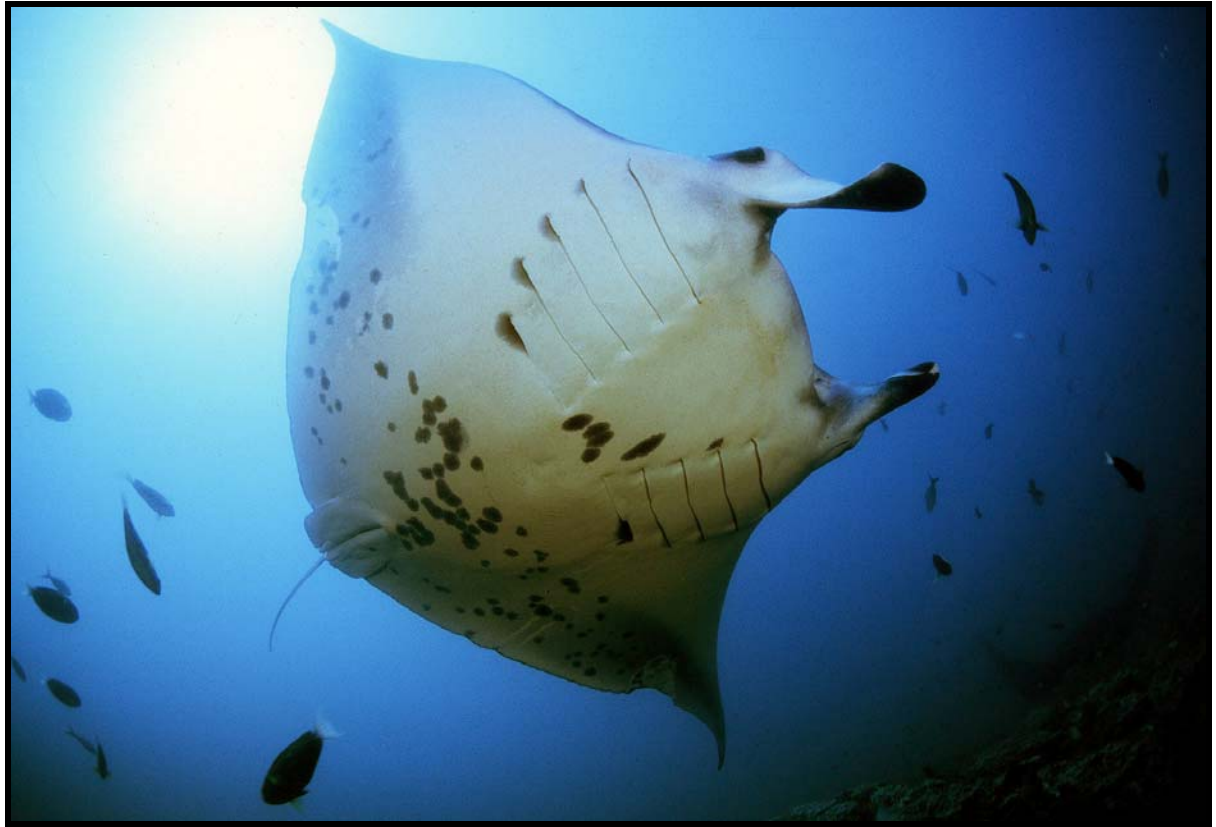# Biology and Population Ecology of *Manta birostris* in Southern Mozambique

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A thesis submitted for the degree of Doctor of Philosophy

at The University of Queensland, Australia in April 2008

The degree is conferred when the thesis has been accepted by the UQ Library

Declaration

I hereby declare that this work is original, except where otherwise acknowledged. The material presented herein has not been submitted in any form for another degree or diploma at any university or institution.

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Andrea Marshall
April 30th, 2008

Statement of contribution by others

Chapter 1

The introduction was my own work. Simon Pierce and my supervisor, Assoc. Prof. Mike Bennett proofread earlier versions of the chapter.

Chapter 2

The general methods chapter was my own work. Simon Pierce and my supervisor, Assoc. Prof. Mike Bennett proofread earlier versions of the chapter.

Chapter 3

This chapter was published in Zootaxa in 2008. I am primary author and Assoc. Prof. Mike Bennett and Simon Pierce are co-authors. I was responsible for sampling and dissection work, data analysis, interpretation of results and writing the manuscript. Simon Pierce assisted in the field with the dissection of the foetus and the formulation of new morphological measurements. Both co-authors also helped with the preparation and revision of the manuscript.

Chapter 4

Dr. Steven Taylor and Dharmadi both assisted with the collection of specimens in Indonesia. Dr. Geremy Cliff supplied specimens from the Natal Sharks board in Durban, South Africa for dissection. Chris Glen assisted with SEM scans. Tom Kashawagi assisted with the data collection for the distribution map. Simon Pierce and my supervisor, Assoc. Prof. Mike Bennett proofread earlier versions of the chapter.

Chapter 5

From October 2005 Simon Pierce periodically assisted me on dives in the field helping to contribute to the photographic database in Mozambique and take genetic samples. Additionally I had four student volunteers assist with field work in 2006 and 2007. Christine Dudgeon assisted with some of the population modeling using program Mark. Christine Dudgeon, my supervisor, Assoc. Prof. Mike Bennett, and two anonymous reviewers from Marine Biology improved upon earlier versions of the chapter.

Chapter 6

From October 2005 Simon Pierce periodically assisted me on dives in the field helping to contribute to the photographic database in Mozambique. Simon Pierce proofread earlier versions of the chapter.

Chapter 7

Simon Pierce assisted with the installation and maintenance of acoustic receivers in Mozambique and tagging of manta rays. I had four student volunteers assist me with data collection and data entry in 2006 and 2007. D. Steven Taylor assisted with the data analysis using Primer. My supervisor, Assoc. Prof. Mike Bennett assisted with cleaning methodology and proofread earlier versions of the chapter.

Chapter 8

Assoc. Prof. Mike Bennett, and two anonymous reviewers from Journal of Zoology improved upon earlier versions of the chapter.

Chapter 9

From October 2005 Simon Pierce periodically assisted me on dives in the field helping to contribute to the photographic database in Mozambique. Additionally I had four student volunteers assist with field work in 2006 and 2007. Christine Dudgeon assisted with some of the population modeling using program Mark. My supervisor, Assoc. Prof. Mike Bennett proofread earlier versions of the chapter.

Chapter 10

The discussion was my own work. Simon Pierce proofread earlier versions of the chapter.

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Moving to Australia many years ago to pursue my educational goals I happened to stumble upon the Bennett Lab. At a time where I didn't know a soul in the country, the members of this lab embraced me as friends and as colleagues. In all my life, I have never met such an honest, supportive, and fiercely intelligent group of people. With you, I learned the real meaning of teamwork and unconditional support. Through this process, I have stumbled many times but you have always been there to help me up. I am so honoured to have known and worked with each of you.

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x

Dedication

To the passionate scientists, the world over, who invest their lives to advance our understanding of the natural world and to those that dedicate themselves to preserving it.

List of publications & presentations relative to this thesis

Peer-Reviewed Publications

Marshall, A.D, Pierce, S.J. and Bennett, M.B. (Accepted Zootaxa) Morphological measurements of manta rays (*Manta birostris*) with a description of a foetus from the east coast of Southern Africa.

Marshall, A.D and Bennett, M.B. (Accepted with corrections Marine Biology) Size and structure of a photographically identified population of manta rays *Manta birostris* (Walbaum) in southern Mozambique.

Kashawagi T, Marshall AD, Bennett MB, Ovenden JR (in review Science) Ecological Speciation in Sympatric Cosmopolitan Manta Ray: Evolutionary and Demographic Consequences.

Marshall, A.D, Compagno, L.J.V. and Bennett, M.B. (In prep for Zootaxa). Redescription of genus *Manta* with the addition of two new species.

Other Publications or Contributions

Marshall AD, Ishihara H, Dudley SFJ, Clark TB, Jorgensen S, Smith WD, Bizzarro JJ (2006) *Manta birostris*. In: IUCN 2006. 2006 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 21 March 2008.

Conference Presentations/ Posters:

Marshall AD, Compagno LJV. Bennett MB. Redescription of the genus *Manta*. Joint Meeting of Ichthyologists and Herpetologists, American Elasmobranch Society, Montreal, Canada July 2008.

Kashawagi, T. Marshall AD, Ovendon J., Bennett MB. DNA evidences for cryptic species boundaries within *Manta birostris*. Joint Meeting of Ichthyologists and Herpetologists, American Elasmobranch Society, Montreal, Canada July 2008.

Marshall AD, Bennett MB. Cleaning behaviour of a photographically identified population of manta rays in southern Mozambique. Joint Meeting of Ichthyologists and Herpetologists, American Elasmobranch Society, Montreal, Canada July 2008.

Bennett MB, Marshall AD. Reproductive ecology of *Manta birostri*s. Joint Meeting of Ichthyologists and Herpetologists, American Elasmobranch Society, Montreal, Canada July 2008.

Marshall, A.D. and Bennett, M.B. Reproductive ecology of *Manta birostris*. Joint Meeting of Ichthyologists and Herpetologists, American Elasmobranch Society, New Orleans, July 12-17th, 2006.

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Abstract

Despite being the world's largest batoid fish, manta rays have been the focus of very little research in the last century. Almost nothing is currently known about their ecology or distribution throughout the world's oceans. Manta rays are considered to be near-threatened to vulnerable by the World Conservation Union's Redlist of endangered species, yet many populations still face threats from target fishing for their fin cartilage, branchial filaments and meat.

The taxonomic history of the genus *Manta* has been questionable and convoluted, with *Manta* having one of the most extensive generic and species synonymies of any living genus of cartilaginous fish. Currently this genus is considered to be monotypic with a single recognized species, *Manta birostris*. Behavioural and morphological data collected during this study showed that there are two distinct, sympatric species of manta ray in Mozambique. This discovery has led to an examination of manta ray distribution worldwide, with the two species hypothesis receiving further support from study sites in the Indian and Pacific Oceans.

This thesis examined large populations of both putative species in Mozambique. Development and application of a photographic-identification methodology allowed identification of individuals based on their unique ventral surface markings (spots). The ability to recognise individuals enabled many aspects of both populations to be examined.

A total of 449 individual rays were identified of *Manta birostris* variant A and 101 individual rays were identified of *Manta birostris* variant B. Both populations exhibited a highly significant female bias in observed sex ratios of 1:3.5 and 1:7.4 respectively. The photographic identification approach was used in studies of population size and structure and to examine the reproductive ecology of these two distinct populations. Population estimates incorporating four years of re-sighting data on *Manta birostris* variant A and five years of data on *Manta birostris* variant B were conducted using the program MARK resulting in super population estimates of 890 and 600 individuals respectively.

The region encompassing the study site was identified as a mating ground for *Manta birostris* variant A based on observations of mating events and fresh pectoral fin tip scars on female rays produced when the male rays bite onto the fins during copulation. The distribution of scars was highly biased, with 99% on the left pectoral fin, indicative of a strong lateralised behavioural trait in this species. No other elasmobranch has been reported to display behavioural lateralisation. The study region also acts as a birthing ground, with individuals giving birth in the summer after a gestation period of approximately one year. Reproductive periodicity in *M. birostris* variant A was most commonly biennial, but a few individuals were seen to be pregnant in consecutive years, confirming an annual ovulatory cycle. The production of a single pup appears to be the normal situation, although observations in the wild as well as during opportunistic dissections of individuals killed in fisheries revealed that two pups are conceived on occasion. Morphometric analysis of late-term foetus (*M. birostris* variant A) was contrasted with measurements taken from adult rays. These are the only detailed measurements on *M. birostris* variant A from the western Indian Ocean.

Predatory scarring and bite injuries on individuals of both variant A and B were consistent with attacks from sharks of various sizes. The frequency and effect of these predatory injuries on the two populations were examined over a three-year period.

Acoustic tags were attached to fourteen rays and their presence/absence at sites around the major inshore reefs was explored to evaluate how the rays utilised their environment on a temporal scale. Cleaning activity of both putative species of manta rays by small fishes occurred on these reefs and was considered to be the main reason why the rays were present at these sites. Parasite removal and wound healing were implicated as the benefits received. Host cleaner fish species partition the manta ray body to avoid interspecific competition while the two putative species appear to partition cleaning habitats, with very little overlap apparent.

This research on manta rays is the first of its kind in African waters. Many aspects of the study have contributed to the limited baseline data currently available for this genus. The study additionally provided sufficient empirical evidence to warrant the separation of these two putative species of *Manta* and a full revision of the genus with the systematic examination of specimens throughout *Manta*'s range. The results of

this study may be useful in this endeavour as diagnostic characteristics have been isolated that may help to differentiate members of this genus. The results of this research study are directly applicable to management strategies for both putative species of manta rays off the East Coast of Africa as well as populations worldwide and have highlighted the potential need for different conservation strategies.

Table of Contents

Chapter 5 Size, structure and survivorship estimates of a photographically identified population of *Manta birostris* variant A in southern Mozambique 70

Chapter 6 The reproductive ecology of *Manta birostris* variant A in southern Mozambique 102

Table of Figures

Chapter 6

Chapter 7

Chapter 9

Table of Tables

Chapter 3

Chapter 4

Chapter 1
Introduction

DISTRIBUTIONS AND GENERAL BIOLOGY

The family Mobulidae (devilrays) is currently divided into two genera, *Mobula* Rafinesque, 1810 and *Manta* Bancroft, 1828. While currently nine species are recognised in the genus *Mobula* (Notarbartolo-di-Sciara 1987b), the genus *Manta* is presently considered to be monospecific, represented by a single species, *Manta birostris* (Walbaum 1792) (Eschmeyer et al. 1983; Homma et al. 1999; McEachran and Aschliman 2004). Regarded to be one of the most evolved and highly derived of all living elasmobranchs (Compagno 1999), *M. birostris* is one of the last additions to the chondrichthyan fauna, with their first recorded appearance in the fossil record only five million years ago in the lower Miocene epoch (Bourdon 1999).

Manta rays exhibit a circumtropical distribution having been identified throughout tropical and semi-temperate regions of the north and south Atlantic, Pacific and Indian Oceans (Bigelow and Schroeder 1953; Last and Stevens 1994; Ebert 2003; Marshall et al. 2006). These pelagic rays are found coastally, as well as off oceanic islands and remote seamounts. The largest of all the living rays, they have been measured to reach disc widths (DW) of up to 7.1 m and estimated to reach up to 9.1 m DW (Bigelow and Schroeder 1953; Last and Stevens 1994; Alava et al. 2002). As a pelagic species, *M. birostris* swims in the water column rather than resting on the bottom like many other batoid species. Anecdotal reports and photographs indicate that they can cease swimming and rest on the seafloor (Notarbartolo-di-Sciara 1987a; Kuiter and Debelius 2006), however the lack of confirmed reports of this behaviour in the wild suggests that this may be a rare occurrence. Like all mobulids, *M. birostris* is predominately planktivorous and is widely known to forage at the surface of the ocean and at depth preferentially targeting zooplankton blooms such as euphausids (Bigelow and Schroeder 1953; Notarbartolo-di-Sciara 1987a; Homma 1999; Ebert 2003). Though manta rays are often observed swimming alone, *M. birostris* has also been documented to exhibit schooling behaviour, with as many as 50 manta rays seen swimming together at one time (Bigelow and Schroeder 1953; Homma 1999).

The typical dorsal colouration of manta rays is dark with white shoulder bars and/or whitish accents, while the ventral colouration is white with darkish spot patterning and edging. Observations worldwide have also revealed the presence of a widely distributed melanistic form of *M. birostris* (Barton 1948; Homma 1999; Rubin 2002;

Ebert 2003). This form is completely black on the dorsal surface with no white accents or shoulder bar colouration and is also predominantly black on the ventral surface with a variably sized white blaze along the midline. This melanistic form is widespread, with sightings reported from most oceans, though not all localities where manta rays occur (Barton 1948; Homma 1999; Ito 2000; Rubin 2002; Marshall et al. 2006). In most locations the melanistic form is rare, although in some regions black manta rays occur with greater frequency comprising up to 50% of observed populations (Barton 1948; Homma 1999; Ito 2000; Rubin 2002). A few sightings and images of albino manta rays have also been reported in some locations, including Indonesia and Malaysia (Ishihara et al. 2001). These rays exhibit an 'inverse' colouration, with an almost entirely pale colouration and black accents instead of white accents (e.g. shoulder bars) on their dorsal surface.

Aggregation sites for manta rays exist along coastlines or island groups that have high primary productivity. Manta rays predictably frequent feeding areas and cleaning stations at these locations. A limited number of long-term photographic surveys have been underway in the last 30 years at such aggregation sites including in the Yaeyama Islands in Japan, the island of Yap in Micronesia, the Maldives, the Hawaiian islands and the Revillagigedo Archipelago in Mexico (Homma et al. 1999; Ito 2000; Clarke 2002; Rubin 2002; Marshall et al. 2006, Kitchen Wheeler 2008). Preliminary observations at these and other aggregation sites suggest that manta rays may be resident to or at least philopatric for specific areas (Notarbartolo-di-Sciara and Hillyer 1989; Homma 1999; Marshall et al. 2006, Dewar et al. 2008; Luiz et al. 2008). However some individuals may still undergo long migrations, with tagged or photographically identified individuals reported to undertake journeys of up to 350 km (Homma 1999).

Few notes or scientific papers have been published on the biology or behaviour of manta rays in the last two decades (Notarbartolo-di-Sciara and Hillyer 1989; Nishida 1990; Last and Stevens 1994; Ishihara and Homma 1995; Alexander 1996; Homma 1999; Yano et al. 1999; Clarke 2002; Dewar 2002; Duffy and Abbott 2003; White et al. 2006; Sleeman et al. 2007; Clark et al. 2008; Kitchen-Wheeler 2008; Marshall et al. 2008; Dewar et al. 2008; Luiz et al. 2008). In spite of these aforementioned contributions, most of the available life history data for *M. birostris* are rough

estimates made from observations at aggregations sites, and fragmentary data from opportunistic dissections (Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953; Homma 1999; White et al. 2006; Marshall et al. 2008). The longevity of this species is unknown and has not been examined, size and age of maturity, the length of their reproductive cycle and natural mortality rates remain unclear. Such basic life history information is crucial to the formulation of effective management strategies for this species.

STUDYING PELAGIC ANIMALS IN THE FIELD

Shark and rays are sensitive to anthropogenic influences due to life history traits, such as low fecundity, slow growth, late age of maturity, and low natural mortality (Dodd 1983; Hoenig and Gruber 1990; Hamlett 1999; Heithaus 2004; Stevens et al. 2005; Garcia 2008). Unlike teleosts, which may recover quickly from periods of over-fishing, sharks and rays typically recover slowly from severe or sustained fishing pressure (Hoff and Musick 1990; Stevens et al. 2005). Consequentially, it is imperative to acquire enough data on their life history parameters, habitat usage, and movement patterns to properly manage and conserve stock populations. Unfortunately, acquiring these data is often difficult, particularly for large, rare or elusive species. For example, while there are numerous reports on the reproductive biology, anatomy, and development of elasmobranchs (Wourms 1977; Dodd 1983; Hamlett 1999, Carrier et al. 2004), very few field studies have been conducted on reproductive behaviour (review in Pratt and Carrier 2001). Currently, most of what we know about reproductive behaviour comes from fortuitous observations by recreational divers, aquarists or biologists of mating activity either in the field or in captive environments (Pratt and Carrier 2005). While these observations provide some insight into various aspects of mating behaviour, they often fail to contribute detailed quantitative information on strategies, social interactions, sexual selection, mating seasonality and success rates. The most comprehensive review of elasmobranch reproductive behaviour to date assembled and analysed a wide range of accounts of reproductive behaviour in order to identify similarities in the behavioural strategies of different species (Pratt and Carrier 2005). Stereotypical behaviours included, harassment and biting behaviours (which were hypothesised to trigger the

cooperation of the female), increased swimming activity in males during breeding seasons, social mating hierarchies, sexual segregation and multiple suitors. While these trends provide only a preliminary understanding of the behavioural ecology of this diverse group of fishes, they can be used to shape the direction of future studies on elasmobranch reproductive behaviour; which includes more focused research on sexual selection, polyandry, philopatry and dominance hierarchies. These topics are of significant interest to biologists as they can affect the reproductive fitness of populations, genetic diversity and gene flow within and between populations (Pratt and Carrier 2005).

Like many shark and ray species, little is currently known about the reproductive ecology of *M. birostris*. Dissections and internal examinations have revealed that manta rays have definitive lipid histotrophic development (Pratt and Carrier 2001; Hamlett et al. 2005), where embryos are nourished in the uterus with a viscous liquid hisotrophe, rich in proteins and lipids (Mitchill 1824; Coles 1916; Wourms 1977; Pratt and Carrier 2001; Hamlett et al. 2005). After an unconfirmed gestation period in the wild, manta rays give birth to live young, most commonly a single large offspring (Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953). Little information is currently available on the reproductive behaviour of either male or female manta rays. However, it has been established that single or multiple male rays will pursue a single female ray during courtship and will often bite the pectoral fin of the female ray to facilitate clasper insertion during copulation (Yano 1999). Manta rays mate belly to belly and copulation is brief, lasting usually less then one minute (Yano 1999). Beyond these limited details, information on mate selection, mating frequency, polyandry, and reproductive periodicity remain unknown.

Determining how a population uses different habitats and how they spend their time or energy budgets is another area of study that requires extensive field observation and experimentation. The term 'home range' was defined by Morrissey and Gruber (1993, pg. 425) as 'the spatially and temporally defined area over which an animal travels while engaged in its normal activities'. Critical habitats are areas within an animal's home range, which the animal frequents to perform important rituals. These can be areas that are visited daily by individuals, such as cleaning stations or foraging grounds. They also may be areas where individuals seasonally congregate to mate and

give birth or corridors that are regularly passed through during migrations. Critical habitats are usually subject to either more frequent use than other sites or have increased numbers of individuals using them. Access to these sites and safety within them may be critical to an individual's fitness and survival. Failure to identify or protect these areas may jeopardise the survival of a population as a whole or their ability to persist in a particular region (Powles et al. 2000).

Many elasmobranch species have distinct home ranges during various stages of their lives (Holland et al. 1993; Morrissey and Gruber 1993; Holland et al. 1999; Hueter et al. 2005). Even species that exhibit migratory behaviours may be philopatric to particular areas. Philopatry is the tendency for individuals to frequent or return to a site or region, and may include areas such as nursery grounds, mating grounds, and specific cleaning and feeding sites (Hueter et al. 2005). Research demonstrating the philopatric nature of some shark species has confirmed that individuals of some species do often return to regions seasonally or cyclically (Hueter et al. 2005). *Manta birostris* may be one such species, with identified individuals regularly occupying critical habitats or aggregation sites during their lifetimes (Homma 1999; Marshall et al. 2006; Kitchen-Wheeler 2002).

Aggregation sites or critical habitats often serve as focal areas for research, with tagging programs employed to quantify habitat usage, demonstrate philopatry and investigate spatial/temporal distributions (Gruber et al. 1988; Casey and Kohler 1992; Holland et al. 1992; Holland et al. 1993; Goldman and Anderson 1999; Silliman and Gruber 1999; Sundström et al. 2001; Latour 2004). Telemetry studies are currently being used to examine everything from the temperature and depth profiles of individual dives to the large-scale or seasonal movements of individuals in studied populations. Tracking technology and methodology has been refined over the years making it possible to track the fine scale movements of animals, define home ranges, and examine the roles of 'critical habitats' (Nelson 1990; Holland et al. 1993; Gruber et al. 1998; Goldman and Anderson 1999; Silliman and Gruber 1999; Pratt and Carrier 2001; Sundström et al. 2001; Voegeli et al. 2001; Hueter et al. 2005). Determining how manta rays use critical habitats and investigating trends in their fine scale movements may prove vitally important to the implementation of effective management strategies.

