binned category of 11-15 m (Table 6.3, Figure 6.5). No relationship was detected between cleaning duration and the cleaning duration of other recorded megafauna cleaning on Angels Ledge or any the other monitored environmental parameters.

Table 6.3. Deviance table documenting the relative importance of the variables included in the GAM model to assess *Mobula kuhlii* cleaning behaviour duration at Angels Ledge, Aliwal Shoal Marine Protected Area, KwaZulu-Natal between 2021-2022.

Variable	Df	% Deviance explained	Pr(>F)	Significance
NULL				
BST	4	20.8	0.0822	
Current direction	8	25.9	0.0389	*
Horizontal visibility (binned)	11	53.3	0.0039	*

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

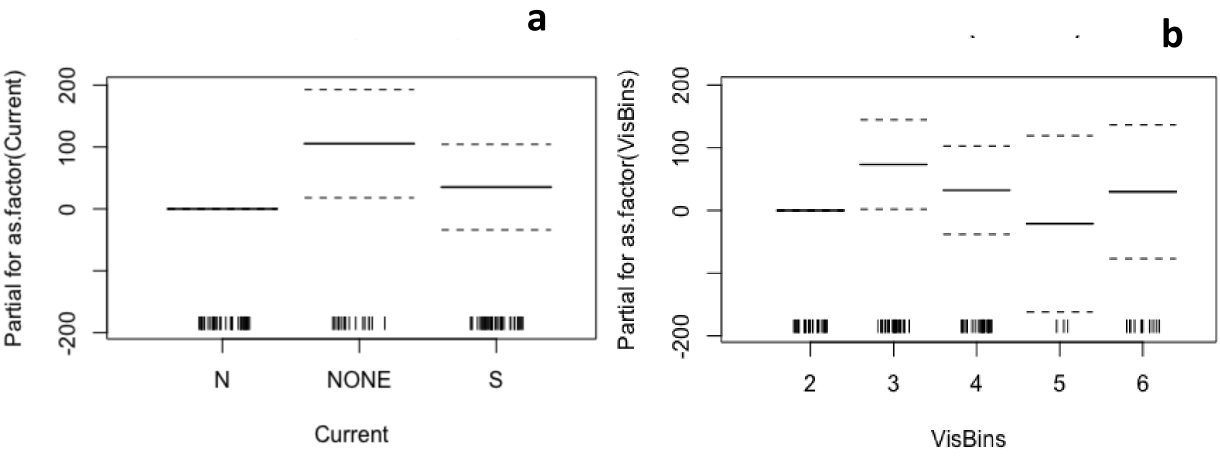


Figure 6.5. Significant predictors (a) current direction and (b) visibility in 5 m increments of *Mobula kuhlii* cleaning duration on Angels Ledge, Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa, between January 2021-March 2022. No footage was recorded of *M. kuhlii* in visibility less than 5 m. The visibility binned into categories ranged from 2: 6-10 m; 3: 11-15 m; 4: 16-20 m; 5:21-25m; and 6:26-30 m.

Mobula kuhlii used a variety of cephalic lobe positions (Figure 2.6). While cleaning, the cephalic lobes were most-commonly both in a furled position (mean = 5.11 times ± 7.40 SD), followed by both being unfurled (mean = 1.63 times ± 2.97 SD), and one unfurled and one furled (mean = 1.56 times ± 2.60). Cephalic lobe positions were changed at a mean of 0.16 times/s (± 0.15 SD position changes/s). *Mobula kuhlii* cephalic lobes were often unfurled simultaneously as one or more *L. dimidiatus* approached their face.

For most of the *M. kuhlii* individuals in the video footage, the sex could not be determined, however, of those successfully sexed (n=30), there were more males (n=22) than females (n=8), with all males being mature (n=22), and most females undetermined, with two individuals confirmed as mature (Figure 6.6). The most prevalent injuries observed were truncated tails (42), scarred pectoral fin (16), complete loss of tail (4), and bent tail (1) (Figure 6.7). Individuals that showed no signs of scarring or injury were observed 17 times. However, the majority of *M. kuhlii* observed were too far away from the GoPro camera to confidently describe injuries, resulting in 70 individuals assigned with unknown injuries. *Mobula kuhlii* was found to clean simultaneously alongside one or more spotted eagle rays, *Aetobatus ocellatus* (total time=207.47 sec) (Figure 6.8), followed by pickhandle barracuda, *Sphyraena jello* (127.73 sec), potato groupers, *Epinephelus tukula* (22.76 sec), and loggerhead turtle, *Carretta caretta* (3.00 sec).