CURRENT THREATS TO MOBULID RAYS AND CONSERVATION

As human pressures have increased worldwide, shark and ray populations have declined in abundance in areas where they were once common (Baum et al. 2003; Baum and Myers 2004). Currently over 21% of the species assessed by the Shark Specialist Group (SSG) of the International Union for the Conservation of Nature (IUCN) are listed as threatened (from vulnerable to critically endangered) (IUCN/SSG unpublished data). Batoids are among the most sensitive and threatened species in the oceans (Last 2007, Garcia 2008) and some sawfish and skate species have been documented succumbing to extreme habitat constrictions and intense fishing pressure (Brander 1991; Casey and Meyers 1998; Simpfendorfer 2000; Stevens et al. 2000).

Mobulid rays may be particularly susceptible to population crashes as their fecundity is amongst the lowest of all elasmobranch species (Dodd 1983; Dulvy et al. 2003; Dewar 2002; Musick and Ellis 2005; Walker 2005; White et al. 2006, Garcia 2008). Used for their meat, skin, and cartilage, these rays are targeted in largely unquantified amounts in many areas of their distribution. Extrapolations from large-scale fisheries data in Mauritania between 2001-2005 suggest that between 120-620 mature mobulid rays were caught as by-catch per annum, an amount that is unlikely to be sustainable over time (Zeeburg et al. 2006). In other areas like Mexico, Indonesia and the Philippines as many as 2,400 mobulid rays (including manta rays) per season have been reportedly caught in directed fisheries and as by-catch (Alava et al. 2002; Dewar 2002; Marshall et al. 2006; White et al. 2006). Target fishing for these rays has increased by an order of magnitude in some areas in the last decade to levels that are deemed unstainable (Dewar 2002). In what was hypothesised to be a direct response to large-scale directed fishing efforts in the Philippines, the size of the local manta ray population crashed, with decreases in reported catches of approximately 95% from one year to the next (Alava et al. 2002). As a result, the local fishing industries in these areas have expanded beyond traditional fishing sites in order to find manta rays to supply Asian demand (Dewar 2002; Alava et al. 2002). Increased rates of harvest are thought to reflect both an increase in fishing effort due to technological advancements in fishing techniques and the increasing demand for mobulid products in Asia, particularly Hong Kong (Dewar 2002; Alava et al. 2002; White et al. 2006). In many surveyed areas, mobulid fishing has shifted from local subsistence fishing to

commercial ventures for trade. Most survey work also suggests that local manta ray populations are being heavily affected by this increase in fishing activity, which has resulted in serial depletions in targeted areas (Dewar 2002; Alava et al. 2002; Marshall et al. 2006). While continued assessment is needed, it is not likely that any mobulid population could sustain long-term or intense fishing pressure and there looms a genuine threat of the localised extinction of certain populations (Marshall et al. 2006).

While *M. birostris* certainly faces many anthropogenic threats, the severely limited body of information on their ecology continues to hamper the accurate assessment of their conservation status. In 2002, this absence of data caused the IUCN to catalogue *M. birsotris* as 'Data Deficient' on their international RedList of threatened species. In 2004, in the absence of solid life history data on this species, *M. birostris* was re-classified as 'Near Threatened' worldwide and 'Vulnerable' in four specific sites: the Gulf of California, the West Coast of Mexico, the South China Sea and the Sulu Sea where directed fisheries and bycatch information was available (Marshall et al. 2006). However, continued assessment is necessary that takes into account the life history parameters of this species.

Unfished manta ray populations in many areas of the world appear to be relatively stable (Marshall et al. 2006). Manta rays do receive limited protection in some marine sanctuaries such as the Komodo National Park, established in 1980, the Revillagigedo biosphere, in Mexico which was instated in early 2002 and in Hawaii, where legislative protection for manta rays from fishing or intentional harm was passed in 2006 (House Resolution No. 30) (Marshall et al. 2006). At large aggregation sites, such as the Hawaiian Islands, the island of Yap, Japan's Yaeyama Islands, Mexico's Revillagigedo Archipelago, and the Maldives, localised efforts exist to protect marine life, such as manta rays, from directed fishing and other anthropogenic pressures (Ishihara and Homma 1995; Alava et al. 2002; Sala et al. 2002; Marshall et al. 2006). In return, stable ecotourism industries have developed based largely around viewing these rays in their natural environment. Marine eco-tourism has proven to be an excellent way of achieving a lucrative, sustainable balance between protecting animal populations and creating an economically viable form of tourism (Homma et al. 1999; Anderson 2002; Clarke et al. 2005). Surveys in the Maldives have reported that the

local economy generates approximately $7.8 million US dollars in combined revenue from manta ray related activities in their islands (Clarke et al. 2005). The potential return to the local community is therefore many orders of magnitude larger than the one time price generated by killing these rays (Homma et al. 1999; Alava et al. 2002; Dewar 2002; Marshall et al. 2006; White et al. 2006).

AIMS OF THIS STUDY IN AFRICA

The African continent is still relatively unexplored. Thirty-two countries contribute to Africa's 40,000+ kilometres of coastline. Presently these countries are contending with both the incredible wealth of available resources and the fragile nature of their coastal ecosystems. Coastal development often leads to the degradation, exploitation, and pollution of marine habitats particularly mangroves, bays, and in-shore coral reefs (Grosholz 2002; de Jonge et al. 2002; Lotze et al. 2006). The adverse impacts of such unsustainable activities can be both swift and irreversible. The loss of critical habitats or regional extinctions of species are some of the immediate and lasting effects of the mismanagement of coastal resources (Lotze et al. 2006).

To date there have been no attempts to study wild populations of *M. birostris* in Africa, particularly along the eastern coastline where they are known to abundantly occur. Mozambique, a country situated along Africa's south-eastern coast boasts one of the longest coastlines of any African nation and yet is relatively undeveloped and unexplored. A fast growing tourism industry, which is in part reliant on the frequent sightings of manta rays, has put the southern coast of Mozambique on the map as a world diving destination. As a result, the development of this coastline has been swift and often hasty without the impacts of such rapid expansion properly assessed.

The aims for this study included:

1) To development standardised morphological measurements for this genus using specimens of *M. birostris* caught as by-catch or in fisheries (Chapter 3).

2) To determine if minimally intrusive photographic techniques could provide the necessary backbone to examine a population of manta rays at a well-known aggregation site in southern Mozambique. (Chapter 5).

10

3) To ascertain population estimates along this stretch of coastline and track population composition seasonally. To determine if this population is closed or open with respect to movement and to establish if individuals are philopatric to specific areas of the coastline (Chapter 5).

4) To opportunistically examine reproductive parameters for both males and females using both field photography and available specimens (Chapter 6).

5) To establish how and when manta rays are using near-shore environments, specifically heavily frequented critical habitats such as cleaning stations (Chapter 7).

6) To describe cleaning interactions and behaviour of both host cleaner fish and manta rays at coastal cleaning stations (Chapter 7).

During the course of this study other topics of interest arose that were simultaneously examined using the same or similar field techniques. They included: the first examination of the predation rates of sharks on an identified population of manta rays (Chapter 8), the separation and comparison of two putative species of *Manta* previously referred to as a single species, *Manta birostris* (Chapter 4), and a full comparative account of the population structure and ecology of the second variant manta ray in Mozambique (Chapter 9). Each chapter is structured to stand-alone, with its own introduction, material and methods, results, and discussion sections. However to prevent unnecessary redundancy, a general methods chapter was constructed that highlights methodology used for most of the studies included in this thesis, as well as provides common site descriptions.

This research on manta rays is the first of its kind in African waters. Additionally many aspects of the study contribute the first information of its kind for the genus *Manta*. The results of this research study are directly applicable to management strategies for manta rays off the East Coast of Africa as well as populations worldwide. To date, no comprehensive management strategy exists for this species in any part of their distribution. For a large, potentially migratory animal, like a manta ray, the formulation of such a plan is essential. The implementation of a management plan in Eastern Africa is of particular importance because of rising fishing pressures from both unregulated international fishing operations and local fisheries. Beyond

local protection in southern Africa, information gleaned from this study will contribute to the limited baseline data currently available for both putative species of *Manta*. Such data may be used to better assess their conservation status or if necessary enhance their eligibility for CITIES (Convention on International Trade in Endangered Species or Wild Fauna and Flora) listing, a means to restrict the international trade of manta ray parts or products.

Chapter 2

General methods

Manta rays off the coast of Inhambane, Mozambique were observed at various inshore sites from May 2003 to March 2008. Data and observations for the study were not collected simultaneously, rather different sections of the study were undertaken during different years and at different times of the year. Each sub-section specifically states the time frame over which particular data were collected.

The primary field site for this study was a 250 m^2 sub-tropical rocky reef with isolated hard corals and patches of soft corals in 20 - 25 m of water, located approximately 22 km south of Inhambane harbour in Southern Mozambique (22.5^o S, 300° E) (Fig. 2.1). This reef is known locally as Manta Reef. A specific route or transect was used to examine the three manta ray cleaning stations (Chapter 7) along this stretch of reef. This transect was approximately 400 m in length and enabled the diver to observe each of the stations in their entirety and thereby count and identify all individual manta rays using these habitats. A second, narrow, reef plateau of approximately 400 m^2 in 25-32 m of water was located 11 km south of the harbour and was also monitored when conditions allowed. This secondary site is known locally as Giant's Castle. The transect for this reef ran east to west and was also approximately 400 m in length. The designated route for this reef also allowed divers to pass by both of the cleaning stations along this stretch of reef and count and identify the individuals using these habitats.

The coastline south of the Inhambane harbour mouth is largely comprised of long sandy surf beaches punctuated by rocky headlands. Both of the study site reefs are located close to shore; Manta Reef being approximately 1.9 km from the nearest point of land and Giant's Castle slightly under 2.3 km. Water temperature off the coast varies seasonally with surface temperature reaching a high of 32° Celsius in the summer months between December and February and dropping as low as 16° Celsius in the winter months between June and August. In addition to the clear seasonal variation in water temperature, there were also quite large variations in monthly, weekly, and daily water temperatures, with both surface and bottom (between 20-30 m) temperatures fluctuating as much as 4-5° Celsius in a 24-hour period.
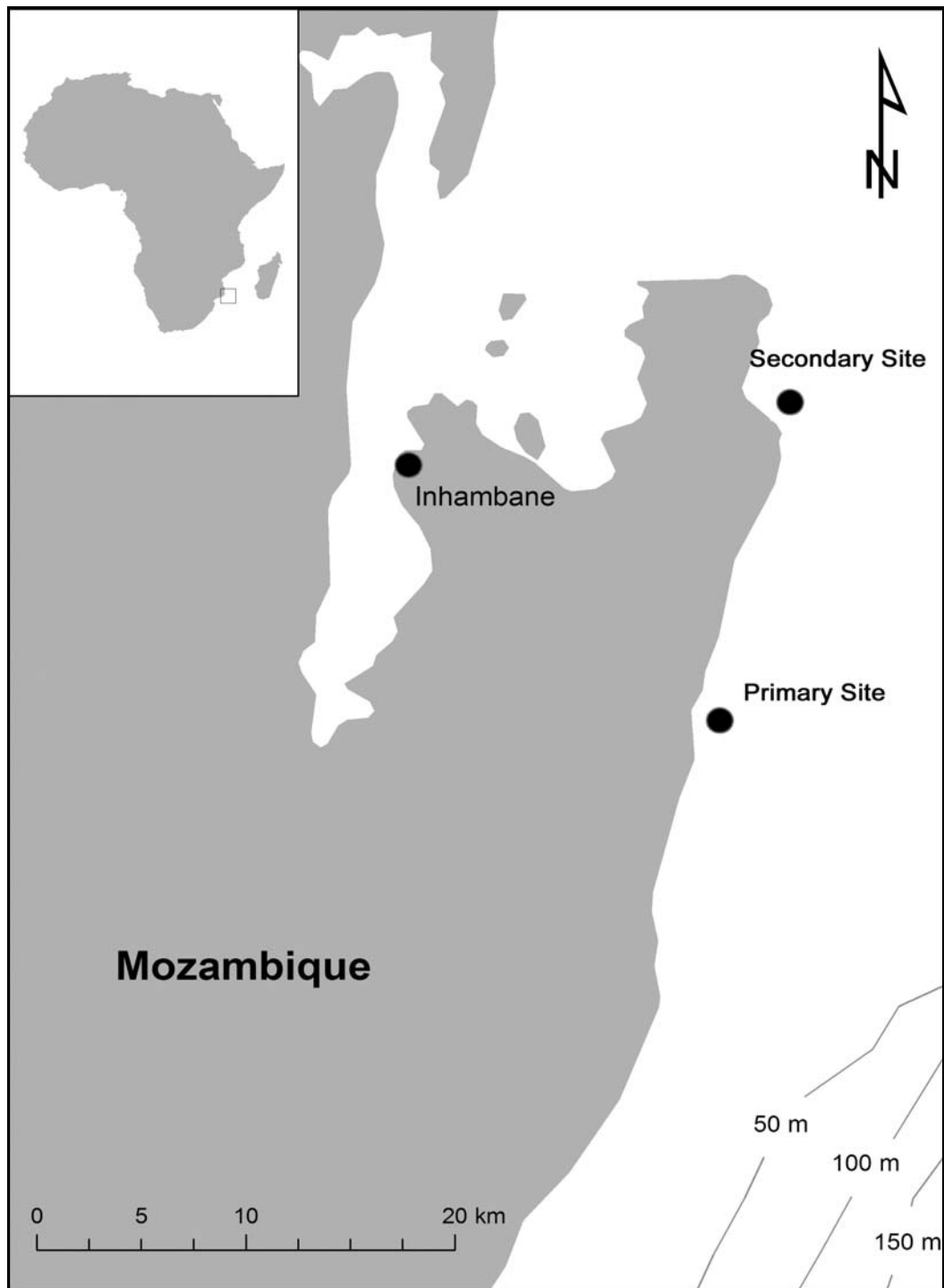
Figure 2.1 Location of study sites off the coast of southern Mozambique

The Mozambican Channel, the body of water between mainland Africa and the island of Madagascar, (Fig. 2.1) is heavily influenced by the Agulhas current system, which penetrates from the north of the channel (de Ruijter et al. 2002). Within the channel, particularly along the eastern Mozambican side, eddies are the major contributor to the southward bound circulation in this region and may account for nutrient rich upwelling along this stretch of coastline (de Ruijter et al. 2002). Many planktivorous species of megafauna utilise this region including whale sharks, humpback whales, and mobulid rays.

The coastal waters off Inhambane were found to be nutrient rich and productive, evidenced by the regular occurrence of both phydoplankton and zooplankton blooms. Planktivorous species of megafauna like the whale sharks and the manta rays were seen to actively feed during the day on zooplankton blooms off many of the rocky headlands that jutted out from the sandy dune coastline. As a result of these regular blooms, the water visibility along the coastline was variable. Horizontal water clarity ranged from 3-30 m throughout the year and would often change dramatically from day to day.

Strong currents were common along the coast both on the surface and at depth. Many of the dive sites could not be dived safely in strong currents and observational dives were occasionally called off as a result of them. Divers and the research vessel also had to contend with surge, large swell and high winds which also sometimes restricted launching or diving. Approximately eight days of every month were called off because of rough seas, dangerous surf launching conditions, or hazardous diving conditions.

Divers were transported to the field sites in a semi-rigid inflatable boat that was launched from the shore. All sites were located using GPS coordinates as none of the reefs along the coastline had marker bouys or moorings associated with them. Observations, data and samples were made or collected exclusively while on SCUBA except for a two week period in November 2006 when divers used fully closed rebreather units (AP Valve Inspiration).

Opportunistically, manta rays caught by local fishermen were examined on the beach before they were processed or before the tide washed them away. Morphological measurements were attempted if rays were not too badly decomposed or dismembered. If time allowed, these specimens were examined both externally and internally and samples were taken. Skin, denticles and tooth band samples were preserved in 70-90% ethanol. Stomach contents and parasite samples were stored in 70% ethanol. Sections of vertebrae extracted from individuals were frozen.

Observations and data collected in the field in Mozambique revealed distinct differences between two 'colour morphs' of *Manta birostris*. Further investigations indicated the broader applicability of these observations. The two putative species are described and contrasted in detail in Chapter 4, but as full taxonomic re-descriptions were still ongoing at the time of submission, they are referred to in the remaining chapters as *Manta birostris* variant A and *Manta birostris* variant B. This thesis focuses on the biology and ecology of *Manta birostris* variant A in southern Mozambique. However, a comparative study on the observed population of *Manta birostris* variant B in southern Mozambique is also included in this thesis (Chapter 9).

In order to collect comparative data, including morphometric and meristic measurements of both putative species, field trips were made to Mexico, South Africa and Indonesia. Trips to the Revillagigedo Archipelago, Mexico were made in November 2006 and 2007. Observations were conducted at both San Benedicto Island (19° 19" N, 110° 49" E) and at a small isolated pinnacle named Roca Pardita (19° 00" N, 112° 04" E) (Figure 2.2). All manta rays observed at this location were *Manta birostris* variant B.

Field trips to Umhlanga Rocks, South Africa were undertaken in June and November 2004 and August 2006. Manta rays incidentally caught in a bather protection netting network off Durban, South Africa, from Richards Bay (28°48'S, 32°6'E) in the north to Port Edward (31°2'S, 30°14'E) in the south were examined courtesy of the Natal Shark's Board (Fig. 2.3). Individual rays that had died in the nets and could not be released were frozen and later thawed prior to measurement and dissection. All manta rays observed at this location were *Manta birostris* variant A.
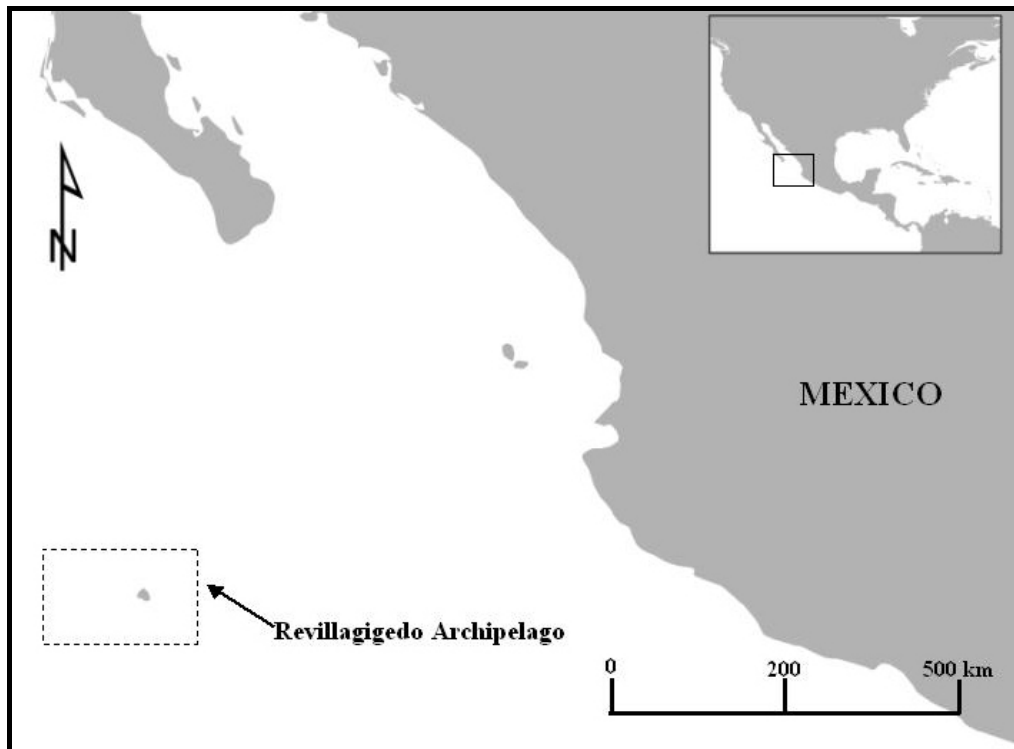
Figure 2.2 Location of study region off the coast of western Mexico

Figure 2.3 Area of bather protection netting network off Durban, South Africa.

A fieldtrip to the islands of Bali and Lombok, Indonesia was made in May 2007. The main landing site on the eastern side of Lombok was Tanjung Luar (8° 47" N, 116° 30" E) on the southeastern side of the island (Figure 2.4). We concentrated our efforts at this fishery as all of the manta ray specimens examined were *Manta birostris* variant B. All manta rays observed were caught south of Tanjung Luar in the southern end of the Alas Strait (the channel between the islands of Lombok and Sumbawa).
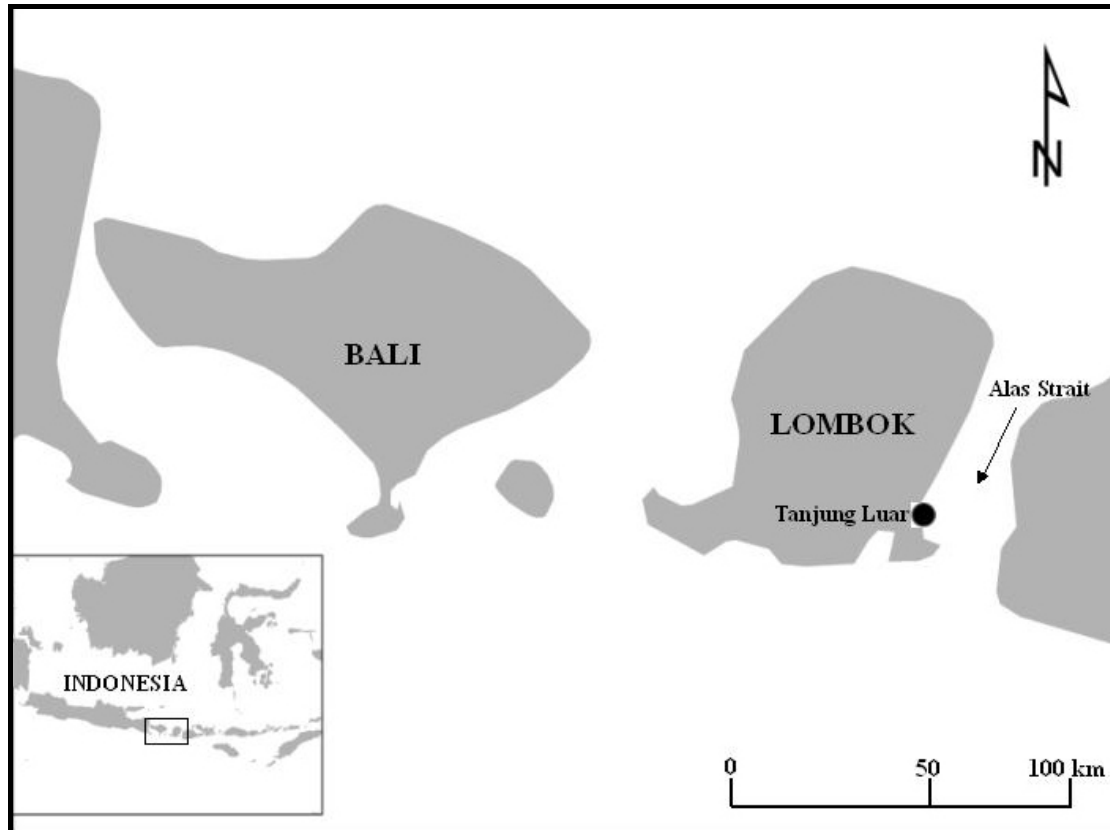
Figure 2.4 Location of landing site and fishing camp, Tanjung Luar, on the south-eastern side of the island of Lombok, Indonesia.

Chapter 3

Morphological measurements of manta rays (*Manta birostris*) with a description of a foetus from the east coast of Southern Africa
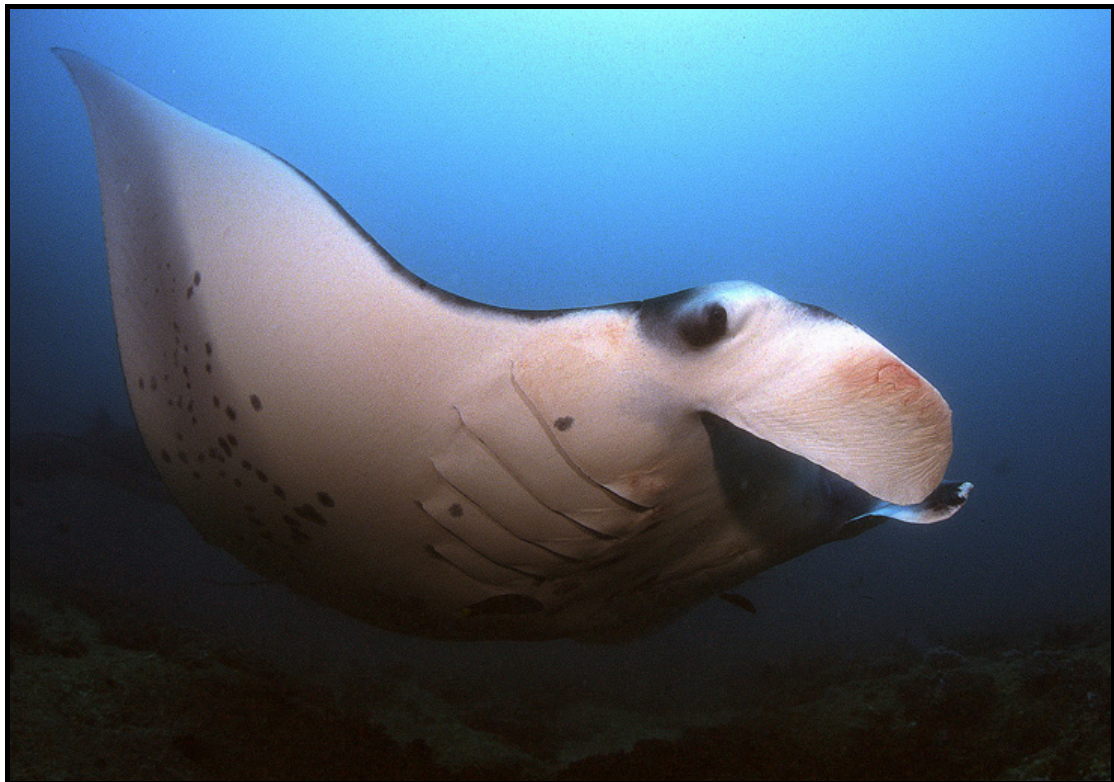
Note: Manuscript was submitted before taxonomic evaluation of putative species was assessed (Chapter 4). All specimens reported in this chapter as *Manta birostris* are *Manta birostris* variant A.

INTRODUCTION

The manta ray (*Manta birostris* Walbaum 1792) is the largest batoid species and one of the largest of all living fish. Data on the life history and ecology of *M. birostris* remain scarce. Few comprehensive morphological descriptions exist for juvenile or adult *M. birostris* (Whitley 1936; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953) and previous descriptions of foetuses have either relied on estimated body sizes and dimensions or have been based on incomplete or non-standard morphometric measurements (Lamont 1824; Lesueur 1824; Müller and Henle 1841; Coles 1916). The most detailed morphological description of a manta ray foetus to date comes from a specimen caught at the Galapagos Islands in 1928 (Beebe and Tee-Van 1941). This study provides the first detailed descriptions of *M. birostris* from the western Indian Ocean, including a near-term foetus, and updates the literature with detailed morphometric measurements appropriate for this species.

METHODS

Three juvenile *M. birostris* specimens were obtained from the Natal Sharks Board (NSB) bather-protection netting network off Durban, South Africa, from Richards Bay (28°48'S, 32°6'E) in the north to Port Edward (31°2'S, 30°14'E) in the south, between April and August 2006. Once landed, specimens were transported to the NSB headquarters, frozen and stored. Specimens were then thawed for 24 hours before dissection. The foetus was obtained from a pregnant female speared by fishers on 5 October 2005 off Paindane beach, Inhambane Province, Mozambique (24º05'568"S, 35º30'304"E). Local fishermen processed the female ray immediately after it was beached, thus it could not be examined. The body cavity had been opened and a single male foetus removed and placed on the sand. The foetus was transported to a local resort freezer for storage and was fully thawed for examination on 15 October, 2005. The carcass was disposed of after dissection.

External morphometric measurements were taken using a measuring tape (± 1 cm) and callipers (± 0.01 cm). Body and liver masses were measured using a spring-balance (± 0.1 kg in Mozambique and ± 1 kg in South Africa). Measurements followed standards used in Notarbartolo-di-Sciara (1987b), Compagno (2001) and Manjaji-Matsumoto and Last (2006) with the addition of three characters. Pectoral fin measurements P1, P2 and P3 were added to describe attributes of the pectoral fins (Table 3.1, Fig. 3.1a). All three of the new measurements were taken from the pectoral fin tip (a), with P1 being the shortest distance from that point to a line connecting the head-pectoral fin junction (b) and the most posterior point of the pectoral fins (c), P2 was the distance a – c and P3 the distance a – b. Due to the curled position of the pectoral fins, disc length (DL), measured from the tip of the snout to the posterior extent of the pectoral fin, was chosen over DW for standardised comparisons between individuals (Notarbartolo-di-Sciara 1987b). Reproductive state was assessed following Walker (2005).

RESULTS

South African Specimens

South African specimens were all described externally before morphological measurements were taken or dissections were made. The dorsal surfaces were black in colouration with two pale patches on the dorsal medial surface (Fig. 3.2a) and on the posterior halves of the pectoral fin tips. The ventral surface of all specimens were cream-coloured with small black spots between the gill slits, centrally above the cloaca and extending outwards for approximately half of the posterior section of the pectoral fin (Fig. 3.2b). All specimens exhibited small, dark, semi-circle shaped patches of colouration posterior to both the fifth gill slits. Light charcoal-coloured streaks were present along the posterior edges of the pectoral fins. All umbilical attachments were entirely closed and whitish in coloration. Tails were thin, with black colouration on the dorsal surface and cream on the ventral surface. No caudal spines were present (Fig. 3.2c), but small depressions were found at the base of the tail. Dermal denticles were evenly distributed across the disc. Eyes with distinctly blue irises. Cephalic lobes rolled spirally outwards (bases of the lobes rolling
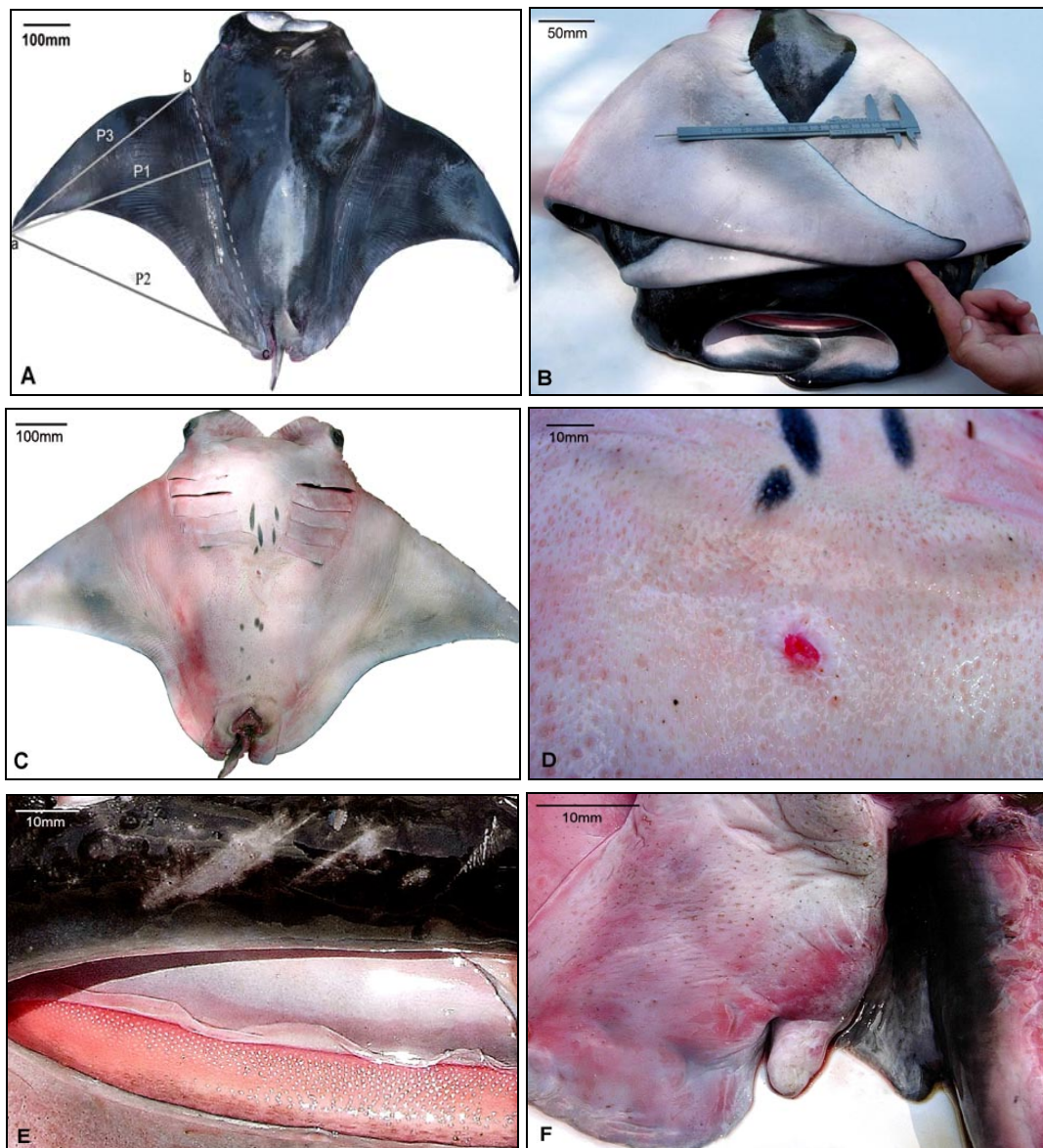
Figure 3.1 Male manta ray (*Manta birostris*) foetus collected from Paindane Beach, Mozambique: (a) dorsal view showing pectoral fin measurements P1, P2 and P3; (b) anterior view showing dorsally folded pectoral fins; (c) ventral view showing natural markings; (d) open umbilical scar; (e) detail of teeth on lower jaw; (f) clasper.

outwards). Lower jaws possessed tooth bands ranging from 58-77% of the width of the mouth. The upper jaw was edentate.

The three South African specimens ranged in size from 2230-2370 mm disc width (DW) and were 71-75 kg in mass. Detailed morphometric measurements are listed in Table 3.1. The single male examined had small, uncalcified claspers. Internal examination revealed that all three rays were immature as they exhibited

undifferentiated reproductive organs. The spiral valves had 44 turns. The livers were bi-lobed, pink in colour with masses of 3.9 kg, 5.3 kg and 6.4 kg (2370 mm DW female, 2230 mm DW female and 2230 mm DW male, respectively).

Mozambican Foetal Specimen

The pectoral fins of the foetus were folded dorsally over the body (Fig. 3.1b). Uncurling the pectoral fins facilitated further observations and morphometric assessment. The ventral surface was cream-coloured with small black spots on the mid-ventral surface from between the gill slits to above the cloaca (Fig. 3.1c). Small charcoal-coloured semi-circular patches were present posterior to both the fifth gill slits (Fig. 3.1c). Faint charcoal-coloured streaks were present along the posterior edges of the pectoral fins. There was a pink tinge to the ventral surface. The dorsal surface was black with paler coloration on the pectoral fin tips (Fig. 3.1a). Other small pale patches on the mid to rear dorsal surface were noted where black pigment had clearly been removed. The anterior margins of the cephalic fins were grey to black in colour, with grey tips on the medial surface. The umbilical attachment was open, 10 mm in diameter and red in colour (Fig. 3.1d). The tail was long and unbroken. No caudal spine was present at the base of the tail. Dermal denticles were patchily distributed on both the dorsal and ventral surfaces of the body, with slightly larger denticles on the ventral surface. Denticles were sparse on the pectoral margins on both surfaces. The lower jaw possessed a fully formed tooth band (Fig. 3.1e).

The foetus measured 1328 mm straight-line disc width (DW) and weighed 15.1 kg. Detailed morphometric measurements are listed in Table 1. The liver mass was 1100 g. The iris was blue in colouration. Claspers were small and uncalcified (Fig. 3.1f). No internal yolk sac was present. The spiral valve had 44 turns.
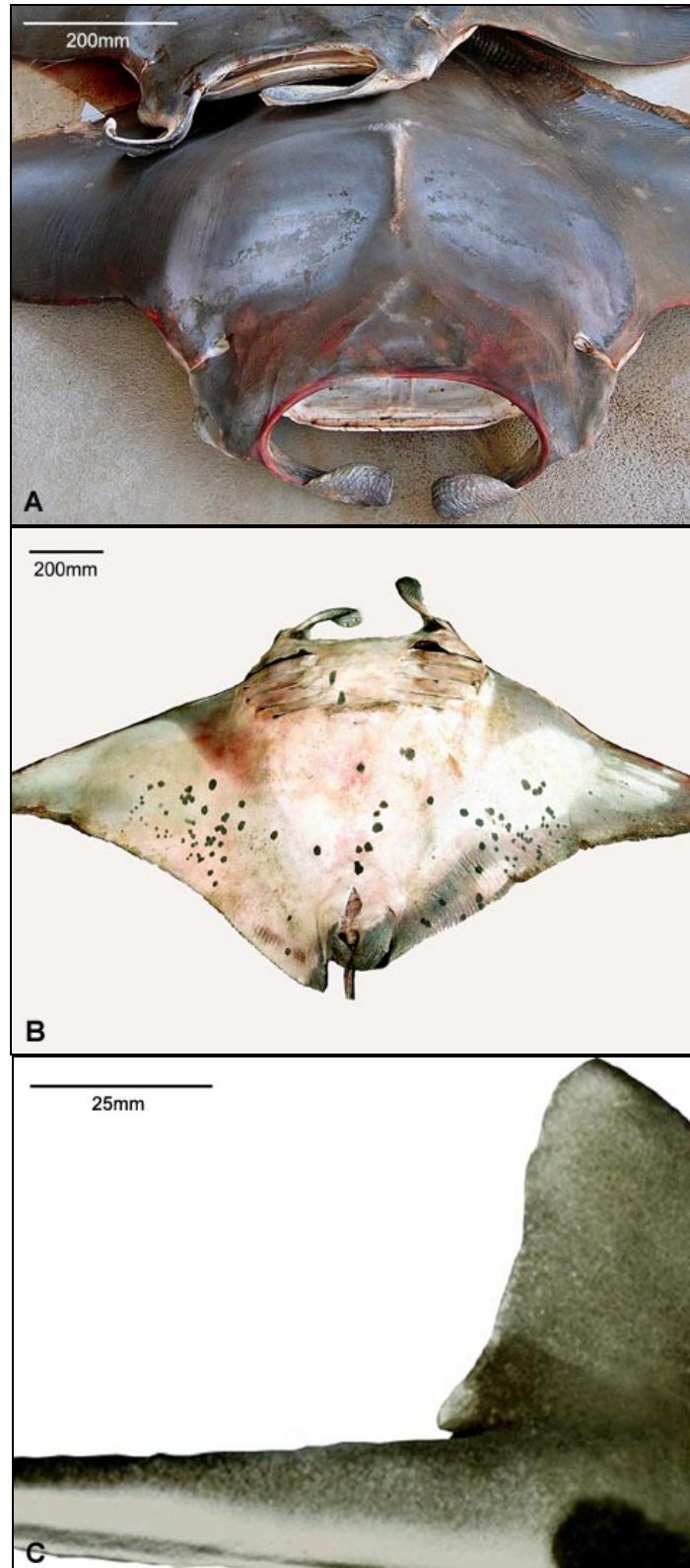
Figure 3.2 Juvenile female manta ray (*Manta birostris*) from Durban, South Africa: (a) anterior dorsal coloration; (b) ventral view showing natural markings; (c) lateral view of dorsal fin and base of tail.

Table 3.1 Morphometric measurements of Mozambican *Manta birostris* foetus and South African specimens.

	Mozambique		South Africa		South Africa		South Africa	
Location	Mozambique		South Africa		South Africa		South Africa	
Sex	Male		Female		Male		Female	
Mass	15.1 kg		75kg		71kg		71kg	
	mm	**%DL**	**mm**	**%DL**	**mm**	**%DL**	**mm**	**%DL**
Disc Length	547	100.00	1000	100.00	1010	100.00	1010	100.00
Disc Width	1328	242.78	2370	237.00	2230	220.79	2230	220.79
Anterior Projection	385	70.38	400	40.00	400	39.60	400	39.60
Rostrum to Pelvic Fin	566	103.47	990	99.00	1000	99.01	1000	99.01
Pre-dorsal Length	472	86.29	870	87.00	860	85.15	860	85.15
Pre-cloacal Distance	442	80.80	850	85.00	850	84.16	850	84.16
Disc Thickness	111	20.29	200	20.00	250	24.75	230	22.77
Pectoral Length 1	482	88.12	930	93.00	930	92.08	900	89.11
Pectoral Length 2	541	98.90	1220	122.00	1150	113.86	1140	112.87
Pectoral Length 3	571	104.39	940	94.00	970	96.04	940	93.07
1st Gill Slit Length	132	24.13	210	21.00	230	22.77	230	22.77
2nd Gill Slit Length	132.7	24.26	240	24.00	241	23.86	245	23.76

Table 3.1 Continued

	mm	%DL	mm	%DL	mm	%DL	mm	%DL
3rd Gill Slit Length	128	23.40	250	25.00	240	23.76	240	23.76
4th Gill Slit Length	110.6	20.22	235	23.50	210	20.79	220	21.78
5th Gill Slit Length	90.7	16.58	180	18.00	175	17.33	180	17.82
Distance Between 1st Gill Slits	114.7	20.97	270	27.00	240	23.76	260	25.74
Distance Between 5th Gill Slits	55	10.05	140	14.00	140	13.86	150	14.85
Rostrum to 1st Gill Slit	106.6	19.49	200	20.00	200	19.80	200	19.80
Rostrum to 5th Gill Slit	212	38.76	435	43.50	425	42.08	430	42.57
Cephalic Fin Length	210	38.39	260	26.00	270	26.73	230	22.77
Cephalic Fin Width	63.7	11.65	120	12.00	125	12.38	120	11.88
Diameter of Eye	18	3.29	24	2.40	30	2.97	20	1.98
Cranial Width	310	56.67	540	54.00	520	51.49	530	52.48
Head Length	180	32.91	350	35.00	330	32.67	320	31.68
Mouth Width	180	32.91	370	37.00	310	30.69	340	33.66
Lower Toothband Length	120.4	22.01	215	21.50	240	23.76	240	23.76
Internarial Distance	175	31.99	340	34.00	310	30.69	310	30.69
Spiracle Length	19	3.47	41	4.10	22	2.18	23	2.28

Table 3.1 Continued

	mm	%DL	mm	%DL	mm	%DL	mm	%DL
Interspiracle Distance	290	53.02	450	45.00	450	44.55	450	44.55
Dorsal Fin Base	73	13.35	170	17.00	145	14.36	160	15.84
Dorsal Fin Height	52.5	9.60	110	11.00	105	10.40	100	9.90
Dorsal Fin Anterior Margin	71.3	13.03	103	10.30	150	14.85	160	15.84
Width Across Pelvic Fin Base	105.5	19.29	220	22.00	200	19.80	210	20.79
Pelvic Fin Length	123.8	22.63	280	28.00	240	23.76	220	21.78
Pelvic Fin Anterior Margin	67.3	12.30	170	17.00	190	18.81	130	12.87
Clasper Inner Length	16.8	3.07	N/A	N/A	160	15.84	N/A	N/A
Tail Length	671	122.67	1130	113.00	980	97.03	1040	102.97
Tail Width	14.9	2.72	21	2.10	15	1.49	16	1.58
Tail Height	16.3	2.98	22	2.20	22	2.18	21	2.08

DISSCUSSION

This study presents the first detailed morphometric measurements for *M. birostris* from the western Indian Ocean. No new descriptions or morphological measurements for the species have been presented since Bigelow & Schroeder's (1953) notes on two Caribbean specimens. The lack of specimens or descriptions available from other areas makes taxonomic questions difficult to resolve. By default, this study provides the first modern description of *M. birostris* using a standard template for external descriptions and measurements.

This study also presents the first description of a foetus from the western Indian Ocean. Like all other accounts, there was only a single embryo extracted from the female (Lamont 1824; Lesueur 1824; Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953). Uniparity is a common pattern in mobulid rays (Notarbartolo-di-Sciara 1987b), with *Manta birostris* appearing to most commonly give birth to a single offspring. The examined foetus was larger than previous foetal specimens examined or estimated of which the largest was approximately 1270 mm DW (Lesueur 1824; Lamont 1824; Müller and Henle 1841; Coles 1916, Beebe and Tee-Van 1941). The foetus was also larger than the smallest free-swimming individuals previously recorded (approximately 1219 mm DW) (Bigelow & Schroeder 1953). The smallest free-swimming individuals recorded in southern Mozambique ranged in size from 1300-1700 mm DW, suggesting that this embryo may be near to term (Marshall unpublished data). The examined foetus was also heavier than previously documented foetuses: 9.1 kg (Lamont 1824) and 12.7 kg (Beebe & Tee-Van 1941). The tail of the Mozambican specimen was considerably shorter than Coles's (1916) report from the Atlantic, which estimated foetal tail length to be up to 2400 mm, but was similar to measurements taken of a foetus collected in Galapagos Island waters whose tail measured 540 mm (Beebe & Tee-Van 1941).

A thorough external examination revealed many similarities to South African specimens examined, particularly in colouration and other external features. The reddish tinge to the ventral surface however may have been an artefact of post-mortem blood pooling and many of the lighter patches on the dorsal surface probably resulted from abrasion during initial extraction or transportation. The foetus's ventral spot patterning (Fig. 3.1c) was similar in arrangement and colour to the patterning

recorded in the South African specimens and in free-swimming manta rays (Fig. 3.2b, Marshall unpublished data). Ventral spot patterns are stable in larger *M. birostris*, remaining unaltered over at least 20 years (Homma et al. 1999). The presence of a distinctive ventral spot arrangement in this foetus establishes that these patterns develop before birth, although it does not confirm that these patterns are invariable from birth.

The morphometric measurements of the foetus show several differences to those of the larger juvenile *M. birostris* caught off the eastern coast of South Africa (Table 3.1). Comparisons between the DW, anterior projection and pectoral lengths 2 and 3 (Table 3.1) suggest that the foetus's pectoral fin tips were more 'swept back' in comparison to the larger individuals, which likely reflects the curled-up position of the foetus while *in utero*. Other than the differences relating to the morphometry of the pectoral fins there were only a few measurements differentiating the foetus from larger juvenile rays (Table 3.1). The tail length was fully intact in the foetus but was damaged and thus shorter in the larger juvenile specimens. Other differences occurred in measurements within the anterior head region of the individual: eye diameter, interspiracle length, and cephalic fin length. These differences may reflect neonate morphology, with certain features appearing disproportionate at this stage of development. While the growth of this foetus may not have terminated at the time the female was captured, it seems likely that it was near-term as parturition within this population normally occurs in the Spring-Summer months, from October to February (Marshall unpublished data).

Chapter 4

Morphological description of two putative

species of *Manta*

INTRODUCTION

The devilrays (Family Mobulidae, Suborder Myliobatoidei, Order Rajiformes), are currently divided into two distinctive genera, *Mobula* Rafinesque, 1810 and *Manta* Bancroft, 1828. The taxonomic history of the genus *Manta* has been convoluted, with *Manta* having one of the most extensive generic and species synonymies of any living genus of cartilaginous fish. In all there have been 10 generic and 25 species synonyms mostly without type specimens (Lamont 1824; Lesueur 1824; Mitchill 1824; Pinchot 1930; Whitley 1936; Beebe and Tee-Van 1941; Fowler 1941; Bigelow and Schroeder 1953). Currently this genus is considered to be monotypic (Eschmeyer et al. 1983; Compagno 1999; Homma et al. 1999; Clarke 2002; McEachran and Aschliman 2004), with a single recognised species, *Manta birostris* (Walbaum 1792). This species has been documented to occur circumglobally as far north as Rhode Island and southern California on the United States east and west coasts, Japan, and the Azores in the northern hemisphere and as far south as Peru, Uruguay, South Africa and New Zealand in the southern hemisphere (Bigelow and Schroeder 1953; Last and Stevens 1994; Duffy and Abbott 2003; Ebert 2003; Milessi and Oddone 2003).