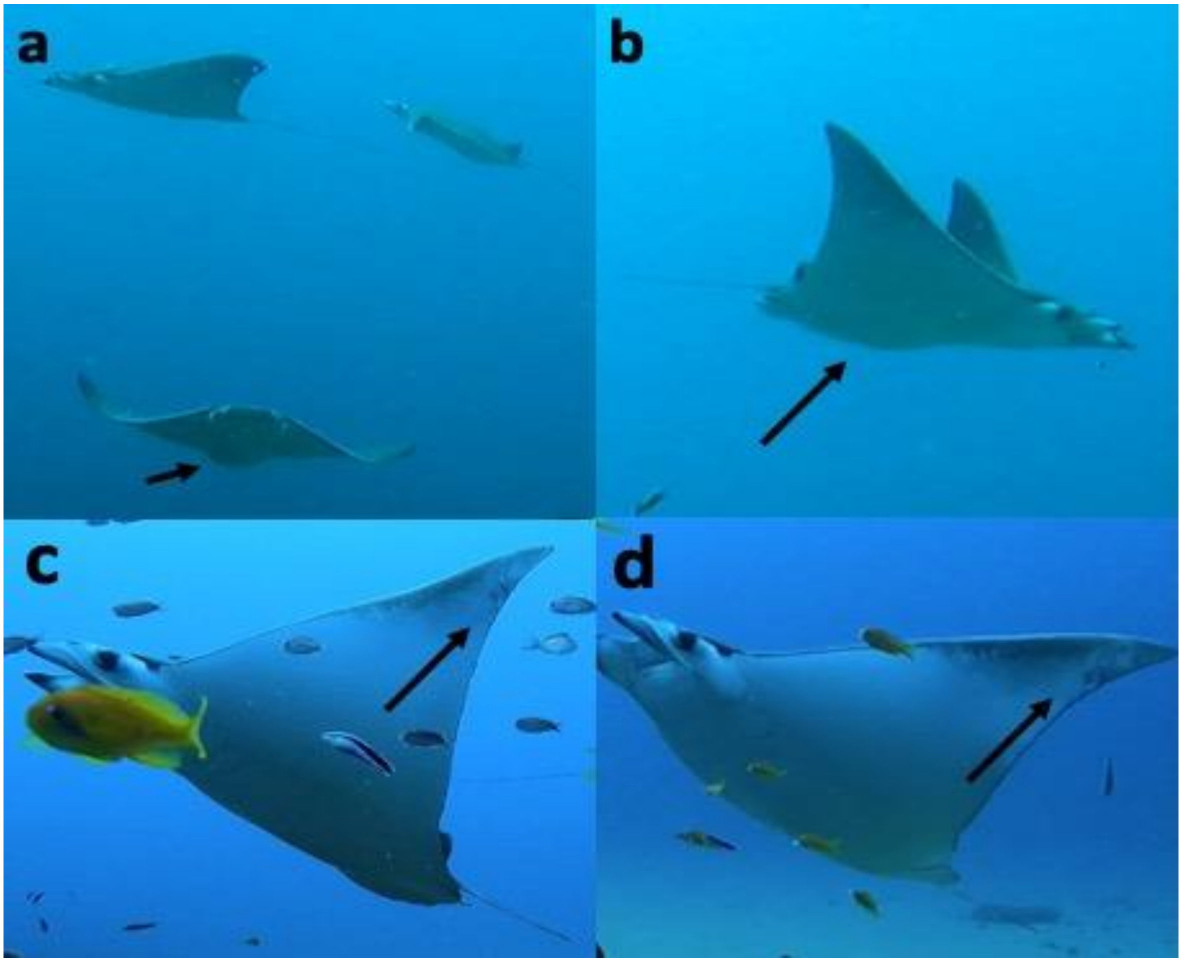


Figure 6.6. Mature females captured on footage cleaning at Angels Ledge, Aliwal Shoal, KwaZulu-Natal, South Africa, including a (a, b) pregnant female and (c, d) females with mating scars.