Opportunistic dissections and incidental catches of manta rays throughout their distribution have only contributed to the confusion with variable reports of maximum disc widths, size at maturity, external colouration patterns, tooth counts and the presence and absence of a tail spine (Lamont 1824; Lesueur 1824; Mitchill 1824; Coles 1916; Gudger 1922; Whitley 1936; Beebe and Tee-Van 1941; Barton 1948; Bigelow and Schroeder 1953; Nishida 1990; Last and Stevens 1994; Homma et al. 1999; Ebert 2003; White et al. 2007; Marshall et al. 2008). Several of these inconsistencies have lead to speculation over the validity of the monospecific status of *Manta* (Campagno 1999) and have highlighted the need for further clarification.

Previous accounts and morphometric measurements of *M. birostris* have been incomplete or used non-standardised methods, often resulting in unclear descriptions that are difficult to interpret or compare to other data (e.g. Lamont 1824; Lesueur 1824; Müller and Henle 1841; Coles 1916). Moreover, like all mobulid rays, specimens of *M. birostris* are poorly represented in ichthyological collections as

individuals are extremely large and specimens are difficult to keep intact, preserve or store in archives (Notarbartolo-di-Sciara 1987b). Most specimens have been misplaced, lost or body casts have been made which fail to depict important colouration patterns or do not include diagnostic characters such as denticles or teeth. The most detailed morphological descriptions of manta rays come from individuals caught in the Galapagos Islands in 1928 (Beebe and Tee-Van 1941), two specimens examined from Bimini, Bahamas, single specimens from the west coast of Florida and New Jersey in the United States (Bigelow and Schroeder 1953), and a single individual caught in the Whitsunday Islands in Australia (Whitley 1936). However, even these more detailed accounts fail to provide sufficient detail for a conclusive or comprehensive worldwide treatment, with most authors recommending further, more critical comparative studies and morphological examinations that go beyond superficial comparisons of colouration (Beebe and Tee-Van 1941; Bigelow and Schroeder 1953).

Certainly the lack of available specimens has stymied efforts to clarify taxonomic issues within this genus. The advent of advanced photographic equipment and sophisticated genetic analyses have modified some of the criteria necessary for taxonomic classification (Frankham et al. 2002; Avise 2004). However, for a species that is typically observed only in the wild it is important that putative species can be differentiated in the field or from photographs, as well as quickly from samples or measurements taken during opportunistic dissections.

This study was initiated after preliminary observations of consistent coloration and behavioural differences between two recognisable 'variants' in the waters off southern Mozambique. Observations of manta rays in the wild in southern Mozambique and the Revillagigedo Archipelago, Mexico in addition to the examination of specimens from small-scale fisheries in Inhambane (Mozambique), Lombok (Indonesia) and bather-protection nets in Durban (South Africa) allowed for broad geographical comparisons of morphology and colouration. The hypothesis that at least two species of sympatric manta rays exist was initially investigated in Mozambique and South Africa using morphological measurements, external characters, size at maturity data, maximum disc width, colouration and behavioural observations. The broader

applicability of preliminary results was then tested in other locations where manta rays were observed in the field or dead specimens from fisheries were dissected.

The splitting of a genus or the identification of a new species is important on many levels, the most obvious of which is the ability for individuals to be accurately identified so as to permit future study. In terms of conservation, the identification of reproductively isolated species can aid in the accurate assessment of a species or population's conservation status. Only after a conservation status has been determined can appropriate monitoring and management strategies be applied. As *Manta birostris* is already listed as a near-threatened species on the IUCN's international redlist for endangered species (Marshall et al. 2006), it is critical that the taxonomic status of this genus is resolved so that different species can be assessed independently from one another and vulnerable populations can be re-examined.

MATERIAL AND METHODS

Morphometric measurements used in this study to describe proportional dimensions followed standards used in Notarbartolo-di-Sciara (1987b), Compagno (2001) and Manjaji (2004), with additional characters added specifically for the genus *Manta* (Chapter 3 / Marshall et al. 2008). Measurements were often taken to the nearest centimetre (cm) for larger measurements, which were later converted to millimetres to facilitate comparisons, and to the nearest millimetre (mm) for smaller measurements. All morphological measurements were expressed as percentage disc length (DL) as sometimes the pectoral fins of the rays had been severed for transport or for processing reasons by fishermen before individuals could be examined. Furthermore, proportional dimensions using DL rather than disc width (DW) allow for comparison to preserved specimens in museums or ichthyological archives that have been stored with the pectoral fins removed or that have been fixed with the pectoral fins curled dorsally (Notobartolo-di-Sciara 1987; Francis 2006).

Meristic measurements, such as tooth counts, were also taken. Lower jaws were removed and tooth counts made at a later time under favourable lighting conditions, with rows counted across the entire length of the tooth band and files (lingual to labial aligned teeth) counted at the midline. Skin samples were taken from the dorsal and

ventral surfaces of the pectoral fin just lateral to the body cavity in the middle of the pectoral fin (Fig. 4.1). When possible total body mass in kilograms (kg) was determined using heavy-duty scales. The industrial scales used were in good working order but were not calibrated and the possibility for small errors in total body mass is acknowledged. In the field only quick internal examinations to determine maturity status were possible following standards in Walker (2005). When possible the number of turns in the spiral valve were also counted and the mass of the liver was determined. Ten body proportions were identified that could be used to separate the two variants of *M. birostris*. These ten proportions are highlighted in Table 4.2. Tissue samples were additionally collected and stored in 70-90% ethanol for subsequent genetic analysis.
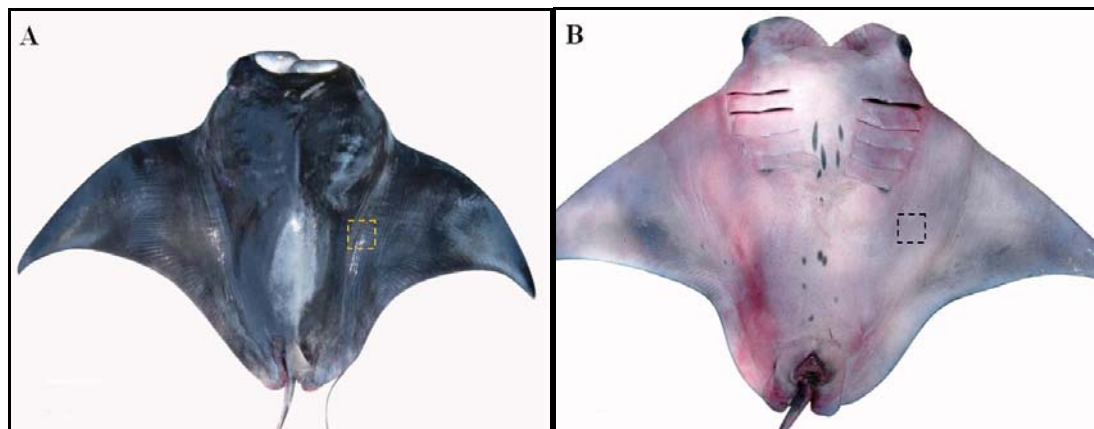
Figure 4.1 Standardised area for skin and denticle samples for both *Manta birostris* variant A and *Manta birostris* variant B.

Teeth and denticles were examined using a JEOL 6460 LA scanning electron microscope (SEM) at the University of Queensland, Australia. Samples were cleaned and rinsed, dehydrated using a graded series of ethanol baths and dried flat in a desiccation cupboard. Approximately 2 mm sections of tooth bands were taken from either side of the midline of each lower jaw examined. Tooth and denticle samples were platinum sputter coated (~15 nm) using an EIKP IB-5 Sputter Coater set at 6mA for five minutes. All SEM images were made using an acceleration voltage of 10 kV. A sagittal plane computed tomography (CT) image of the calcified mass and embedded spine (located at the base of the tail of some specimens) was made at the University of Queensland.

Photographs of colouration and morphological features are presented at the end of the result sections to facilitate comparisons between the two variant manta rays. Photographs from the four field sites (Inhambane, Mozambique; Durban, South Africa; Lombok, Indonesia and the Revillagigedo Archipelago, Mexico) are compared along with additional images from other locations, including the Yaeyama Islands and Ogasawra Islands (Japan), Stradbroke Island and Exmouth (Australia) and Yap (Micronesia).

A map depicting the regional distribution for the two variant manta rays was generated using data points collected from approximately 100 known aggregation sites worldwide. Images (n = 2231) from the author's own photographic library plus the libraries of 10 professional photographers were used in addition to the private libraries of other researchers, scuba diving centres, tourists, manta ray catalogues/databases, magazines/books, museum specimens, and publications. Only clear, good quality images in which individual rays could be identified using the key generated for this genus were used for this study.

RESULTS

***Manta birostris* variant A**

Material examined

Juvenile male caught in bather protection nets on 11 April, 2006 off Margate beach, Durban, South Africa (2230 mm DW, mass 71 kg).

Juvenile female caught in bather protection nets on 17 July 2006 off Karridene beach, Durban, South Africa (2370 mm DW mass, 75 kg).

Juvenile female caught in bather protection nets on 28 April, 2006 off Sunwich Port beach, Durban, South Africa (2330 mm DW, mass 71 kg).

Mature male caught in Mozambique on 15 January, 2004 off Paindaine Beach, Inhambane, Mozambique (3420 mm DW)

Juvenile male caught in bather protection nets on 14 June, 2004 in Umhlanga Beach, Durban, South Africa (2520 mm DW, mass 107 kg).

Juvenile female caught in bather protection nets on 21 June, 2004 off South Port, Durban, South Africa (2440 mm DW, mass 101 kg).

Juvenile male caught in bather protection nets on 10 August, 2004 off Durban, South Africa (2320 mm DW, mass 85 kg).

Juvenile male caught in bather protection nets on 15 September, 2004 in South Broom, Durban, South Africa (2470 mm DW, mass 105 kg).

Near-term male foetus caught in Mozambique on 15 October, 2004 in Paindane Beach, Durban, South Africa (1328 mm DW, mass 15 kg).

General description

Body compressed laterally, lozenge-shaped disc with elongated, pointed pectoral fins. Disc approximately 2.2 – 2.4 times as broad as it is long. Maximum disc width size approximately 5500 mm. Head distinct from body, with pectoral fins originating adjacent to each spiracle. Large, transverse, terminal mouth, with cephalic lobes present. Eyes positioned laterally on the side of the head. Slender whip-like tail approximately 123% of disc length if intact. No caudal spine present at base of tail, although specimens may have a slight depression on the dorsum of the tail immediately posterior to the posterior margin of the dorsal fin. Small, knob-like dermal denticles evenly distributed on both the dorsal and ventral surfaces. Ventral surface has slightly larger denticles.

Morphometrics

See Table 4.1 for measurements of *M. birostris* var. A. Individuals shown are those which the complete set of morphological measurements were taken. See Table 4.2 for a morphometric comparison of these individuals to individuals of *M. birostris* var. B.

Colouration

Dorsal surface black in overall colouration (Fig. 4.2a). Pale 'V' shaped patch typically stretches anteriorly from the insertion point of the dorsal fin. Pale colouration may be present along the distal margin of the pectoral fins. Pale to white coloured shoulder bars, with or without dark spots within them, present on each side of the dorsal supra-branchial region. Anterior margin of shoulder bar initially emanates posteriorly from

spiracle before curving medially, a diagnostic feature of *M. birostris* colouration (Fig. 4.3a-h). Towards the midline, the colouration again begins to radiate out posteriorly continuing down over the supra-branchial region in variably sized shoulder patches. Anterior distal side of the shoulder bar may present as an anterior facing hook. Colouration around and within mouth is white or cream. Ventral surface predominantly cream to white in colouration with variable dark markings. Blue-grey to black spots of variable size can occur across most of the ventral surface (Fig. 4.4a-h). The most diverse spot patterns typically occur medially to the five pair of gill slits, centrally on the abdomen and across the posterior half of the pectoral fins (Fig. 4.2b, 4.4a-h). A small black semi-circular spot of variable size is located immediately posterior to the fifth gill slit on each side of the body. Pale to dark charcoal-coloured bands are present on the posterior edge of each pectoral fin, typically stretching mid-way down the length of the fins from the pectoral fin tip (Fig. 4.2b). A melanistic form of *Manta birostris* variant A occurs which is almost entirely black on both the dorsal and ventral surfaces with a variably sized white blaze along the mid-line (Fig. 4.5a,b). The distinctive ventral spot patterning of *Manta birostris* variant A is often still visible through the black colouration on the abdominal region and between the gill slits (Fig. 4.5a,b).

Dentition

Terminal mouth with tooth band on lower jaw comprising 54.2 – 77.4% of total jaw width (Fig. 4.6a). Tooth band containing 6 – 8 rows of small cusped teeth (approximately 1 – 2 mm in length) and 142 – 182 files across entire width of the tooth band (Fig. 4.6b,c). Total tooth counts range from 918 – 1456 for entire tooth band. Morphology of individual teeth variable and may be dimorphic between sexes (Fig. 4.6d,e). General tooth morphology is shown in figure 4.6(d-h). Each tooth has a bulbous root that is embedded in the dental ligament, a freestanding stalk that ends in a curved cusp that forms the occlusal surface and is oriented to face the lingual side of the jaw (Fig. 4.6f,h). Teeth in the tooth band do not overlap (Fig. 4.6c). Upper jaw edentate with no enlarged denticle bands present.

Denticles

Denticles are small, non-overlapping and uniformly distributed along the dorsal and ventral surfaces (Fig. 4.7a,e). Each denticle comprises a stellate base (which is

embedded in the skin, Fig. 4.7f-h) with a dorso-laterally elongated emergent knob (Fig. 4.7a,e,f,h). Denticles on the ventral surface are larger than those on the dorsal surface, but all are of similar overall morphology (Fig. 4.7g,h).

Size

The smallest individuals observed in the wild were approximately 1500 m DW and a single examined near-term foetus was 1300 mm DW (Chapter 3). Dissected specimens of *M. birostris* var. A measured up to 3420 mm DW but estimates of the largest individuals sighted in southern Mozambique were slightly over 5 m DW. Size at maturity may vary slightly throughout its range, but males in southern Africa mature at approximately 3 m DW (Chapter 6), with the only mature male specimen examined in southern Mozambique, 3430 mm DW. Females in southern Africa mature at approximately 3900 m DW (Chapter 6).