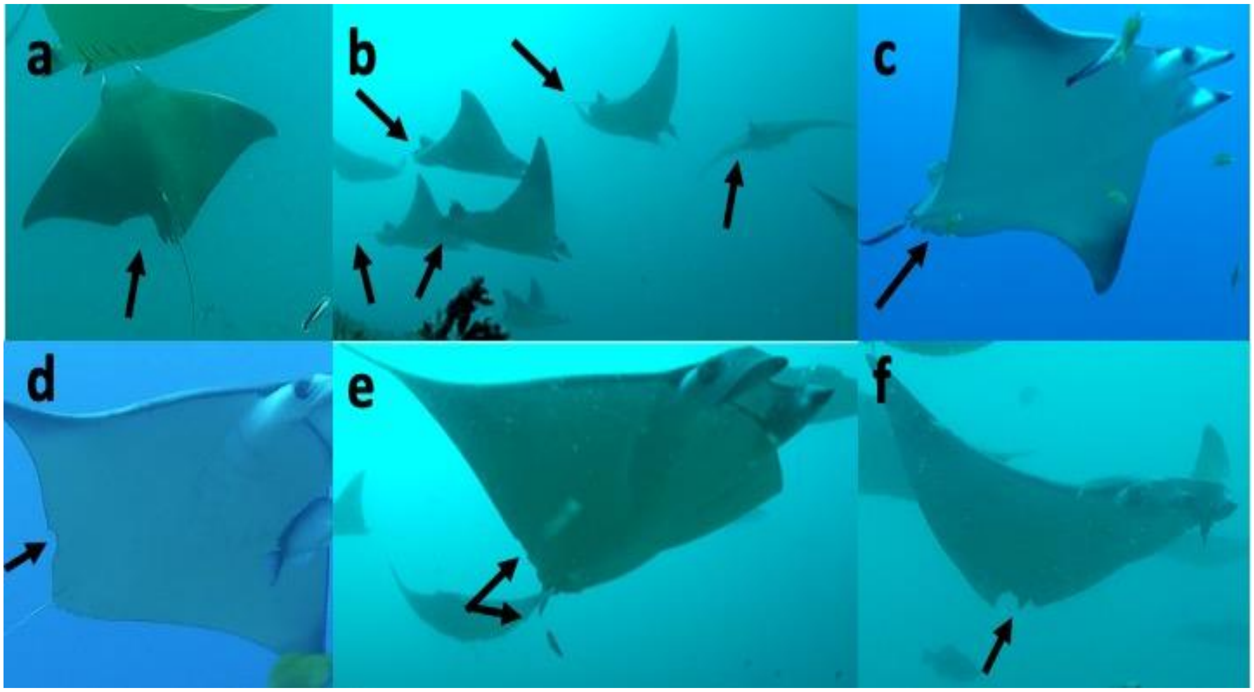


Figure 6.7. *Mobula kuhlii* injuries captured at Angels Ledge, Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa between 2020-2022. These include the (a) pectoral fin, (b) loss of the tail, (c) damage to the pelvic fins, (d) small predatory scars, (e) truncated tails, and (f) loss of reproductive organs.

Figure 6.8. A shortfin devil ray, *Mobula kuhlii* and a spotted eagle ray, *Aetobatus ocellatus*, being cleaned simultaneously on a station at Angels Ledge, KwaZulu-Natal, South Africa.

6.2. Discussion

Mobula kuhlii were significantly more likely to be encountered in a sea surface temperature range of 24-26°C, which is typical for summer (December-January) in KZN (Smit *et al*. 2013), supporting the other findings of this thesis whereby summer months displacy increased mobulid sightings and/or presence (Chapters 4,5). A group of >150 *M. kuhlii* was encountered in December 2021, with many of these individuals exhibiting feeding behaviour, confirming that the reef within the MPA supports both feeding and cleaning sites for the species. While *M. kuhii* were encountered at all recreationally-visited dive sites, individuals predominantly cleaned at Angels Ledge and nearby locations including Bay of Caves and Kyles Reef. The majority of cleaning activity took place along a very specific section of the reef/shoal supporting findings of other mobulid studies which found that individuals were highly selective and showed affinity to specific sites for cleaning (Chapter 3, Couturier *et al*. 2012, Murie and Marshall 2016).

Unlike manta ray hosts which support cleaning from a variety of cleaner species (Marshall 2008), only *L. dimidiatus* were recorded cleaning *M. kuhlii*, with all body patches being cleaned and a significant preference for the pelvic fins, dorsal body, and ventral head surfaces. Different cleaner fish have been documented to clean different parts of large bodied hosts, with certain fish species targeting specific parasites (Marshall 2008, Murie *et al*. 2020). *Labroides dimidiatus* is known to preferentially target caligid copepods (*Pupulina cliffi*) (Grutter 1997) and in KZN, *M. kuhlii* has been found to have high density loads of this ectoparasite (Lebepe and Dippenaar 2013). Thus, it is essential for *M. kuhlii* to visit cleaning stations such as these found on Aliwal Shoal MPA to maintain their health.

These findings support other studies on mobulids that show individuals feeding, cleaning and exhibiting courtship in close proximity (Chapter 4, Stewart *et al*. 2016, Stevens *et al*. 2018, Germanov *et al*. 2019) with environmental conditions dictating suitable times for each activity (Rohner *et al*. 2013). In this study, *M. kuhlii* were recorded to clean significantly longer in the absence of current, which has been documented in other studies on mobulid cleaning behaviour (Barr and Abelson 2019, Murie *et al*. 2020). As such, it is possible that *M. kuhlii* exhibit a trade-off between cleaning and feeding, foraging at times when conditions are favourable with high abundances of plankton and cleaning when these densities are low, however, more research will be required to investigate this.

Mobula kuhlii rarely cleaned alone, being with at least one or more individuals in close proximity (1 m or less) with each other 87% (n=4,395 sec). Mobulids are known to be social elasmobranchs (Notarbartolo di Sciara 1987, Perryman *et al*. 2019, Perryman *et al*. 2022b) and Kitchen-Wheeler (2013) hypothesised that the presence of an established *M. alfredi* individual at a cleaning station may bring in other individuals, who will then imitate their behaviour. *Mobula alfredi* are well-studied for their sociality, and have been documented to clean in specific groups (Perryman *et al*. 2019, Perryman *et al*. 2022b) and exhibit 'following' behaviour (Perryman *et al*. 2021), reflecting a possible learned ritual. Further, the distinct contrasting dark and light colouration on the cephalic lobes of *Mobula* spp. has been suggested to aid in social signalling (Notarbartolo di Sciara 1987) which may occur at *M. alfredi* cleaning stations (Perryman *et al*. 2021). *Mobula kuhlii* recorded in this study displayed similar cephalic lobe positions to *M. alfredi* (Perryman *et al*. 2021), as well as similar 'posture' positions while cleaning (Marshall 2008). As *M. kuhlii* cleaning behaviour is similar to other mobulids such as *M. alfredi* it is possible that cleaning behaviour, like mating behaviour is retained across the mobulid species.

Mobula kuhlii uncommonly cleaned at the same cleaning station at the same time with other megafauna species (7% of the time) and never with another mobulid species. Manta rays (*M. alfredi*, *M. birostris*) have been seen cleaning at the Pinnacles dive site on the Aliwal Shoal MPA and spotted ragged tooth sharks, *Carcharias taurus*, have been seen cleaning at the dive sites Cathedral and Chunnel (Carpenter, pers. obsv) both species having a larger total length or disc width than *M. kuhlii*. Many cleaning studies have reported on larger individuals being preferred by cleaner fish, and this is attributed to them likely having more parasites on a greater surface area (Grutter *et al*. 2005, Oliver *et al*. 2011, Kitchen-Wheeler 2013). However, 57% of the time that *M. kuhlii* cleaned with another megafauna species this was with one or more *A. ocellatus*, the first description of these species simultaneously cleaning at the same cleaning station. It is possible that the cleaning station habitat was partitioned by *M. kuhlii* to avoid competition with larger, more dominant species, with adult *A. ocellatus* being only slightly larger or the same size (White *et al*. 2010) as adult *M. kuhlii*.

Visiting cleaning stations is essential for an individuals' health, helping to control parasite loads (Clague *et al*. 2011), and promote recovery from injury (Strike *et al*. 2022). Mobulid populations are impacted naturally by predation (Marshall and Bennett 2010b, Strike *et al*. 2022), or unnaturally by fishing line, mooring rope, net entanglement (Deakos *et al*. 2011, Couturier *et al*. 2012, Carpentier *et al*. 2019), and boat strikes (Strike *et al*. 2022). The injuries documented in this study indicate the potential of both natural and anthropogenic impacts on *M. kuhlii*.

Researching cleaning behaviour at established cleaning stations by using high-definition video recording devices like GoPros has allowed for these complex interactions to be studied in detail. *Mobula kuhlii* are elusive and difficult to monitor and video analysis helps to capture interactions which can be later reviewed in slow motion. Limitations due to video quality and distance impeded the ability to capture and analyse all cleaning behaviour and therefore cleaning interactions may be underestimated. These challenges could be addressed in future studies by using a larger set-sup of Remote Underwater Videos, higher quality cameras or even 360° camera systems which could allow for greater fields of view. Further research should also focus on incorporating acoustic and/or satellite telemetry to confirm the movements of *M. kuhlii* in and outside this MPA. Being able to accurately describe the patterns of use of these critical inshore habitats may provide the information needed to more effectively safeguard this endangered species.

CHAPTER 7: First observations of shortfin devil ray (*Mobula kuhlii*) courtship behaviour

RESULTS AND DISCUSSION

The limited knowledge about the shortfin devil ray (*Mobula kuhlii*) has resulted in a global lack of courtship behaviour documented on video.

7.1. Results

Here, I present the first-ever observations of courtship behavior in the Endangered shortfin devil ray (*Mobula kuhlii*) within the Aliwal Shoal Marine Protected Area (MPA) in KwaZulu-Natal, South Africa. The recorded instances of courtship, termed 'mating trains,' captured on video in November 2020 and January 2021, reveal common courtship behaviors such as males trailing a lead female, rapid speed bursts, swerving, and avoiding the female. These findings confirm the occurrence of M. kuhlii mating in the Aliwal Shoal MPA's habitat.

7.1.1 Observations of Mobula kuhlii courtship behaviour

Three courtship events were recorded on video (DOI: 10.2989/1814232X.2022.2158131). Of these, one event involved a lead female that was pregnant and near-term. Six components of *M. kuhlii* courtship were observed (Figure 7.1). All events occurred in shallow waters of less than 30 m depth, during daylight between 08:40 and 15:34, in sea surface temperatures (SST) of 22-25 °C, in water with an estimated horizontal visibility of 15-25 m, and during times of dense slicks of zooplankton on the surface. All *M. kuhlii* observed in mating trains were estimated to be 1.0-1.2 m disc width (DW). Courtship behaviour was observed at three sites: ‘Bay of Caves’, ‘North Sands’, and ‘Outside Edge’. The supplementary material for this study can be found online in the African Journal of Marine Science, which includes raw video footage of the encounters of *Mobula kuhlii* courtship.