Habitat and distribution

Commonly sighted inshore, within a few kilometres of land. Found around coral and rocky reefs as well as along productive coastlines with consistent upwelling, island groups, atolls and bays. The worldwide photographic survey of individuals at different aggregation sites yielded a preliminary distribution of *M. birostris* var. A (Marshall and Kashawagi, unpublished data). This variant is widespread in the Indian Ocean, with images and sightings of *M. birostris* var. A from the Red Sea in the north to Durban, South Africa in the south, and from mainland Thailand in the north to waters off Perth, Australia in the south. In the eastern and south Pacific *M. birostris* var. A occurs from the Yaeyama islands, Japan in the north to the Solitary Islands, Australia in the south and is sighted as far east as French Polynesia south of the equator and the Hawaiian islands north of the equator (Fig. 4.8).
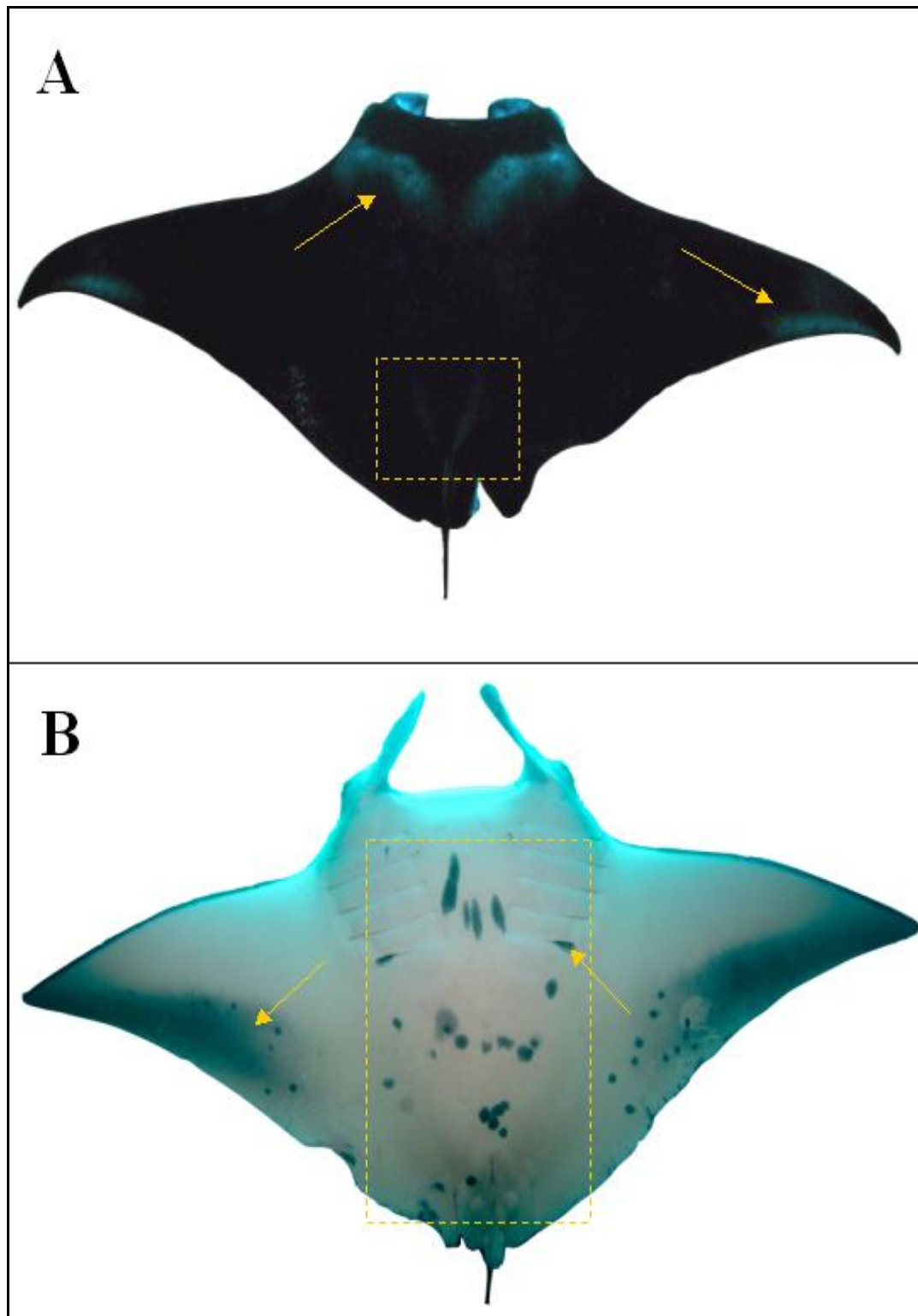
Figure 4.2 Natural colouration patterns in *Manta birostris* variant A: (a) dorsal surface, box showing faint 'V' shaped marking anterior to dorsal fin arrows showing location and shape of shoulder patches and pectoral fin colouration; (b) ventral surface, box showing region of highest spot density and distribution, arrows showing small semi-circular spots posterior to the fifth gill slits and colouration on the posterior edge of the pectoral fins.
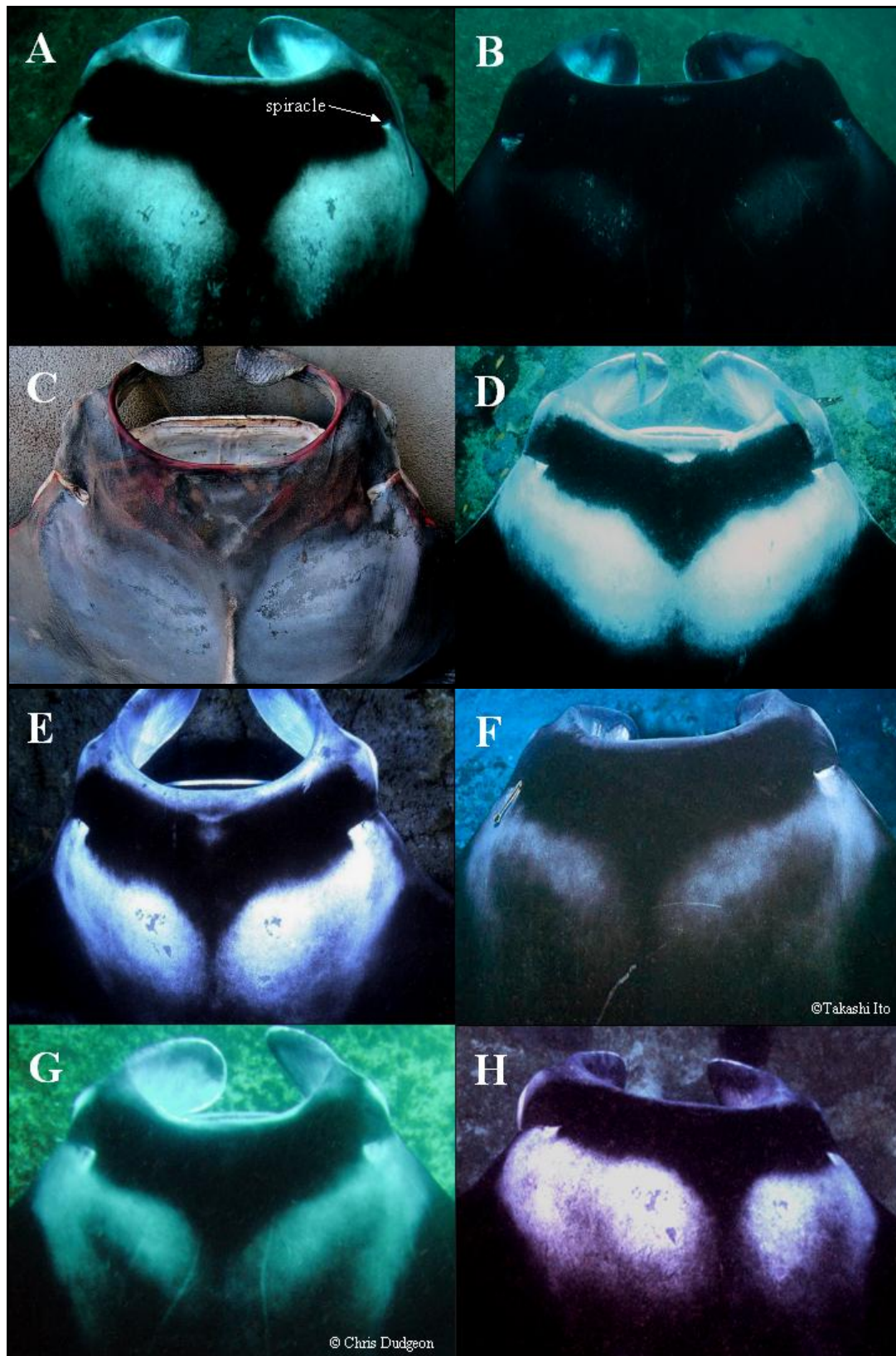
Figure 4.3 Variation in dorsal supra-branchial shoulder bar markings on *Manta birostris* variant A shown on individuals from: (a) Inhambane, Mozambique; (b) Inhambane, Mozambique; (c) Durban, South Africa; (d) Inhambane, Mozambique; (e) Inhambane, Mozambique; (f) Yaeyama Islands, Japan; (g) Stradbroke Island, Australia; (h) Inhambane, Mozambique.
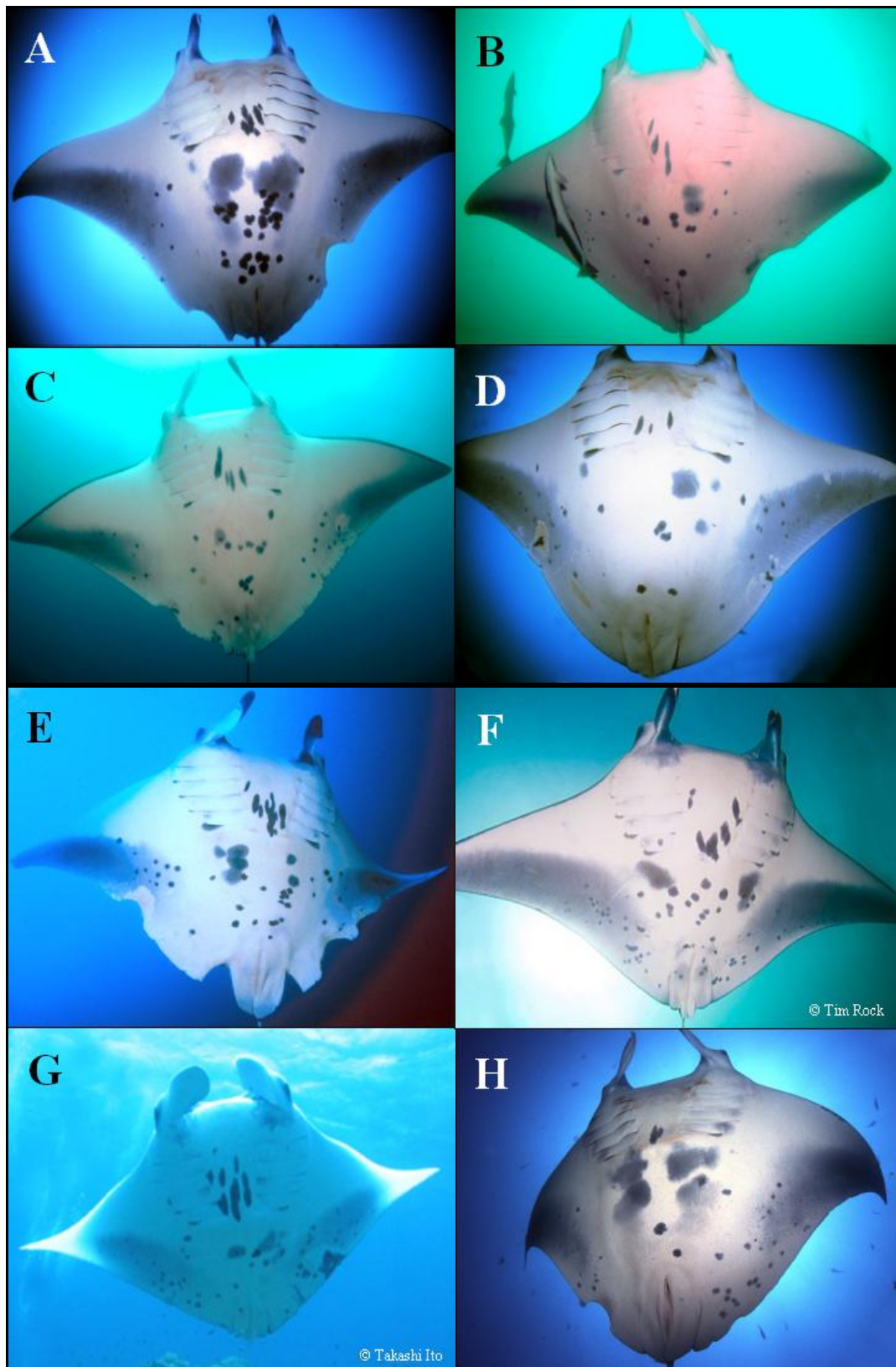
Figure 4.4 Variation in ventral markings on *Manta birostris* variant A from: (a) Inhambane, Mozambique; (b) Inhambane, Mozambique; (c) Inhambane, Mozambique; (d) Inhambane, Mozambique; (e) Inhambane, Mozambique; (f) Yap, Micronesia; (g) Yaeyama Islands, Japan; (h) Inhambane, Mozambique.
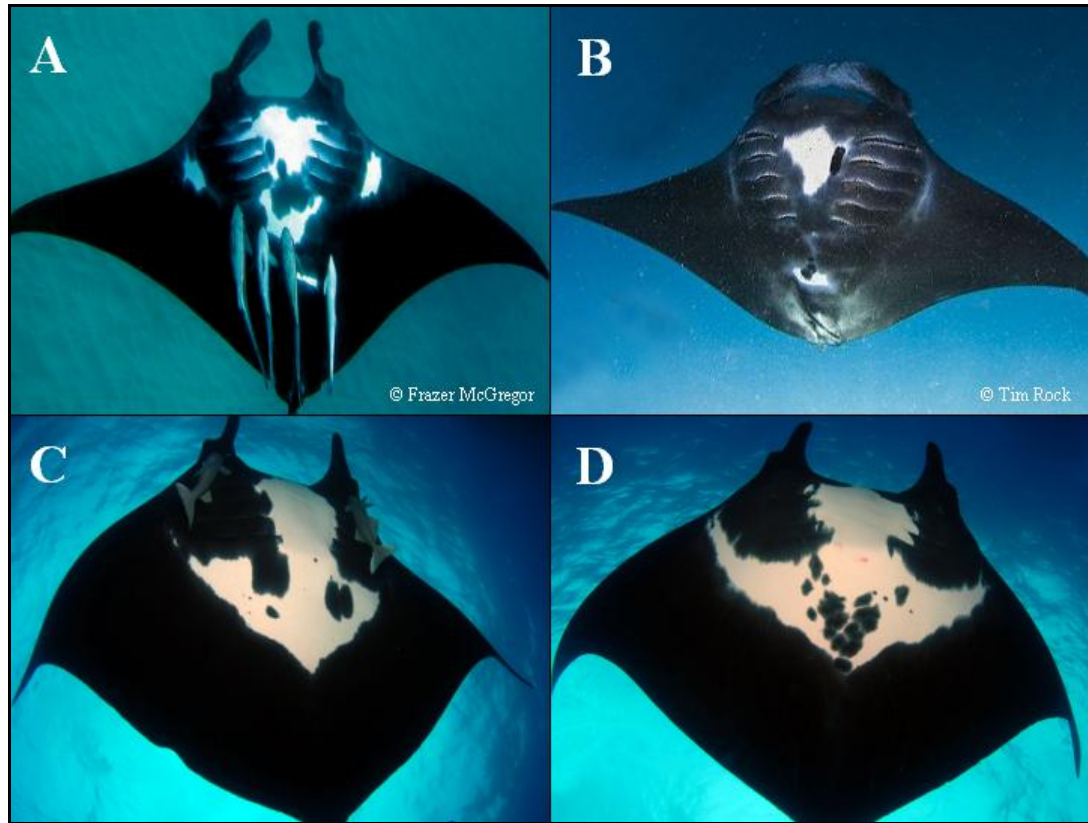
Figure 4.5 Melanisic form in genus *Manta*: *Manta birostris* variant A (a) Ningaloo Reef, Australia; (b) Yap, Micronesia; and *Manta birostris* variant B (c-d) Revillagigedo Archipelago, Mexico.
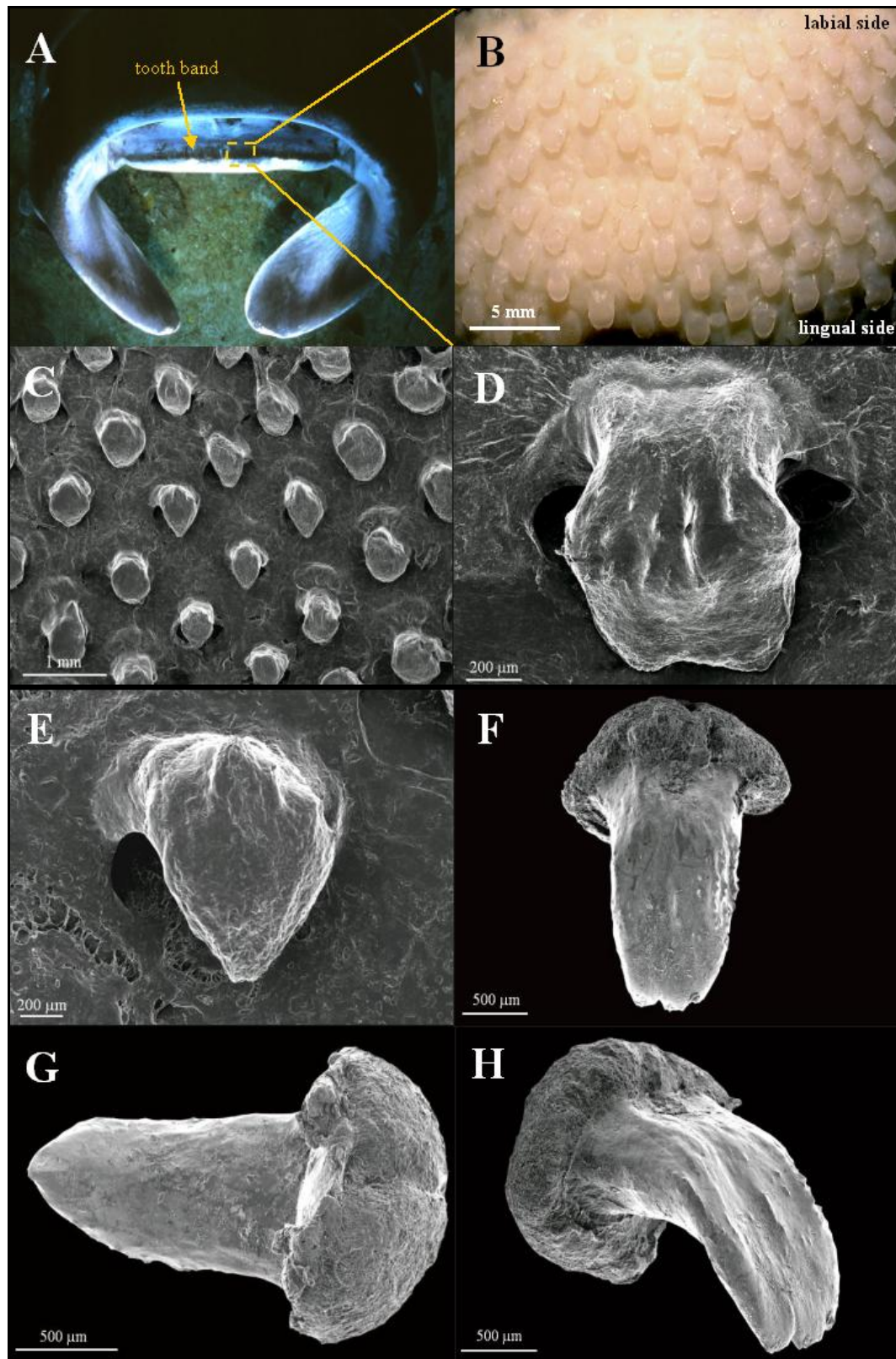
Figure 4.6 Dentition and tooth morphology in *Manta birostris* variant A: (a) lower jaw with elongated tooth band on lower jaw; (b) section of teeth in band; (c) embedded teeth of male ray; (d) view of single embedded female tooth; (e) view of single embedded male tooth; (f) labial view of single male tooth; (g) lingual view of cusp and bulbous root of male tooth; (h) dorso-lateral view of male tooth showing detail of occusal surface and tooth cusp.
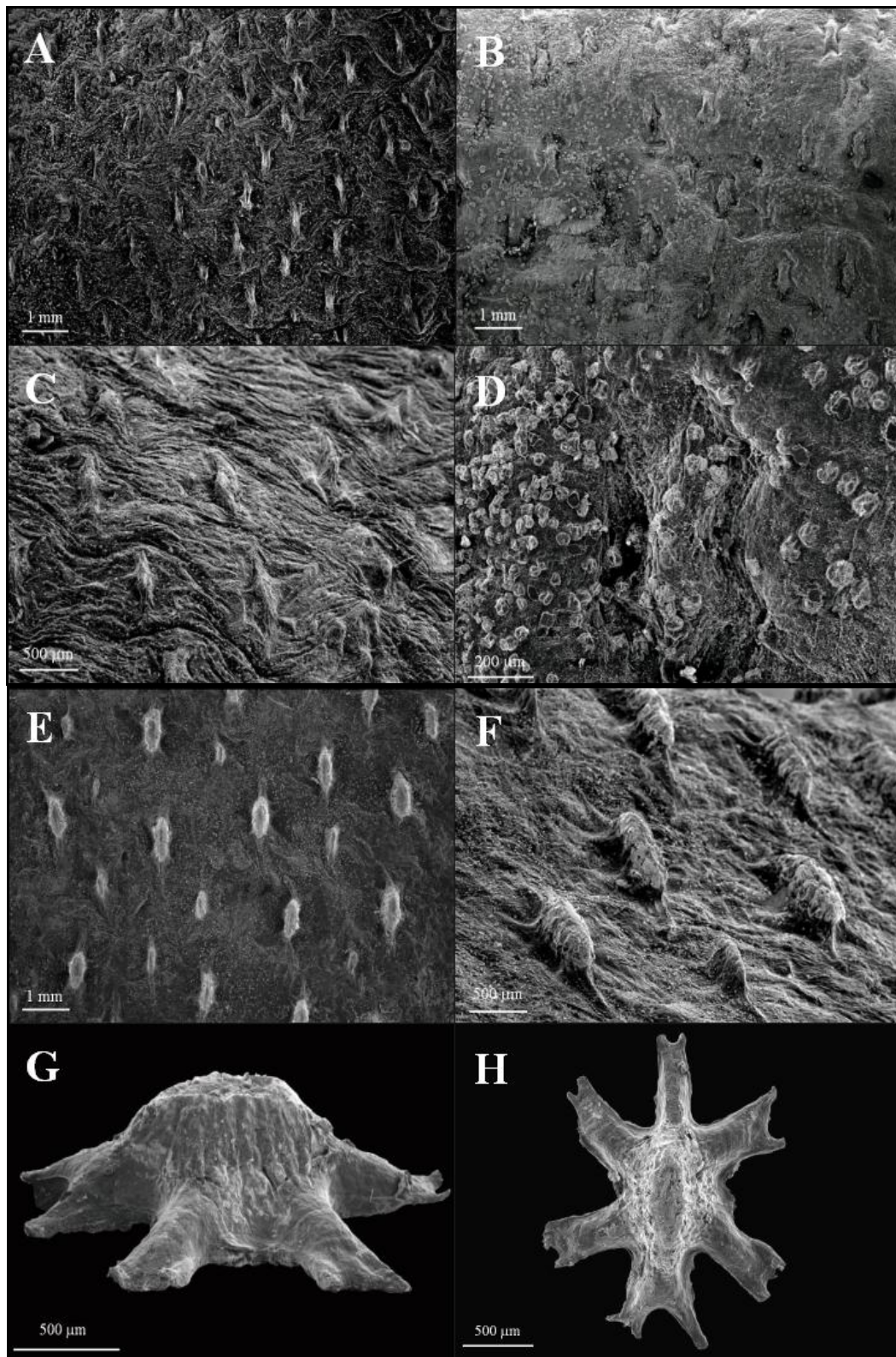
Figure 4.7 Skin and denticle morphology in *Manta birostris* variant A: (a) superior view of dorsal skin of male ray; (b) superior view of dorsal skin of female ray; (c) close-up tilted view of embedded denticles in dorsal surface of male ray; (d) morphology of a single, heavily embededed dorsal denticle of female ray; (e) superior view of ventral skin of male ray; (f) close-up of embedded denticles in dorsal surface; (g) morphology of ventral denticle lateral view; (h) morphology of ventral denticle superior view.
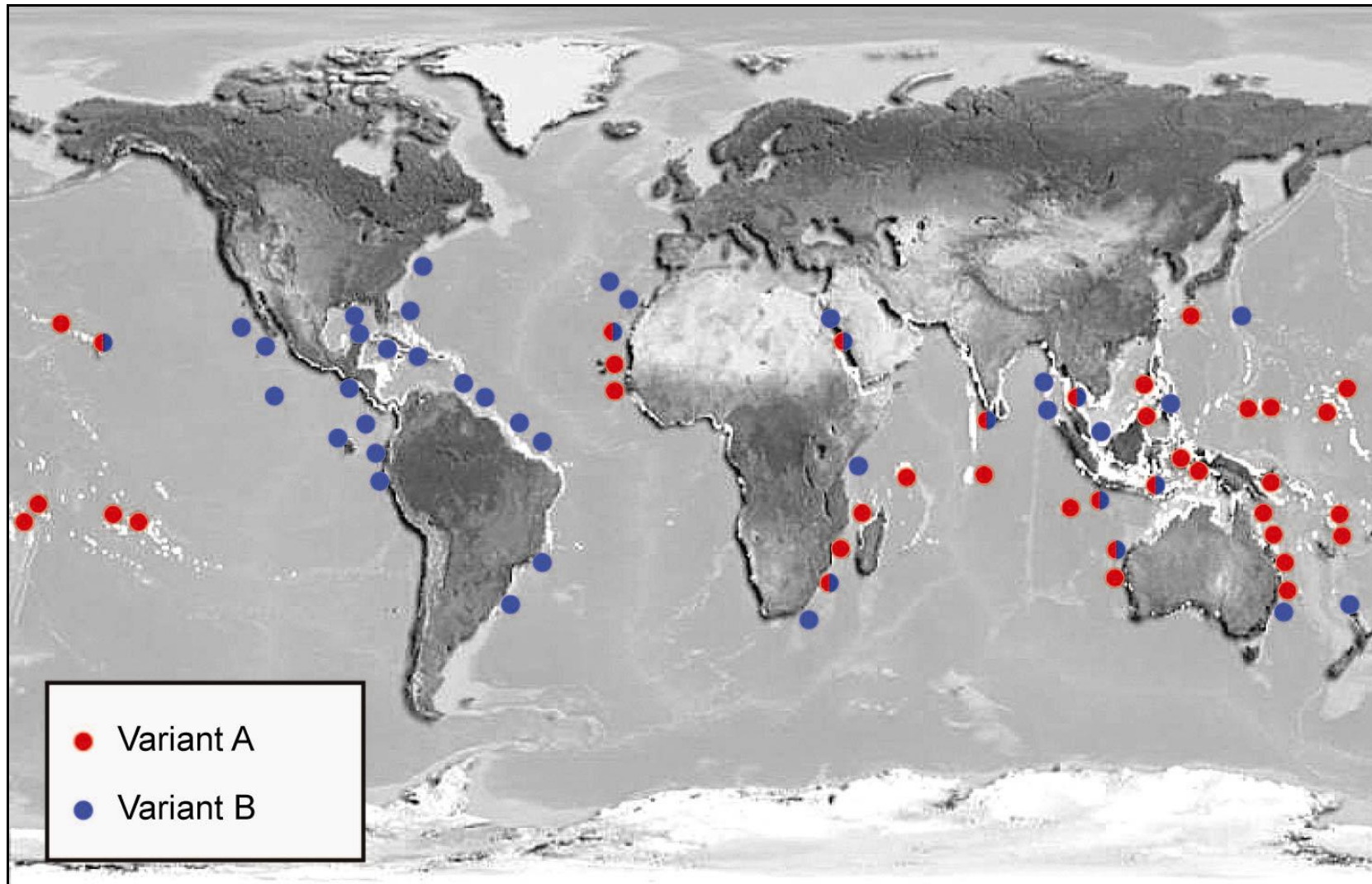
Figure 4.8 Worldwide distribution of *Manta birostris* variant A and *Manta birostris* variant B

Table 4.1 Morphometric measurements of *Manta birostris* variant A (South Africa) and *Manta birostris* variant B (Lombok, Indonesia).

Location	South Africa	South Africa	South Africa	Lombok	Lombok	Lombok	Lombok
Sex	Female	Male	Female	Female	Female	Female	Male
Disc Width (mm)	2370	2330	2330	3765	3800	3568	3850
	%DL	**%DL**	**%DL**	**%DL**	**%DL**	**%DL**	**%DL**
Disc Width	237.00	220.79	220.79	228.88	228.23	227.26	215.69
Anterior Projection	40.00	39.60	39.60	36.78	35.74	35.67	34.73
Rostrum to Pelvic Fin	99.00	99.01	99.01	101.52	101.32	100.76	100.28
Pre-dorsal Length	87.00	85.15	85.15	83.89	82.28	79.62	79.55
Pre-cloacal Distance	85.00	84.16	84.16	79.03	82.28	76.11	78.15
Disc Thickness	20.00	24.75	22.77	23.10	28.83	29.30	25.21
Pectoral Length 1	93.00	92.08	89.11	92.16	90.09	90.76	85.71
Pectoral Length 2	122.00	113.86	112.87	112.46	110.51	112.74	102.52
Pectoral Length 3	94.00	96.04	93.07	98.78	97.30	94.27	96.92
1st Gill Slit Length	21.00	22.77	22.77	20.97	22.52	22.29	21.01
2nd Gill Slit Length	24.00	23.86	23.76	24.01	24.02	23.57	22.13
3rd Gill Slit Length	25.00	23.76	23.76	22.98	23.48	22.29	21.29
4th Gill Slit Length	23.50	20.79	21.78	20.36	20.30	19.75	18.82
5th Gill Slit Length	18.00	17.33	17.82	15.50	14.83	14.97	15.52
Distance Between 1st Gill Slits	27.00	23.76	25.74	25.65	24.92	23.25	23.53
Distance Between 5th Gill Slits	14.00	13.86	14.85	12.58	12.61	12.42	11.76
Rostrum to 1st Gill Slit	20.00	19.80	19.80	18.30	19.22	19.75	17.65

Table 4.1 Continued

	%DL	%DL	%DL	%DL	%DL	%DL	%DL
Rostrum to 5th Gill Slit	43.50	42.08	42.57	44.07	42.94	42.99	41.46
Cephalic Fin Length	26.00	26.73	22.77	27.36	28.23	28.34	26.89
Cephalic Fin Width	12.00	12.38	11.88	13.37	14.11	11.46	11.76
Diameter of Eye	2.40	2.97	1.98	3.95	4.50	4.20	3.47
Cranial Width	54.00	51.49	52.48	58.36	56.76	58.28	51.82
Head Length	35.00	32.67	31.68	32.83	34.29	32.48	31.09
Mouth Width	37.00	30.69	33.66	38.30	37.54	36.94	34.17
Lower Toothband Length	21.50	23.76	23.76	24.80	24.44	25.73	25.21
Internarial Distance	34.00	30.69	30.69	34.04	31.83	32.48	30.81
Spiracle Length	4.10	2.18	2.28	2.92	2.98	3.69	3.31
Interspiracle Distance	45.00	44.55	44.55	50.46	48.05	47.13	45.10
Dorsal Fin Base	17.00	14.36	15.84	13.37	12.91	13.69	12.89
Dorsal Fin Height	11.00	10.40	9.90	8.81	7.87	8.34	8.40
Dorsal Fin Anterior Margin	10.30	14.85	15.84	14.29	13.21	13.38	12.89
Width Across Pelvic Fin Base	22.00	19.80	20.79	16.72	18.32	17.20	17.09
Pelvic Fin Length	28.00	23.76	21.78	25.41	22.52	24.20	24.93
Pelvic Fin Anterior Margin	17.00	18.81	17.82	13.37	12.31	11.46	10.92
Clasper Inner Length	N/A	15.84	N/A	N/A	N/A	N/A	24.65
Tail Length	113.00	97.03	102.97	89.06	N/A	89.17	N/A
Tail Width	2.10	1.49	1.58	1.58	N/A	1.53	N/A
Tail Height	2.20	2.18	2.08	1.64	N/A	1.66	N/A

Table 4.2 Comparison of morphometric measurements of two putative species of *Manta* from Durban, South Africa and Lombok, Indonesia presented as proportion of total disc length (%DL), with highlighted results indicating higher values and non-overlapping minimum and maximum proportional measurements between *Manta birostris* variant A and *Manta birostris* variant B (no foetal specimens were used).