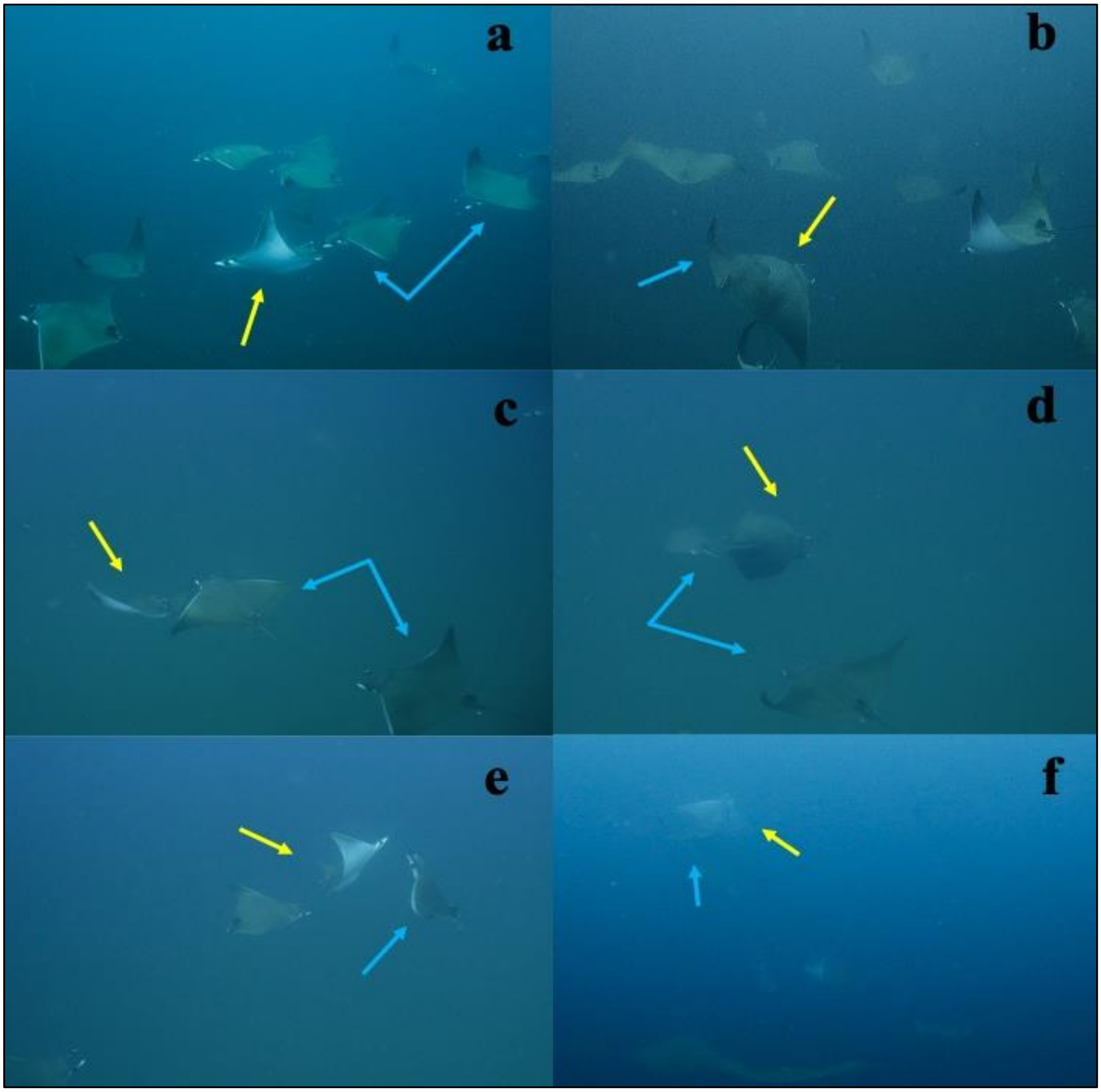


Figure 7.1. Courtship behaviour of shortfin devil rays, *Mobula kuhlii*, observed at ‘Bay of Caves’ within the Aliwal Shoal Marine Protected Area, KwaZulu-Natal, South Africa, on 17 November, 2020. The blue arrows point to males and the yellow arrow signifies the lead female who is heavily pregnant (a) and avoids male attempts to bite her pectoral fin (b) by rapid speed bursts (c), swerving sharply (d), and when encountered by males on the ventral side (e), swimming vertically towards the surface (f). Photos by Michelle Carpenter.

7.2. Discussion

These results provide the first information on courtship in *M. kuhlii*, supporting the contention that KZN is important habitat for mobulid reproductive behaviour. This behaviour included components such as mating trains of multiple males following one female, close pursuit with faster-than-average swimming speeds, female avoidance, rapid direction change (‘veering’ or ‘swerving'), female-male contact, and male grasp attempt (Yano *et al*. 1999, Sobral 2013, Duffy and Tindale 2018, McCallister *et al*. 2020). The behaviours observed in this study are consistent with the observations of Stevens *et al*. (2018), specifically the categories described as ‘Initiation’, ‘Endurance’, and ‘Evasion’. In *M. alfredi*, these categories involve males following the movements of a single female, rapid chase by 1–26 males in single file, and the female making abrupt turns at an increased swimming speed (Stevens *et al*. 2018). Copulation in mobulids entails the closest male biting on the left pectoral fin of the female and subsequently positioning himself abdomen-to-abdomen with the female (Yano *et al*. 1999, Marshall and Bennett 2010). Prior to biting, the male will position close to the female’s dorsal surface while in pursuit (Stevens *et al*. 2018). Several attempts were made by the males to grasp the female’s pectoral fin on the dorsal surface, but copulation itself was not observed.

On one occasion a pregnant female was observed engaged in a courtship event, representing 33% of observations. Despite the small sample size, this is consistent with reports from the Maldives, where 12% of *M. alfredi* mating trains included a near-term pregnant female (Stevens *et al*. 2018). Mating in elasmobranchs may be facilitated by olfaction (Johnson and Nelson 1978, Chapman *et al*. 2003). This has been reported across several mobulid species, indicating that pregnancy signals a reproductively receptive female who likely secretes chemical cues (Deakos 2010, Marshall and Bennett 2010, Duffy and Tindale 2018). These observations suggest that mating in *M. kuhlii* occurs immediately following birth, which is in agreement with studies on *M. mobular* and other batoids (Chapman *et al*. 2003, Duffy and Tindale 2018).