	Manta birostris **variant A**				*Manta birostris* **variant B**			
	Min	**Max**	**Mean**	**N**	**Min**	**Max**	**Mean**	**N**
Disc Width	214.79	237.00	224.87	7	215.69	230.15	226.04	5
Anterior Projection	39.60	54.08	43.93	5	34.73	36.78	35.73	4
Rostrum to Pelvic Fin	99.00	101.02	99.79	5	100.28	101.52	100.87	5
Pre-dorsal Length	80.99	101.02	88.85	7	79.55	83.89	81.34	4
Pre-cloacal Distance	76.52	85.00	82.02	7	76.11	82.28	78.89	4
Disc Thickness	20.00	24.75	22.51	3	23.10	29.30	26.61	4
Pectoral Length 1	89.11	93.00	91.40	3	85.71	92.16	89.68	4
Pectoral Length 2	112.87	122.00	116.24	3	102.52	112.74	109.56	4
Pectoral Length 3	93.07	96.04	94.37	3	94.27	98.78	96.82	4
1st Gill Slit Length	20.00	22.77	21.39	5	20.97	22.29	21.70	4
2nd Gill Slit Length	21.82	24.00	23.08	5	22.13	24.02	23.43	4
3rd Gill Slit Length	20.91	25.00	22.97	5	21.29	23.48	22.51	4
4th Gill Slit Length	18.37	23.50	20.71	5	18.82	20.36	19.81	4
5th Gill Slit Length	14.28	18.00	16.40	5	14.83	15.52	15.21	4
Distance Between 1st Gill Slits	21.43	27.00	23.95	5	23.25	25.65	24.34	4
Distance Between 5th Gill Slits	10.71	14.85	12.86	5	11.76	12.61	12.35	4

Rostrum to 1st Gill Slit	15.31	20.00	18.25	5	17.65	19.75	18.73	4

Table 4.2 Continued

	Min	Max	Mean	N	Min	Max	Mean	N
Rostrum to 5th Gill Slit	40.00	43.50	41.79	5	41.46	44.07	42.87	4
Cephalic Fin Length	22.77	26.73	25.11	5	26.89	28.34	27.70	4
Cephalic Fin Width	8.67	12.38	10.99	5	11.46	14.11	12.68	4
Diameter of Eye	1.98	4.08	3.01	5	3.38	4.50	3.90	5
Cranial Width	50.00	54.00	51.78	5	51.82	58.36	56.30	4
Head Length	30.61	35.00	32.17	5	31.09	34.29	32.67	4
Mouth Width	30.69	37.00	34.53	5	34.17	38.30	36.74	4
Lower Toothband Length	19.90	23.76	21.96	5	24.44	25.73	25.05	4
Internarial Distance	30.61	34.00	31.38	5	30.81	34.04	32.29	4
Spiracle Length	2.18	4.10	2.85	3	2.92	3.69	3.22	4
Interspiracle Distance	44.55	45.00	44.70	3	45.10	50.46	47.68	4
Dorsal Fin Base	13.91	17.00	14.82	7	12.89	13.69	13.17	5
Dorsal Fin Height	9.09	11.44	10.08	7	7.60	8.81	8.20	5
Dorsal Fin Anterior Margin	10.30	15.84	13.66	3	13.21	14.29	13.44	4
Width Across Pelvic Fin Base	19.80	22.00	20.86	3	16.72	18.32	17.33	4
Pelvic Fin Length	21.78	28.00	24.51	3	22.52	25.41	24.27	4
Pelvic Fin Anterior Margin	17.00	18.81	17.88	3	10.92	13.37	12.02	4
Tail Width	1.49	2.10	1.72	3	1.53	1.58	1.56	2
Tail Height	2.08	2.20	2.15	3	1.64	1.66	1.65	2

***Manta birostris* variant B**

Material examined

Mature male caught in gill net on May 13th, 2007 in the Alas Strait south of TanJung Luar, Lombok (3850 mm DW).

Juvenile female caught in gill net on May 13th, 2007 in the Alas Strait south of TanJung Luar, Lombok (3765 mm DW).

Juvenile female caught in gill net on May 13th, 2007 in the Alas Strait south of TanJung Luar, Lombok (3800 mm DW).

Juvenile female caught in gill net on May 13th, 2007 in the Alas Strait south of TanJung Luar, Lombok (3568 mm DW).

Mature female caught in gill net on May 13th, 2007 in the Alas Strait south of TanJung Luar, Lombok (4695 mm DW).

General description

Body compressed laterally, lozenge-shaped disc with elongated, pointed pectoral fins. Disc approximately 2.2-2.3 times as broad as it is long. Maximum disc width over 7000 mm. Head distinct from body, with pectoral fins originating adjacent to each spiracle. Large, transverse, terminal mouth, with cephalic lobes present. Eyes positioned laterally on the side of the head. Slender whip-like tail. Reduced caudal spine predominantly encased in a calcified mass present on the dorsum of tail immediately posterior to the dorsal fin. Dermal denticles situated on long laterally raised ridges in the dermis that extend down the length of both the dorsal and ventral surfaces. Ventral surface has slightly larger denticles.

Morphometrics

See Table 4.1 for complete measurements of *Manta birostris* variant B. See Table 4.2 for morphometric comparison to *Manta birostris* variant A.

Colouration

Dorsal surface black in overall colouration, with large, white shoulder bars, with or without black spots within them, in the supra-branchial region (Fig. 4.9a). Distinct pale to white 'V' shaped patch, of variable size, extends anteriorly from the anterior insertion point of the dorsal fin (Fig. 4.9a). White shoulder bars, present on each side of a dark midline, are distinct and approximately triangular in shape with posterior facing hook on the anterior distal side (Fig. 4.10a-h). Anterior edge of shoulder bars runs medially from spiracle in an approximately straight line parallel to the edge of the mouth, a diagnostic character of this species (Fig. 4.10a-h). Colouration around and within mouth is charcoal gray to black. Ventral surface cream to white with dark grey to black spots and patches only on the abdominal region posterior to the 5th gill slit openings and anterior to the anterior opening of the cloaca (Fig. 4.9b), a diagnostic feature for *M. birostris* var. B. Spots do not occur medially between the five gill slits or on the pectoral fins lateral to the body cavity (Fig. 4.11a-h). Medium to large black semi-circular spots posterior to the fifth gill slits are always present. Charcoal-coloured margin present along the posterior edges of the pectoral fins typically stretches entire length of each pectoral fin (Fig. 4.11a-h). A melanistic form occurs which is almost entirely black on both the dorsal and ventral surfaces with a variably-sized white blaze along the ventral mid-line. However, distinctive spot patterns are still often visible along the midline, with spots centralised on the abdominal region and absent medially between the gill slits (Fig. 4.5c,d).

Dentition

Terminal mouth with tooth band on lower jaw comprising 64.76 – 69.65% of total jaw width (Fig. 4.12a). Tooth band containing 12 – 16 rows of small cusped teeth (approximately 1.5 mm in length) and 220 – 250 files across entire width of the band (Fig. 4.12b,c). Total tooth counts range from 3000 – 4000 for entire tooth band. Morphology of individual teeth variable and may be dimorphic between sexes (Fig. 4.12d,e). General tooth morphology is shown in figure 4.12(d-h). Each tooth has a bulbous root, which is embedded in the dental ligament, a freestanding stalk that ends in a curved cusp that forms the occlusal surface and is oriented to face the lingual side of the jaw (Fig. 4.12f-h). Teeth in the tooth band slightly overlap (Fig. 4.12c). Tooth band absent in upper jaw but two irregular bands of enlarged denticles (Fig. 4.13a-d)

extend along upper jaw a distance equivalent to the length of the lower tooth band. Morphology of these enlarged denticles is shown in figure 4.13(e-h).

Caudal spine

Spine with serrated lateral edges embedded in a large mass of highly mineralised cartilage, similar to that described for *Mobula japonica* (Notobartolo-di.Sciara 1987) (Fig. 4.14a-g). Calcified mass with embedded spine located on the dorsum of tail immediately posterior to dorsal fin (Fig. 4.15a). Calcified mass with embedded spine lacks attachment via collagenous connective tissue to tail and detaches if skin is removed (Fig. 4.15a-d). Spine appears to have an enameloid exterior and is slender in shape, approximately 3.5% of the width of the calcified cartilage mass (Fig. 4.14e). Tip of spine projects approximately 3 mm out from the surrounding mass (Fig. 4.14e). A sagittal plane CT scan clearly shows the spine embedded one third of the way into the calcified mass (Fig. 4.14g). Visual examinations in the field suggest that the size of the calcified mass is positively correlated with disc width. The calcified masses extracted from the two rays examined (male 3850 mm DW / 1785 mm DL and female 3765 mm DW / 1645 mm DL), were similar in overall shape and were 5.98% and 6.69% of the total DL of the rays respectively.

Denticles

Prominent dermal denticles present on both the dorsal and ventral surfaces are arbitrarily distributed along vertical ridges in the skin (Fig. 4.16a-h). Denticles on the dorsal and ventral surfaces are oriented in an antero-posterior direction (Fig. 4.16a-c,e) and are similar in appearance and distribution, with slightly larger denticles on the ventral surface. Denticles have pronounced bifid cusps (Fig. 4.16d,g) that give the skin a much rougher texture than that of *M. birostris* var. A. The morphology of the two prominent denticle forms on both the dorsal and ventral surfaces are shown in Fig. 4.16 (d,g,h).

Size

Dissected specimens of *M. birostris* var. B measured up to 4695 mm DW but estimates of the largest individuals sighted in the field (southern Mozambique and Mexico) were slightly over 6000 mm DW. *Manta birostris* variant B reaches disc

widths of at least 7000 mm DW, with anecdotal reports up to 9100 mm (Compagno 1999; Alava et al. 2002).

Size at maturity for male *M. birostris* var. B may vary slightly throughout its range, but males in southern Africa appeared to mature at approximately 4000 mm DW (n = 3; Chapter 9). In the Revillagigedo Archipelago, Mexico seven immature males were observed in the field but all were less than 3800 mm DW. In Indonesia the only mature male examined was 3850 mm DW. Additional fisheries data from Lombok, Indonesia suggest male *M. birostris* var. B mature at 3750 mm (White et. al. 2006).

Size at maturity for female *M. birostris* var. B may also vary slightly throughout its range. In Indonesia, female *M. birostris* var. B up to 3800 mm DW were immature. The only examined mature female in Indonesia was 4695 mm DW. Additional fisheries data from Lombok, Indonesia suggest that females mature at 4130 mm (White et. al. 2006). The only mature females observed or examined (n = 2) in southern Mozambique were in excess of 5000 mm DW.

Habitat and distribution

Manta birostris variant B occurs in tropical, sub-tropical and temperate waters around the globe. Commonly sighted along productive coastlines with regular upwelling, island groups and particularly offshore pinnacles and seamounts (Compagno 1999). Also reported from river mouths and estuaries off the southern and south-eastern coasts of the United States (Bigelow and Schroeder 1953; Compagno 1999). A worldwide photographic survey of individuals at different aggregation sites from 2001-2008, a rigorous internet search, and contributed photographs from the libraries of professional photographers, dive centers, and other researchers around the globe has yielded a preliminary distribution of *M. birostris* var. B (Marshall and Kashawagi, unpublished data). *Manta birostris* variant B has been documented to occur as far north as southern California and Rhode Island on the United States west and east coasts, the Ogasawara Islands, Japan, the Sinai Peninsula, Egypt and the Azore Islands in the Northern Hemisphere and as far south as Peru, Uruguay, South Africa and New Zealand in the Southern Hemisphere. In some locations, including Mozambique *M. birostris* var. B occurs in sympatry with *M. birostris* var. A (Fig. 4.8), but in these areas *M. birostris* var. A and B typically exhibit different habitat use and movement patterns (Chapter 9).
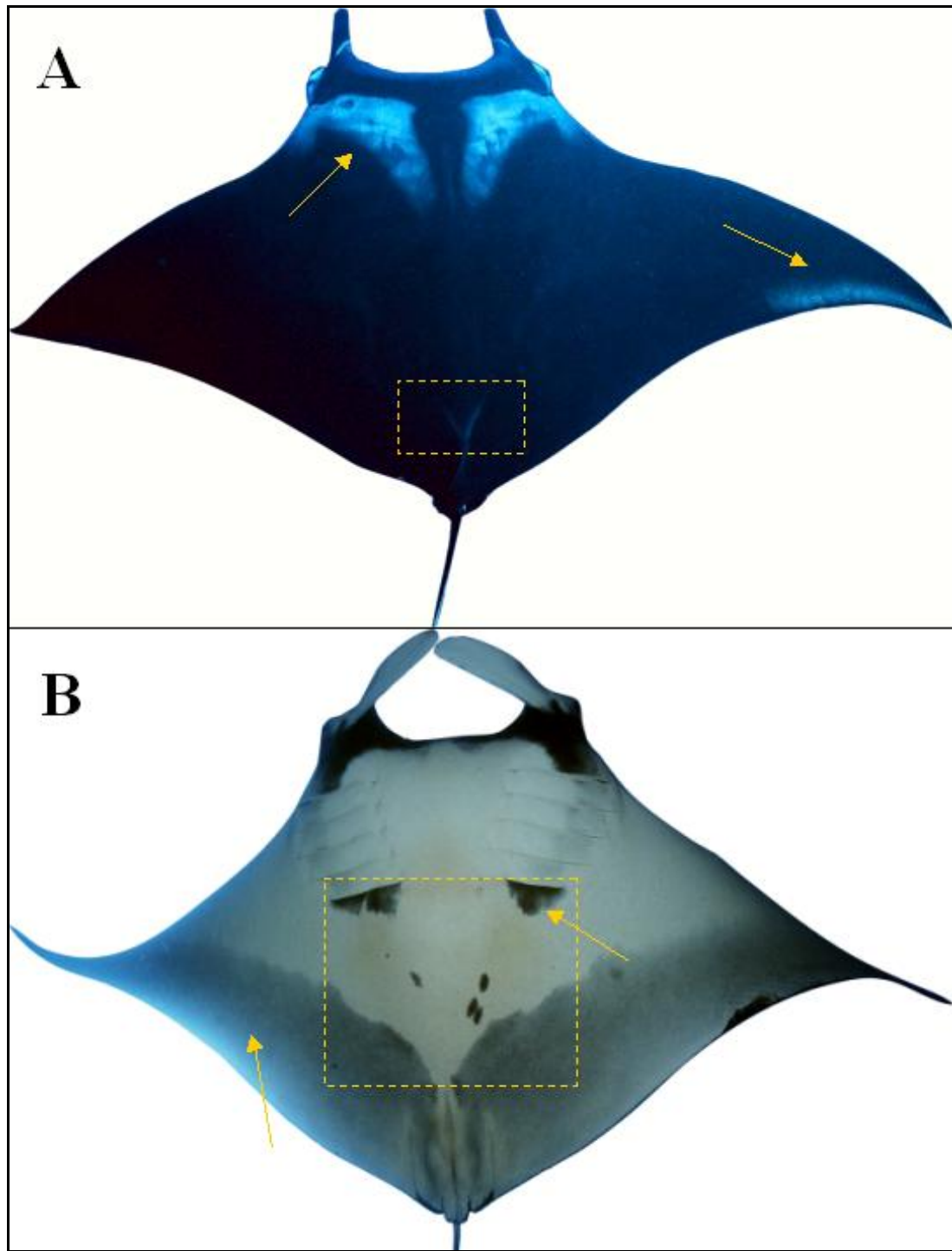
Figure 4.9 Natural colouration patterns in *Manta birostris* variant B: (a) dorsal surface, box showing 'V' shaped marking anterior to dorsal fin arrows showing location and shape of shoulder patches and pectoral fin colouration; (b) ventral surface, box showing region of highest spot density and distribution, arrows showing large semi-circular spots posterior to the fifth gill slits and colouration on the posterior edge of the pectoral fins.
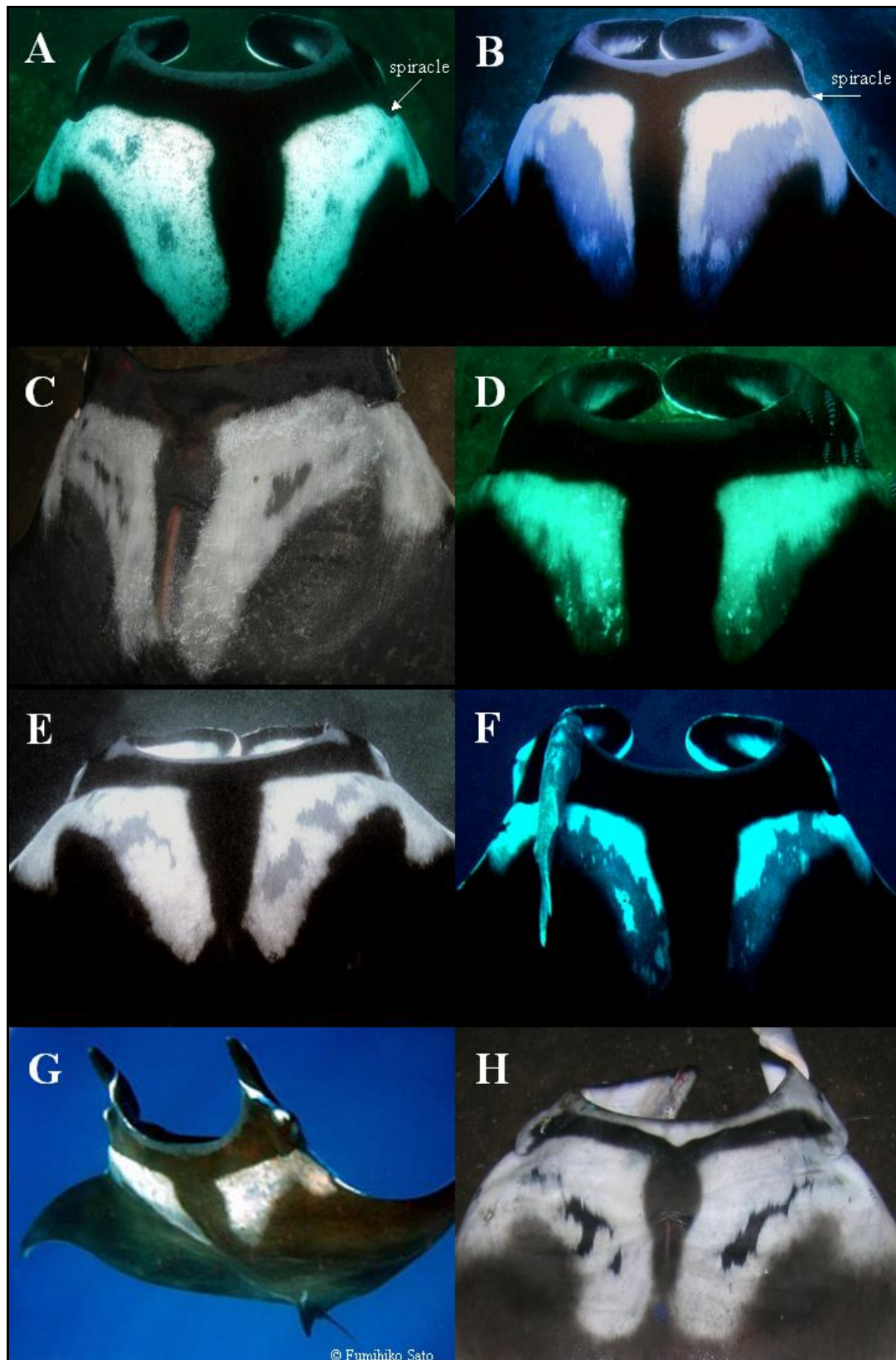
Figure 4.10 Variation in dorsal supra-branchial shoulder bar markings on *Manta birostris* variant B shown on individuals from: (a) Inhambane, Mozambique; (b) Inhambane, Mozambique; (c) Lombok, Indonesia; (d) Inhambane, Mozambique; (e) Brothers Islands, Red Sea; (f) Revillagigedo Archipelago, Mexico; (g) Ogasawra Islands, Japan; (h) Lombok, Indonesia.
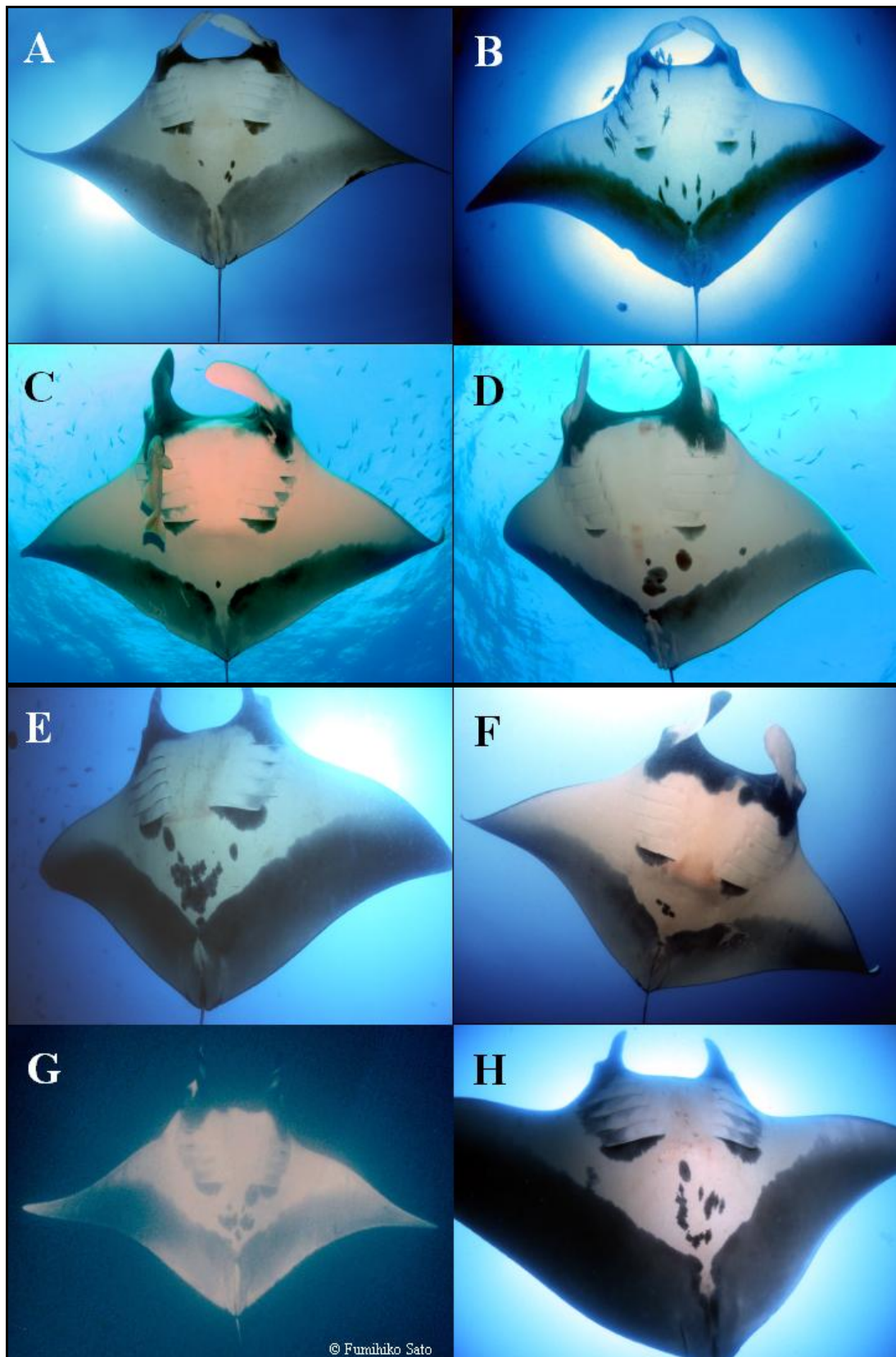
Figure 4.11 Variation in ventral markings on *Manta birostris* variant B from: (a) Inhambane, Mozambique; (b) Inhambane, Mozambique; (c) Revillagigedo Archipelago, Mexico; (d) Revillagigedo Archipelago, Mexico; (e) Inhambane, Mozambique; (f) Inhambane, Mozambique; (g) Ogasawra Islands, Japan; (h) Inhambane, Mozambique.
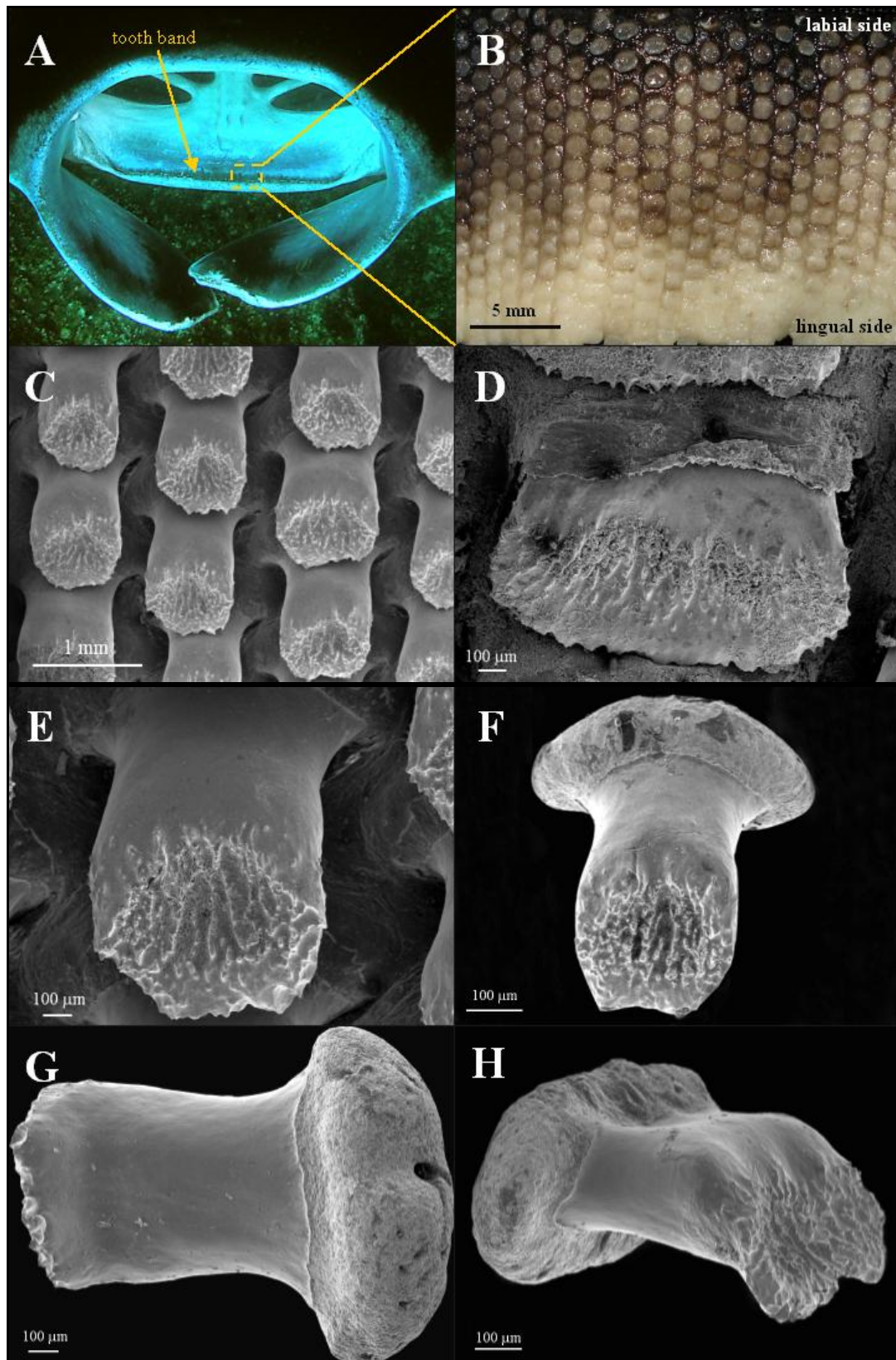
Figure 4.12 Dentition and tooth morphology in *Manta birostris* variant B: (a) lower jaw with elongated tooth band on lower jaw; (b) section of teeth in band; (c) embedded teeth of male ray; (d) view of single embedded female tooth; (e) view of single embedded male tooth; (f) labial view of single male tooth; (g) lingual view of cusp and bulbous root of male tooth; (h) dorso-lateral view of male tooth showing detail of occusal surface and tooth cusp.
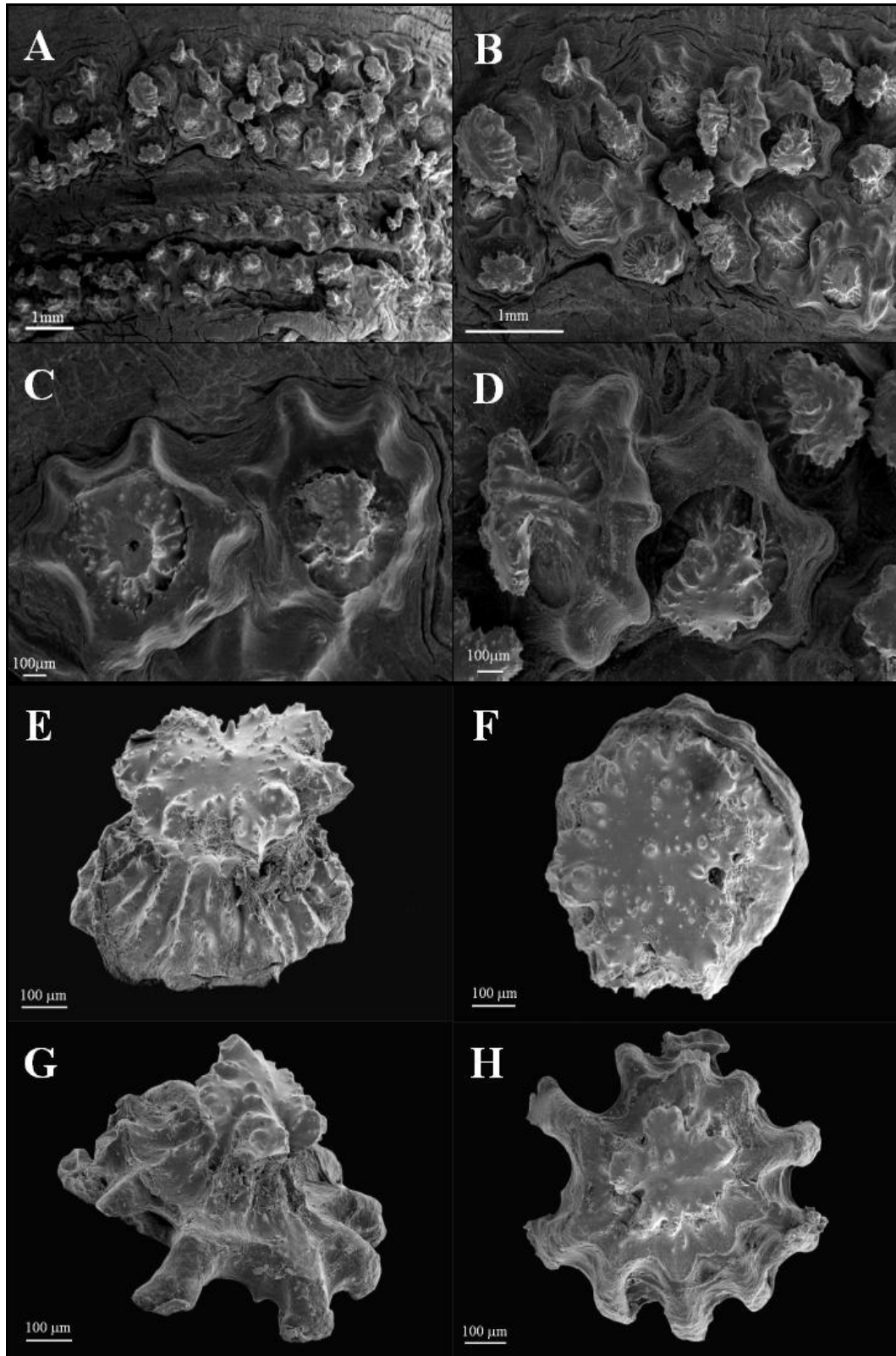
Figure 4.13 Enlarged denticle bands and denticle morphology in the upper jaw of *Manta birostris* variant B: (a) view of double band of enlarged denticles; (b) denticles in a single band; (c) two embedded denticles, one intact and one with the cusp broken showing portion of the stellate base embedded into the dermis; (d) denticles replacement process; (e) denticle morphology lateral view; (f) denticle morphology superior view; (g) additional denticle morphology lateral view; (h) additional denticle morphology superior view.
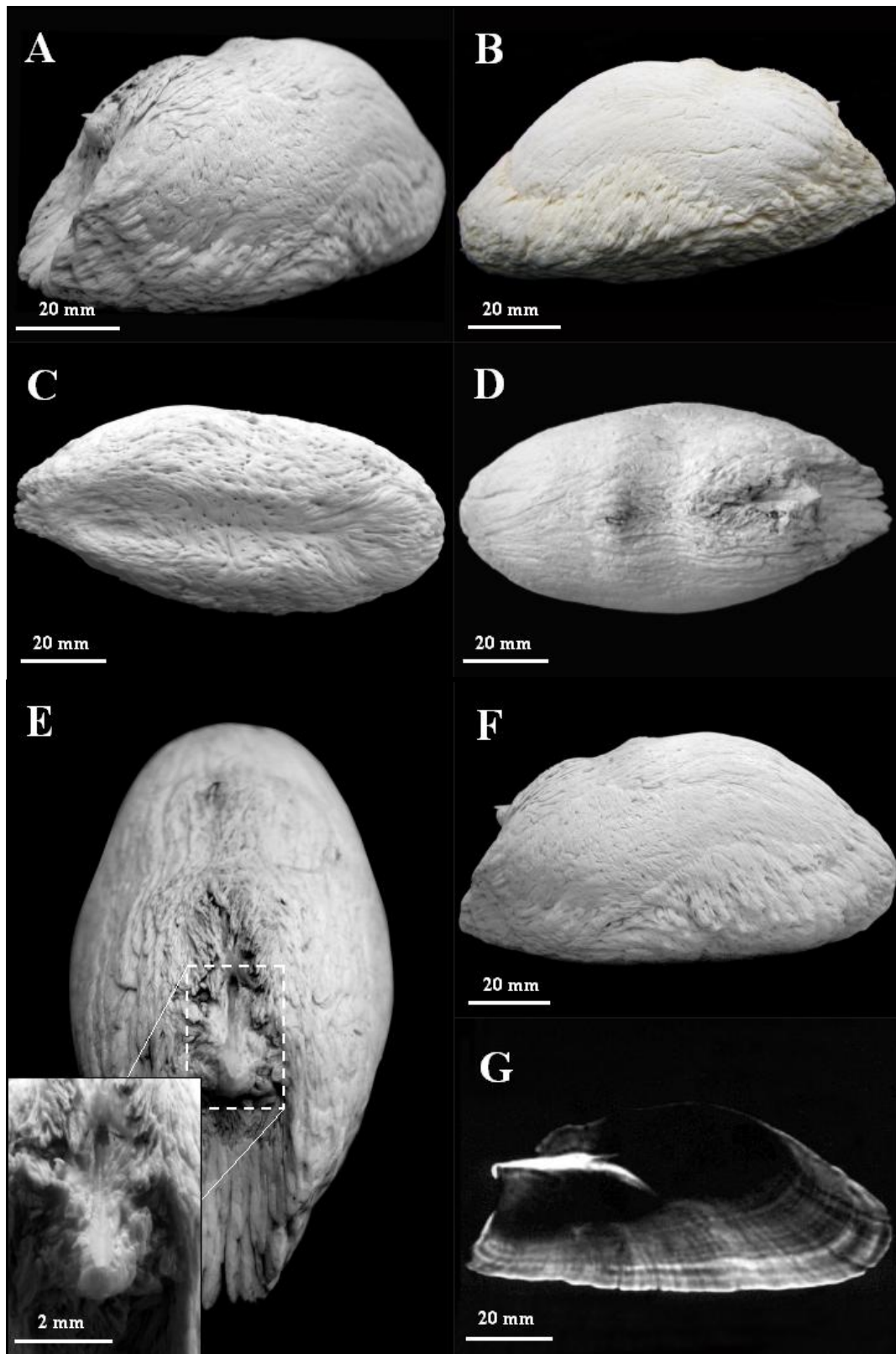
Figure 4.14 Views of calcified mass with embedded spine from *Manta birostris* variant B (entire structure was 106.7 mm total length, 42 mm wide, and 46.7 mm in height and has a mass of 112.5 grams and a density of 1.324 g/cm^3): (a) oblique posterior lateral view; (b) oblique anterior lateral view; (c) ventral view; (d) dorsal view; (e) posterior view with embedded spine, (inset) emerging spine tip; (f) lateral view; (g) sagittal plane CT scan showing the embedded spine.
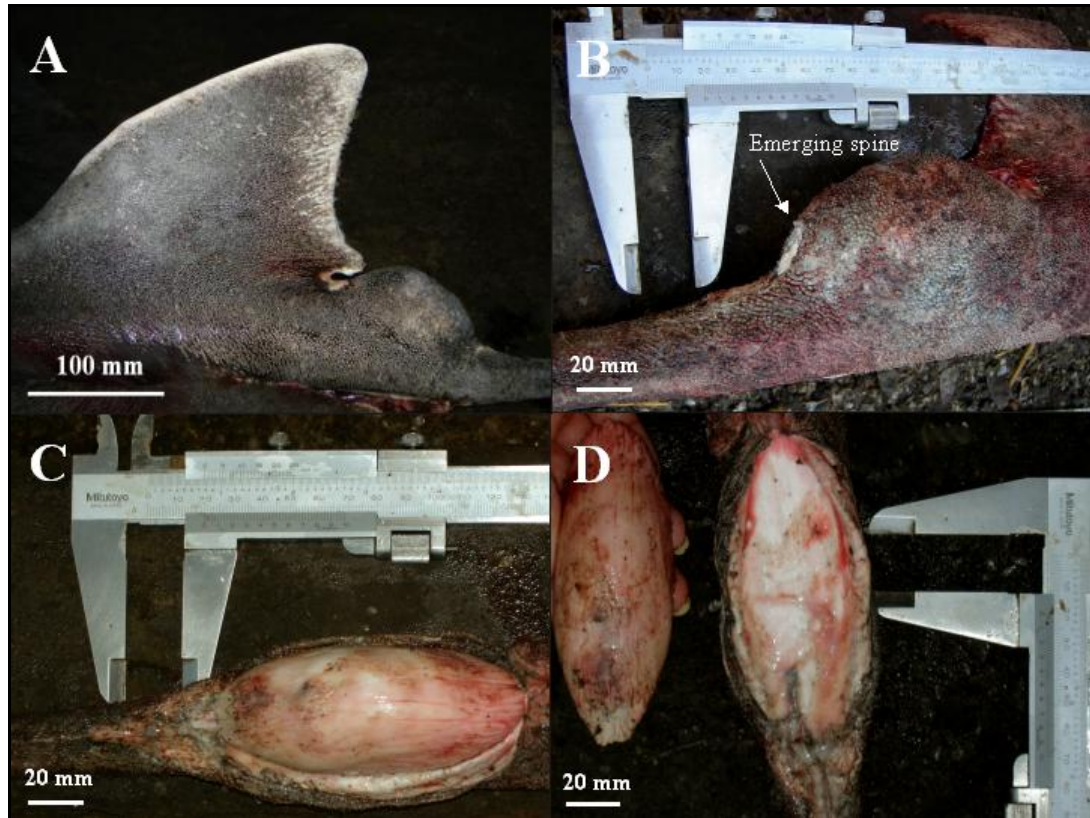
Figure 4.15 Sequence of spine removal from the tail of *Manta birostris* variant B: (a) lateral view of the dorsal fin and calcified mass with embedded spine; (b) close-up of intact specimen with tip of spine emerging from skin; (c) superior view after the skin was peeled back to expose the calcified mass; (d) resting place of the calcified mass, which sits unconnected to the underlying connective tissue or vertebrae.
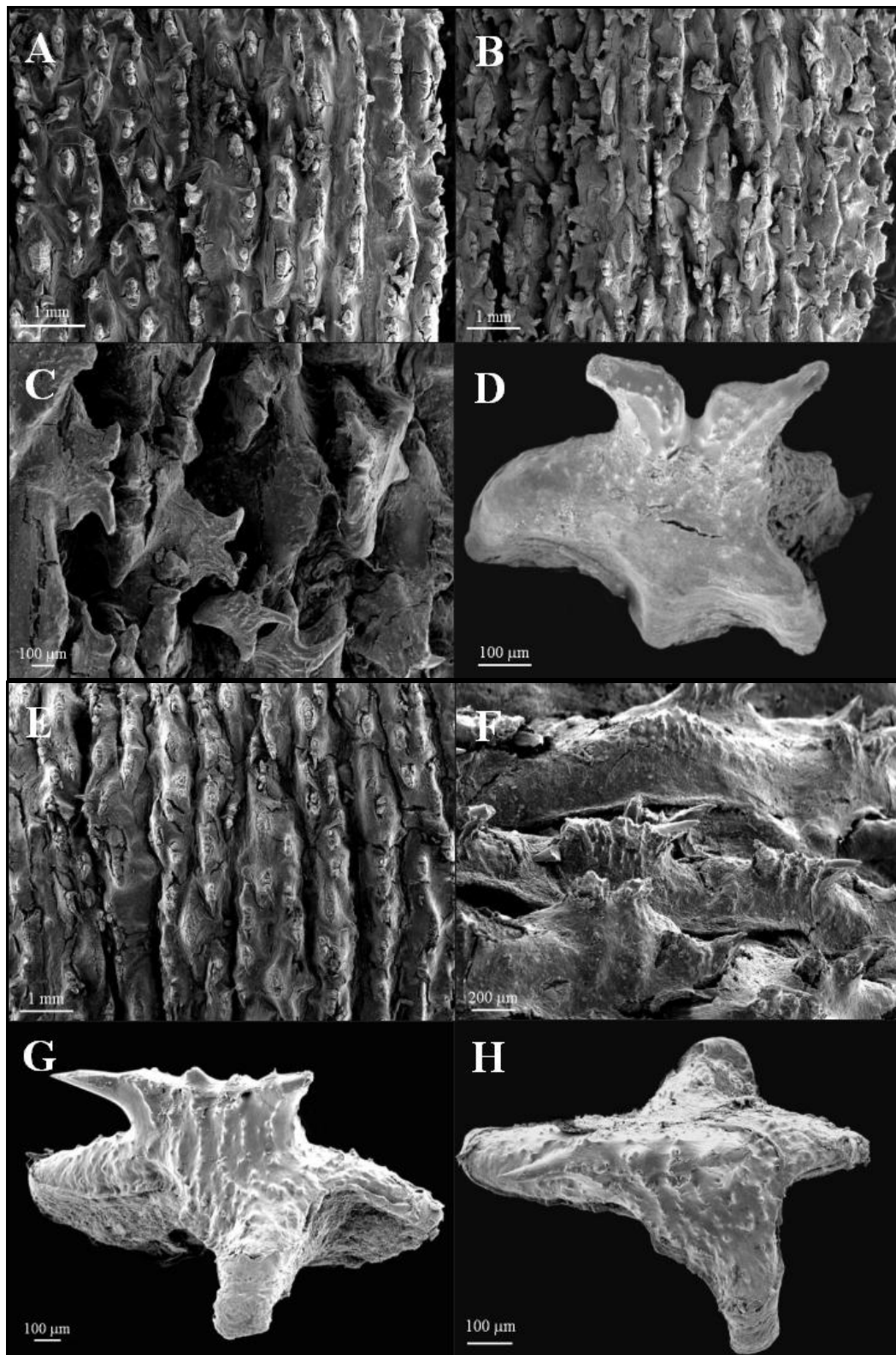
Figure 4.16 Skin and denticle morphology in *Manta birostris* variant B: (a) superior view of dorsal skin in male ray; (b) superior view of dorsal skin in female ray; (c) close-up of embedded denticles in dorsal surface of a male ray; (d) morphology of a single dorsal denticle in a male ray; (e) superior view of ventral skin in male ray; (f) close-up of embedded denticles in vental surface of a male ray; (g) morphology of secondary type of denticle, lateral view; (h) morphology of secondary type of denticle, superior view.

KEY FEATURES DIFFERENTIATING THE TWO PUTATIVE SPECIES

Manta birostris **variant A**

1) Pale to white coloured shoulder bars present on each side of the dorsal supra-branchial region. Anterior margin of shoulder bar initially emanates posteriorly from spiracle before curving medially. Towards the midline, colouration again begins to radiate out posteriorly continuing down over the supra-branchial region in variably sized shoulder patches. Anterior distal side of the shoulder bar may present as an anterior facing hook.

2) Distinctive dark spots on the ventral surface of disc are present across the posterior half of the body and medially between the five adjacent gill slits. Small semi-circular spot extends posteriorly from each of the most posterior gill slits.

3) No caudal spine or calcified mass on tail.

4) Skin on both dorsal and ventral surfaces embedded with small non-overlapping, evenly spaced denticles with stellate bases and laterally elongated knob-like (lacking cusps) structures projecting from skin.

5) Dental ligament with small cusped teeth on the lower jaw measuring roughly 22% of total disc length with approximately 6-8 rows, 142-182 files across entire width of the tooth band. Total tooth counts of 900-1500 for entire tooth band. Top jaw lacks rows of enlarged denticles.

Manta birostris **variant B**

1) White-coloured shoulder bars present on each dorsal supra-branchial region on each side of a dark midline. Shoulder bars are distinct and approximately triangular in shape with posterior facing hook on the anterior distal side. Anterior edge of shoulder bars runs medially from spiracle in an approximately straight line parallel to the edge of the mouth.

2) Distinctive dark spots on ventral surface of disc located over abdominal region. No spots present medially between the five adjacent gill slits. Prominent semi-circular spot extends posteriorly from each of the most posterior gill slits. Charcoal-coloured margin typically present on posterior edges of pectoral fins that extend the entire length of each pectoral fin.

3) Caudal spine mostly encased in a calcified mass present on the dorsum of the tail immediately posterior to the dorsal fin.

4) Skin forming distinct vertical ridges and furrows along the entire length of the dorsal and ventral surfaces densely embedded with overlapping, multicuspid denticles.

5) Dental ligament embedded with small cusped teeth on the lower jaw measuring roughly 25% of total disc length with approximately 12-16 rows, 220-250 files across entire width of the band. Total tooth counts of 3000-4000 for entire tooth band. Upper jaw contains at least two rows of enlarged denticles that span the same width of the upper jaw as the tooth band on the lower jaw.