In this study *Mobula kuhlii* courtship events were observed in November and January, further supporting mobulid presence in KZN waters during summer, likely associated with the seasonal increased primary productivity (Chapters 4, 5, and 6, Woodson *et al*. 2012, Guastella and Roberts 2016). *Mobula alfredi* sometimes engage in courtship whilst on surface-feeding grounds (Armstrong *et al*. 2016), whereas *M. birostris* have been observed courting within a deep (50–80 m) thermocline layer where zooplankton was trapped (Stewart *et al*. 2019). Reproductive activity appears to be seasonal in mobulids (Marshall and Bennett 2010, Duffy and Tindale 2018, Stevens *et al*. 2018). In southern Mozambique, *M. alfredi* reproductive activity peaks in austral summer (October–January) at Tofo (Marshall and Bennett 2010), yet 90 km further south, at Závora, it occurs during winter and austral summer (July–November) (Carpenter *et al*. 2022). *Mobula tarapacana* courtship was observed in April–May at the Saint Peter and Saint Paul Archipelago, Brazil (Mendonça *et al*. 2020), and in autumn (March) for *M. mobular* in New Zealand (Duffy and Tindale 2018). Hence the seasonality of mobulid reproduction evidently varies by species and location.

Despite some sampling in KZN in 2021–2022, *M. kuhlii* courtship was rarely encountered. When mating trains were encountered, observation times were short, and the high swimming speeds of *M. kuhlii* individuals inhibited the distinguishing of mating scars on lead females. More research is needed, therefore, to capture the full cycle of *M. kuhlii* mating in KZN and to determine how widely South African waters are used for reproduction. Nevertheless, these results confirm the presence of a mating area for this species within KZN waters and further highlights the importance of Aliwal Shoal.

CHAPTER 8

CONCLUSIONS

Effective mobulid conservation requires a synergistic approach combining research, ecotourism, education, public awareness, and management efforts.

8.1. Assessment of mobulid habitat use in southern Africa

This thesis presents the first comprehensive investigation of mobulid aggregation sites in Závora, Mozambique and in the KwaZulu-Natal Province, South Africa, utilising a variety of research methods. This research has made significant contributions, including the first assessment of reef manta ray (*Mobula alfredi*) abundance in Závora, identification of manta ray (*M. alfredi*, *M. birostris*) aggregations in South Africa, analysis of spatial-temporal trends in catches of manta rays (*M. alfredi*, *M. birostris*) in KZN, and novel descriptions of cleaning and mating behaviours of shortfin devil rays (*M. kuhlii*) in South Africa. These findings reveal mobulid habitat use patterns that can inform the development of effective conservation strategies. The thesis concludes with recommendations for management and further research to enhance the conservation of these threatened species based on the described spatio-temporal patterns in this study.

8.1.1. Identifying critical sites for mobulids

Critical habitats were identified for three mobulid species along the southern African coast. These included Závora, Mozambique (*M. alfredi,* Chapter 3), the iSimangaliso Wetland Park, South Africa (*M. alfredi*, Chapter 4), the southern border of KZN, South Africa (*M. alfredi*, *M. birostris*, Chapter 5), the Aliwal Shoal MPA (*M. kuhlii,* Chapters 6-7), and possibly Ballito, KZN (*M. birostris*, Chapter 4). The majority of these sites were found to be cleaning stations, which serve health, social, and reproductive purposes for mobulids (Couturier *et al*. 2012). The potential juvenile area (*M. alfredi* and/or *M. birostris*) found between Hibberdene and Mzamba, KZN (Chapter 5), and possibly extending to Port St. John's, Eastern Cape (Marshall *et al.* 2022, Chapter 4), also constitutes critical habitat, due to the importance of nursery habitats for populations. Further research should be conducted at these identified sites for long-term monitoring on these threatened species and to better understand habitat use at these aggregations and the potential nursery for juvenile manta rays (*M. alfredi*, *M. birostris*).

8.1.2. Spatial-temporal patterns in habitat use

Mobulids exhibited seasonal patterns in southern Africa, which has been found to be typical along continental coastlines, such as Australia (Armstrong *et al.* 2020a). Peak season for the *M. alfredi* aggregation at Red Sands in Závora, Mozambique occurred in winter (July-November) which is characterised by high primary productivity at this time, indicative of upwelling (Quartly and Srokosz 2004). Further, abundance estimates varied greatly each year, with 2017 and 2020 being much higher than other years. Such fine-scale and large-scale temporal patterns were also observed in KwaZulu-Natal, South Africa, where manta ray (*M. alfredi*, *M. birostris*) catch was significantly higher in summer (December-February), but varied greatly year to year, and *M. kuhlii* was encountered at Aliwal Shoal significantly more during summer sea surface temperatures of 24-25°C. Summer in KZN is associated with higher primary productivity (Lamont and Barlow 2015), attributed to increased riverine input and upwellings. The *M. alfredi* migrations between southern Mozambique and KZN found in prior studies, and in this thesis, further demonstrate that this species, and possibly other mobulids, exploit a wide range of habitat. These fine-scale temporal patterns are likely a result of seasonal productivity shifts in a given year, whereas large-scale patterns could be attributed to regional changes in currents and/or upwelling, or climate systems, such as El Nino Southern Oscillation (ENSO) or dipole effects, the latter which affect plankton patchiness and distribution (Folt and Burns 1999, Quartly and Srokosz 2004, Beale *et al*. 2019).

Social and reproductive activity were observed at cleaning stations and/or surface feeding aggregations. This included the observations of mature individuals including pregnant females, and courtship behaviour of *M. alfredi* at Red Sands, at surface feeding aggregations in the iSimangaliso Wetland Park, and of *M. kuhlii* at Aliwal Shoal. Most animal species reproduce during a time of food abundance and/or after great food intake (Dunn and Hannon 1992, Zabel and Taggart 1989, Kolluru and Grether 2004). In southern Mozambique, the continental shelf is near to shore, and in the iSimangaliso Wetland Park, KZN, there are several submarine canyons. Subamrine canyons are known areas of high biodiversity crucial in supporting primary productivity and spawning by pelagic and benthic teleost species (Fernandez-Arcaya *et al*. 2017, Sink *et al*. 2006). Zooplankton can be trapped at depth, whereby manta rays exploit these layers, particularly during the night (Braun *et al*. 2014, Burgess *et al*. 2016). Thus, it is possible that at Závora and in the ISWP, *M. alfredi,* individuals may exploit the deep scattering layer at night, and return to the shallows (e.g., cleaning stations or surface feeding aggregations) for cleaning, courtship, and thermoregulation (Braun *et al*. 2014, Couturier *et al*. 2018, Barr and Abelson 2019, Harris and Stevens 2021). Therefore, individuals may travel along the southern African coast utilising various sites over contrasting times due to ontogenetic variations in movement and site use such as reproductive periodicity, or as part of a social network, that coincide with following food sources (Perryman *et al*. 2019, Venables *et al*. 2021). However, further telemetry research (e.g., acoustic or satellite) is needed to confirm these hypotheses.

8.2. Mobulid conservation in southern Africa

Given the conservation concern for the mobulid populations in both Mozambique and South Africa (Peel 2019, Rohner *et al*. 2013, Tibiriçá *et al*. 2011, Venables 2020, Marshall *et al*. 2022) and the established international (CITES 2013, CMS 2015, IUCN 2022) and national protection laws (Notice No. 40875 under No. 476 of the Biodiversity Act, 10 of 2004,2017, Boletim da República 2020), it is essential to focus on priority habitats such as those found in this thesis, where mobulids might be at risk. A decline in the abundance of manta rays (*M. alfredi, M. birostris*) was detected in KZN, which began in the late 1990s, which agrees with studies in Mozambique that show populations to be declining (Rohner *et al*. 2013, Venables 2020), and around the world (Croll *et al*. 2016). This can be attributed to targeted fishing (O'Malley *et al*. 2016), and incidental capture from gill nets (Marshall 2008), trawling nets (Fennessy 1994), and the bather protection programme (Dudley and Simpfendorfer 2006). With one of the lowest population growth rates measured in elasmobranchs any fishing of these species is unsustainable (Dulvy *et al*. 2014). The *M. alfredi* population in southern Africa is considered to be the most threatened in the world (Rohner *et al*. 2017, Venables 2020) because of the fishing that occurs in both Mozambique and South Africa. A combination of research, management, and education efforts would help facilitate their conservation into the future.

8.3. Conclusions

The findings of this thesis demonstrate the importance of understanding mobulid habitat use and visitation patterns in southern Africa. Some critical sites that were described for mobulids this thesis are located in already existing MPAs (e.g., ISWP for *M. alfredi*, Aliwal Shoal MPA for *M. kuhlii*). However, some mobulid aggregations remain completely unprotected (e.g., Závora for *M. alfredi*, Ballito for *M. birostris*), are impacted inside the MPA (e.g., in the Aliwal Shoal MPA from the Scottburgh bather protection nets), with large knowledge gaps on how these populations move in and outside MPAs and their home ranges. The protection of critical habitats, including those used for cleaning and feeding, ensures the long-term conservation of these vulnerable species. Given the pressing need to protect mobulid populations, future research and conservation efforts are recommended for the southern Africa region.

8.3.1. Recommendations for research

Future research on mobulids in southern Africa should focus on several areas to improve the understanding of their populations, movements, habitat use, and ecology. Acoustic telemetry would capture information on mobulid extensive range and how often they travel beyond marine protected area (MPA) borders. The use of habitat outside the borders of the Aliwal Shoal MPA by *M. kuhlii* and the potential connection of this aggregation with the Mozambique population may be determined by acoustic telemetry studies on this species, especially given that it is not suitable for photo-ID. Further, satellite telemetry studies to capture mobulid migratory routes and behaviour should be conducted, particularly *M. birostris*, which likely spends considerable time offshore, challenging traditional in-water studies' efficacy in tracking its movements. The application of such movement studies holds particular significance in South Africa, a country where no telemetry studies on mobulids have been published thus far.

To gain a comprehensive understanding of population dynamics of manta rays along the southern Africa coast, the South Africa and Mozambique databases should be merged, including the Bazaruto Archipelgao, Tofo, Závora, and South Africa, to identify shared individuals. Subsequently, a thorough analysis using robust population modeling, building upon the results from Venables (2020) and Chapter 2 of this thesis, would offer the most accurate depiction of *M. alfredi* population abundance and temporal changes along the southern African. Similar studies should be conducted on *M. birostris*, however, fewer photo-identified individuals in the databases could be attributed to their distinct movement patterns. Further, an important knowledge gap involves locating and validating juvenile habitats, particularly in southern KZN and the Eastern Cape (Marshall *et al*. 2022). Exploring whether these habitats are linked to larger populations of *M. alfredi* and *M. birostris* that use waters from both South Africa and Mozambique is a crucial aspect of merging the manta ray databases across southern Africa.

Since certain findings within this thesis suggest that temporal changes in zooplankton may contribute to the observed patterns in mobulid habitat utilisation in southern Africa, further feeding ecology studies are recommended. Stable isotope analyses of *M. alfredi* and *M. birostris* in the Seychelles (Peel *et al*. 2019) and Ecuador (Burgess *et al*. 2016), respectively, revelead unique trophic roles and feeding ecology of these species, including both emergent and mesopelagic zooplankton. These studies can be applied to southern Africa, such as *M. alfredi* in Závora and the ISWP, *M. birostris* in Ballito and the Aliwal Shoal MPA areas, and *M. kuhlii* in the Aliwal Shoal MPA. Understanding if mobulids in the region occupy similar trophic roles and how these patterns might be affected by bathymetry and oceanic processes (e.g., currents, upwelling, eddies) may collectively contribute to more accurate predictions of annual and seasonal habitat use, and how these might evolve on a broader scale in response to climatic shifts. Integrating these suggested future research endevours, together with the findings of this thesis and other studies across Southern Africa, will facilitate a holistic approach to the conservation of these threatened and iconic species.

8.3.2. Recommendations for conservation

In light of the findings presented in this thesis, a series of recommendations are proposed to enhance conservation efforts for mobulid species in the southern African region. Notably, the conservation initiatives proposed in southern Mozambique, despite the country's comparatively lower resources, exemplify potential tactics to be applied in South Africa. National protection of three mobulid species (*M. alfredi*, *M. birostris*, and *M. kuhlii*) was officiated in Mozambique in 2021 (Boletim da Republica 2021), however, *M. kuhlii* lacks protection in South Africa. Listing *M. kuhlii* as a protected species in South Africa would result in a tailored management strategies for its preservation, given that this species is globally Endangered (Rigby *et al*. 2020), has critical habitat within KZN as found in this thesis, and has had significant sightings declines in Mozambique (Rohner *et al*. 2017). In addition to species-specific measures, broader forms of conservation management can be implemented to protect mobulids and other important elasmobranchs.

The establishment of Important Shark and Ray Areas (ISRAs) at locations in southern Africa will enhance elasmobranch and mobulid conservation by focusing conservation efforts on specific areas that are critical habitat for these species. An ISRA is a designated geographical area that possesses substantial ecological importance due to factors such as breeding, feeding, or migration (IUCN 2022). By identifying and safeguarding these areas, ISRAs contribute to the effective management and protection of elasmobranchs. The findings from this thesis have already been incorporated into ISRA proposals, notably in the cases of the Aliwal Shoal MPA (‘The Greater Aliwal Shoal’) and the ISWP (‘Maputaland’). Once established, ISRAs will contribute to habitat protection, population recovery, reduced bycatch, ecosystem health, research, public awareness, policy advocacy, sustainable tourism, collaborative efforts, and long-term monitoring of these species.

Currently, South Africa's MPA network safeguards approximately 5% of the continental Exclusive Economic Zone (EEZ) through 38 MPAs (Department of Forestry, Fisheries, and the Environment 2021, Protected Areas Act 2014). The WildOceans’ systematic conservation plan for South Africa (Faure-Beaulieu *et al*. 2023) advocated for the need to increase 'no-take zones,' which bans all directed fishing methods. For instance, the *M. kuhlii* cleaning stations are located outside of the existing no-take zone within the Aliwal Shoal MPA, and therefore might be impacted by entanglement (Marine Living Resources Act No. 18 of 1998, Government Gazette No. 26433, South Africa 2004). Similar concerns extend to *M. birostris*, with just 14% of its home range in South Africa currently having any form of protection, with *M. alfredi* and *M. kuhlii* being unknown (Faure-Beaulieu *et al*. 2023). The lack of habitat protection is further compounded by MPAs that include the bather protection nets within their borders (e.g., Aliwal Shoal MPA, Trafalgar MPA), which inadvertently capture mobulids, emphasising the neeed for careful consideration of MPA definitions to avoid unintended impacts. The removal of the bather protection nets during peak mobulid season in KZN, in the summer months (Dec-Feb), or the use of sustainable alternatives (e.g., drones, Artificial Intelligence cameras, electromagnetic barriers, or manned searches) is recommended. As such it is imperative to expand the MPA network in South Africa, specifically targeting no-take zones that ban all types of direct and indirect catch, to meet the Ocean Economy and Sustainability Goals set by the United Nations for 2020. In conclusion, these concerted efforts, coupled with the continued research and management tactics advocated in this thesis, hold the potential to help drive the recovery of mobulid populations in southern Africa.

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