***Manta birostris* variant A**
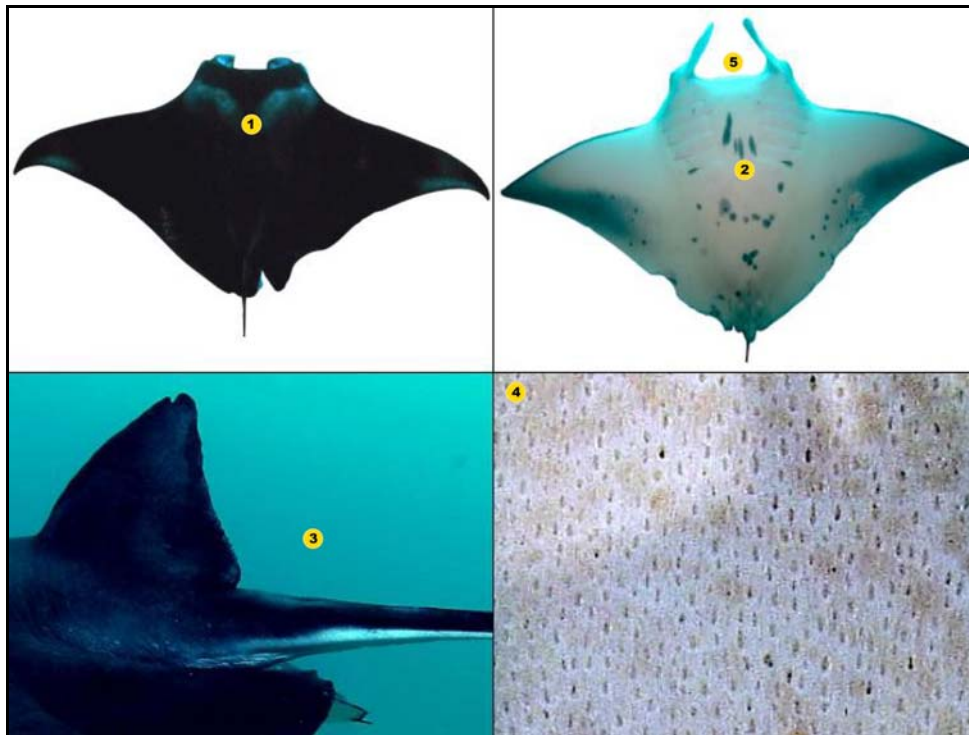
***Manta birostris* variant B**
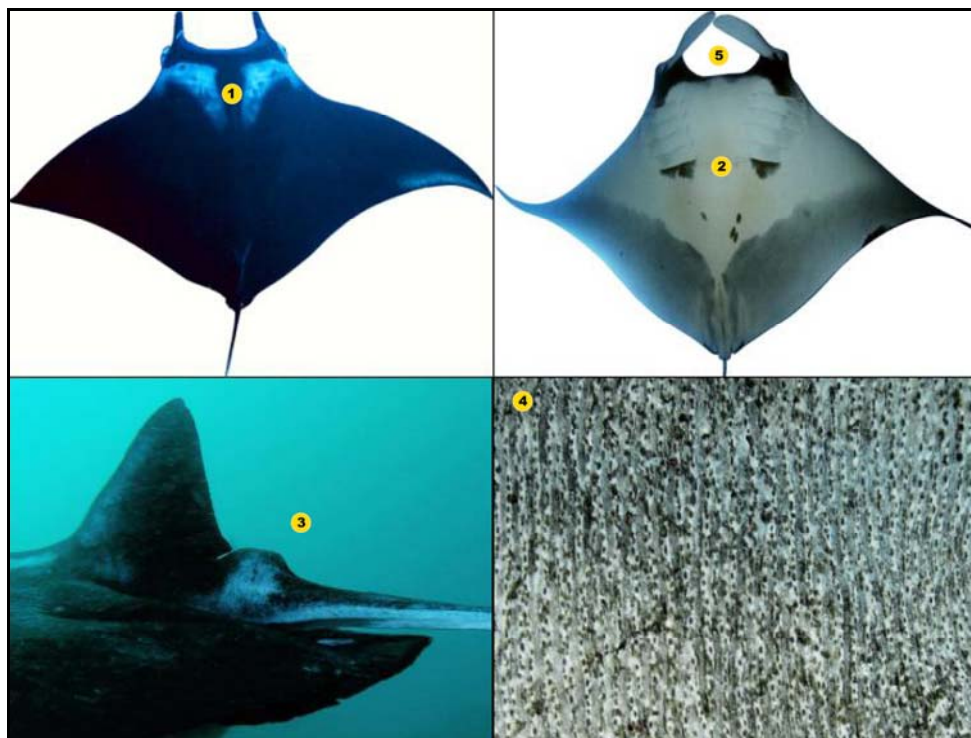
Figure 4.17 Key features used to differentiate *Manta birostris* variant A from *Manta birostris* variant B in the field: (1) supra-branchial shoulder bar shape (2) ventral spot distribution and colouration (3) presence or absence of caudal spine (4) appearance of skin and denticles (5) dentition.

DISCUSSION

The intention of this study was to highlight differences in the two putative species of *Manta* occurring in sympatry in Mozambique and give a preliminary account of their distribution throughout the world's oceans. Additionally a key was constructed which highlights many of the conspicuous and diagnostic features of these two putative species using data collected throughout their respective geographical ranges.

Based on morphometric measurements, several different external characteristics including coloration, dentition, denticle and spine morphology as well as size at maturity, maximum disc width, behaviour and geographic distribution, it is proposed that the genus *Manta* is not monospecific and should consist of at least two species. Additionally a separate worldwide genetics study has been conducted that supports the splitting of the genus *Manta* by demonstrating that reproductive isolation does exist between forms (Kashawagi, Marshall, Bennett and Ovendon in prep.).

Previous studies that examined the natural colouration of manta rays in the Pacific and Atlantic oceans determined that ventral and shoulder bar colouration had no recognizable patterns that allowed for geographical separation (Clarke 2002). Furthermore, Clarke (2002) stated that the results of regional photographic surveys suggested that there was little systematic or taxonomic relevance to colouration as a character in this species. Results from the current study indicate that this conclusion is not valid, mainly because the examination of colouration differences by geographical region would not have been sufficient, as these two putative species have sympatric distributions in many parts of their range.

The current study provides sufficient empirical evidence to warrant the separation of these two putative species of *Manta* and a full revision of the genus with the systematic examination of specimens throughout *Manta*'s range. The results of this study may be useful in this endeavour as diagnostic characteristics have been isolated that may help to differentiate members of this genus in the field, in preserved museum specimens, in photographs and in historical records. One of the limitations of this study was time and sample size. While many individuals were examined in the field, far fewer specimens were available for detailed dissection work. Future research should include a more rigorous morphological analysis of individuals of different size classes, particularly mature individuals across a broad geographic range. While a

wide-scale genetic survey of photographically identified individuals of both *Manta birostris* variant A and *Manta birostris* variant B has already been completed outside of this thesis, continued genetic sampling would be beneficial in other regions. Additionally, genetic analysis on a finer scale at specific aggregation sites could provide more clarity on issues of relatedness, speciation and hybridization.

Chapter 5

Size, structure and survivorship estimates of a photographically identified population of *Manta birostris* variant A in southern Mozambique
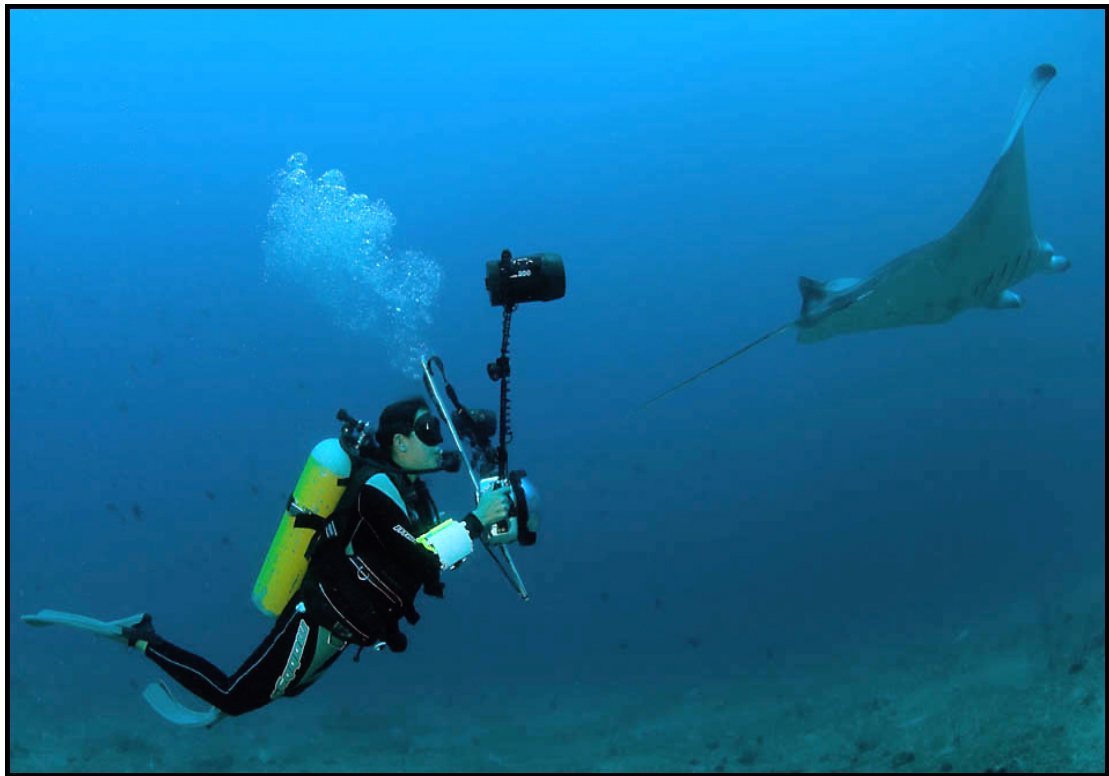
INTRODUCTION

The conservation status of pelagic elasmobranch megafauna remains unclear. This is mainly due to the fact that the vast geographic areas that these species inhabit and the corridors through which they travel are largely unexplored (Amorim 2000; Sims 2000; Martin 2007; Stevens 2007). Subtropical hotspots have been identified worldwide as areas rich in species density and diversity (Worm et al. 2005), with aggregation sites for pelagic megafauna often present and conspicuous (Stevens 2007). These areas offer some of the best opportunities to gather data on species whose population sizes or distributions are otherwise unclear or unknown, a fact which ultimately hampers the assessment and management of their populations and fisheries (Bonfil 2002; Baum et al. 2003).

Manta birostris, the largest batoid fish in the world, is a pelagic ray with a wide-ranging distribution throughout most of the world's tropic and sub-tropic oceans (Briggs 1960; Last and Stevens 1994; Compagno 1999). These large rays are most commonly found around island groups, seamounts and other productive coastal areas, with aggregations of these rays being encountered by SCUBA divers in shallow bays, tidal channels, offshore pinnacles and at cleaning stations (Last and Stevens 1994; Homma et al. 1999; Anderson 2002; Marshall et al. 2006; Dewar et al. 2008). Despite being large and easy to approach, very little information is known about the biology, life history, and ecology of manta rays. Specifically, information is lacking on their population sizes, population structure and demography, and movement patterns.

Mark and re-capture studies using conventional tagging methods are not ideal for species like *M. birostris* as the required effort is often high, tag return often low and the application of tags may run the risk of disturbing local study populations (Minta and Mangel 1989; Kohler and Turner 2001; Castro and Rosa 2005). Additionally, because there no industrial fisheries for manta rays and artisanal fisheries are often challenging to work with, tag recovery from these sources may be particularly difficult. *Manta birostris* is also likely a long-lived species, which may additionally hamper the compilation of useful information, as data collection may be required over extended periods of time and wide spatial scales, with tag-fouling and tag-shedding presenting fundamental problems (Kohler and Turner 2001; Feldheim et al. 2002a).

As an alternative to tagging, visual sight-resight techniques using natural markings or scars, present a non-intrusive, potentially more accurate way to collect similar data (Pollock 1974; Hiby and Hammond 1989; Stevick et al. 2001; Evans and Hammond 2004; Castro and Rosa 2005; Auger-Méthé and Whitehead 2007). While it has some similar limitations to conventional tagging methods such as limited temporal and spatial coverage, researchers that involve commercial and recreational divers/operations through public programs can often use photo identification in an efficient and long-term manner. As a result, photo-identification (Photo-ID) has become an increasingly common and accepted technique, with several aquatic studies having confirmed its accuracy as a research tool (Würsig and Jefferson 1990; Stevick et al. 2001; Auger-Méthé and Whitehead 2007; Bansemer and Bennett 2008). Photographic sight-resight techniques, however, are not commonly employed in elasmobranch research as many shark and ray species lack suitable natural markings (Castro and Rosa 2005; Porcher 2005; Meekan et al. 2006). Photo-ID provides an effective, low-cost alternative to conventional marker tagging and may be particularly appropriate when examining critically endangered species or populations (Bansemer and Bennett 2008).

While research using photographic sight-resight techniques on elasmobranchs is still in its infancy, studies to date have examined spotted eagle rays (*Aetobatus narinari*), using the distinctive patterns on the dorsal surface of the body (Corcoran and Gruber 1999); white sharks (*Carcharodon carcharias*), using unique shapes and spot patterns on dorsal fins (Anderson and Goldman 1996; Klimley and Anderson 1996; Bonfil et al. 2005) and body patterning and scars (Domeier and Nasby-Lucas 2006); basking sharks (*Cetorhinus maximus*) using dorsal fin shape (Sims et al. 2000); nurse sharks (*Ginglymostoma cirratum*) and black tip reef sharks (*Carcharhinus melanopterus*) using distinctive marks and scars on the body and fins (Castro and Rosa 2005; Porcher 2005); and whale sharks (*Rhincodon typus*) (Arzoumanian et al. 2005; Meekan et al. 2006) and spotted raggedtooth sharks (*Carcharias taurus*) (Van Tienhoven et al. 2007; Bansemer and Bennett 2008) using natural spot patterns, coupled with marks and scarring. Species that are suitable or good candidates for photo-ID tend to posses one or more common characteristics: relatively small number of animals in a population or group; distinctive markings present in a high proportion of individuals; individuals large in overall size; and in many cases concern over a

species conservation status precludes more invasive studies. Manta rays have unique ventral markings that allow for individual identification. Although a comprehensive study is absent from the literature, photographically identified individuals have been monitored in various locations including the Maldives (Guy Stevens, pers. comm.), Hawaii (Tim Clark and Mark Deakos pers. comm.), Mexico (Robert Rubin, pers. comm.), Brasil (Luiz et al. 2008), New Zealand (Duffy and Abbott 2003), Australia (Frazer McGregor and Mike Bennett pers. comm.), Yap (Bill Acker pers. comm.), and Japan, where twenty-years of unpublished observations have confirmed the longevity of these natural markings (Homma et al. 1999).

This study aims to promote the use and standardisation of photo-ID techniques for *M. birostris* variant A as a means to study this species in a non-intrusive way. Applying this technique, we report on the structure, and seasonality of a photographically identified population of *M. birostris* variant A in southern Mozambique. Additionally using photographic sight-resight data, estimates of the apparent survival and capture probabilities were constructed and yearly and super population estimates (estimate of the total number of individuals using the examined area) were ultimately produced. These numbers can be valuable when considering the conservation status of a population. This is the first time that super population estimates for a photographically identified population of manta rays has been produced.

METHODS

The two field sites examined in this study supported three and two major cleaning stations for manta rays respectively (Chapter 2 and 7), with *M. birostris* variant A using both sites year-round. The primary field site, Manta Reef, was examined more regularly (76% of total dives) as this reef was the shallower of the two reefs (affording divers longer bottom time), had more cleaning stations and did not require a drift ascent which could compromise the safety of the divers. Scuba dives to the two examined reefs were conducted between 8:00 am and 3:00 pm, although, as a result of weather and launching conditions, the majority of dives took place between 8:30 am and 12:30 pm. Field seasons which started in the month of May each year and ended the following April were completed in 2003-2004, 2004-2005, 2005-2006, and 2006-2007 and will henceforth be referred to as Year 1, Year 2, Year 3, and Year 4.

Photographs and measurements

During each encounter, a picture of the ventral surface of the manta ray was taken and, if possible, one was also taken of the dorsal surface and any other identifying characteristics/markings (Fig. 5.1a-h). The sex of the animal was determined through the presence or absence of male reproductive organs (claspers) located on the pelvic fins (Fig. 5.1c,e). Male reproductive state was assessed through visual (Fig. 5.1c,d) and on occasion tactile examination of the claspers following Walker (2005) (Chapter 6). During tactile examination, the claspers of male manta rays hovering over the reef while being cleaned were quickly manipulated using an un-gloved hand. This technique could not be used for juvenile manta rays as the claspers were too small, but worked with sub-adult and mature individuals. Pregnancy was determined through observations of distended stomachs in female mantas (Fig. 5.1f).

Once the appropriate pictures were taken, a size estimate of the animal was obtained. Size estimates were achieved using one of three methods. Disc width (DW), the distance between the tips of the pectoral fins, was often estimated based on known lengths of divers swimming immediately above or below the animal as it hovered above the reef. Between 2003 and 2005, estimates were also made using a measuring tape, as rays hovered above cleaning stations (Chapter 6). Instead of attempting to measure the entire animal, divers measured from the midline of the manta ray to the tip of one pectoral fin and doubled the value for a total DW estimate. This method was found to slightly decrease the margin of error inherent in measuring such a large animal underwater. Starting in the 2006 field season, a custom-made bracket, supporting two fixed lasers projecting parallel beams of light (50 cm apart), was attached to an underwater camera housing. Employing this system, when photographs of the dorsal or ventral surface of the manta ray were taken flat-on, two small red dots, 50 cm apart, were visible in the resulting image (Fig. 5.1a) allowing the approximate size of the manta ray to be extrapolated. The system was calibrated on the morning before use to ensure that the lasers had not moved and were providing parallel beams. All three measurement techniques were employed by the same individual to reduce recorder bias. Additionally, all inherent errors in measuring large animals in the field affected the measurements of both male and female rays. As this study employed different techniques and methods for obtaining disc width estimates, size classes were examined and compared, rather than exact measurements. Based
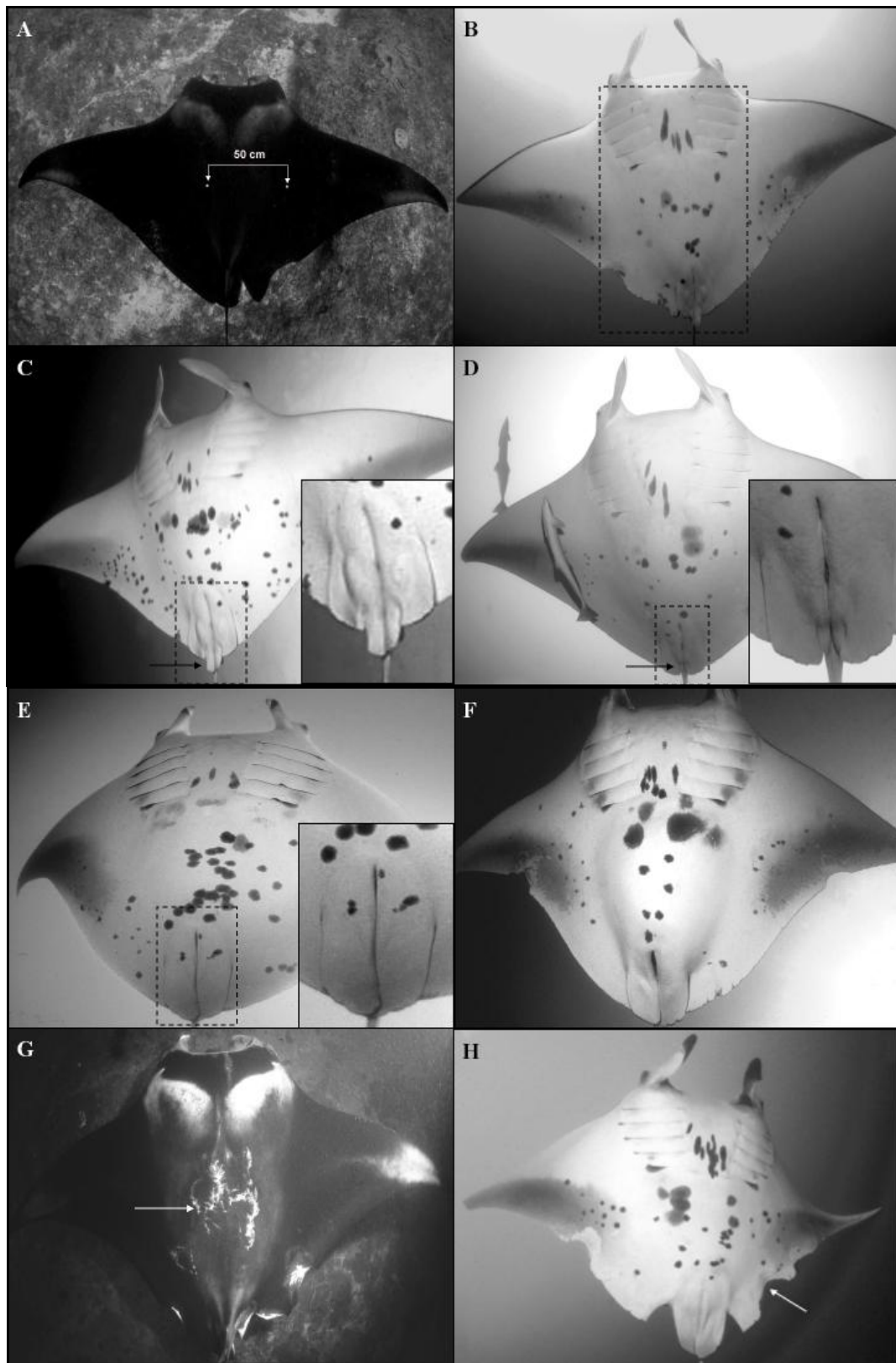
Figure 5.1 Distinctive markings on *Manta birostris* variant A: (a) dorsal surface with laser spots indicating a 50 cm distance; (b) ventral spot patterning showing standardised area for ID shots; (c) a mature male with boxed area showing enlarged claspers; (d) an immature male with boxed area showing juvenile claspers; (e) a female with boxed Area showing pelvic fins; (f) pregnant female; (g) natural markings and bite mark scars on the dorsal surface; (h) distinctive bite marks from sharks on the body of a female ray.

on the smallest and largest individuals encountered, four size class categories were used: <3m DW, 3.0-3.9m DW, 4.0-4.9m DW and >5m DW.

Identifying marks

Distinctive patterns of spots and patches on a manta ray's ventral surface (Fig. 5.1b-f,h) were used to distinguish between individuals. Spot patterns are present in near-term embryos and neonate individuals (Marshall et al. 2008; Chapter 3) and occur in both male and female rays. Images of the spot patterning were captured with still cameras and occasionally video cameras, with or without an artificial light source while SCUBA diving or snorkeling. When photographing individuals, images of both the dorsal and ventral surfaces were taken when possible. Although natural markings on manta rays sometimes occurred across the entire ventral surface of the animal (Fig. 5.1c,h), it was considered appropriate to standardise an area of the body for photographic identification and comparison. For this study, the region was defined as a rectangle-shaped area extending posteriorly from the anterior margins of the anterior gill slits to and including, the pelvic fins (Fig. 5.1b). While some individuals had natural spot markings across the entire ventral surface (Fig. 5.2a,b), others lacked spots in between the gill slits (Fig. 5.2c) or had few spots on the abdomen (Fig. 5.2d). The combination of both of these regions and the inclusion of the pelvic fins was ultimately chosen for standardisation because it was easy to define, was not distorted by the degree of pectoral fin flexion, consistently had the most distinctive patterns of any area on the ventral surface, and showed the sex of the animal. At least one good quality image of this area was required for the identification of each manta ray.

The majority of manta rays (>75%) had distinctive injuries to their bodies or fins (Fig. 5.1g,h) that were attributed to boat strikes, entanglement and predominantly shark attacks (Chapter 8). While the shape or the colour of an injury or scar might potentially alter over time, the presence or absence of a scar, bite mark or amputation was often used to further confirm the identification of an individual whose ventral surface had already been matched. Likewise the coloration and shape of patterns on the dorsal surface (Fig. 5.1a,g) were also used to confirm re-sightings.

In addition to maintaining a photographic image database (Fig. 5.3), a detailed black and white composite sketch was created of each manta ray identified which incorporated all known information on that animal into one dorsal and ventral view

(Fig. 5.4). Sketches were consistently updated with additional information about the animal (e.g. new bite marks or scars). As the most complete and up-to date information of the individual was available on the sketch, they were primarily used when making a re-sighting. Whenever a new image was matched to an existing sketch, it was checked against the definitive image of that individual in the photographic database to verify its identification.
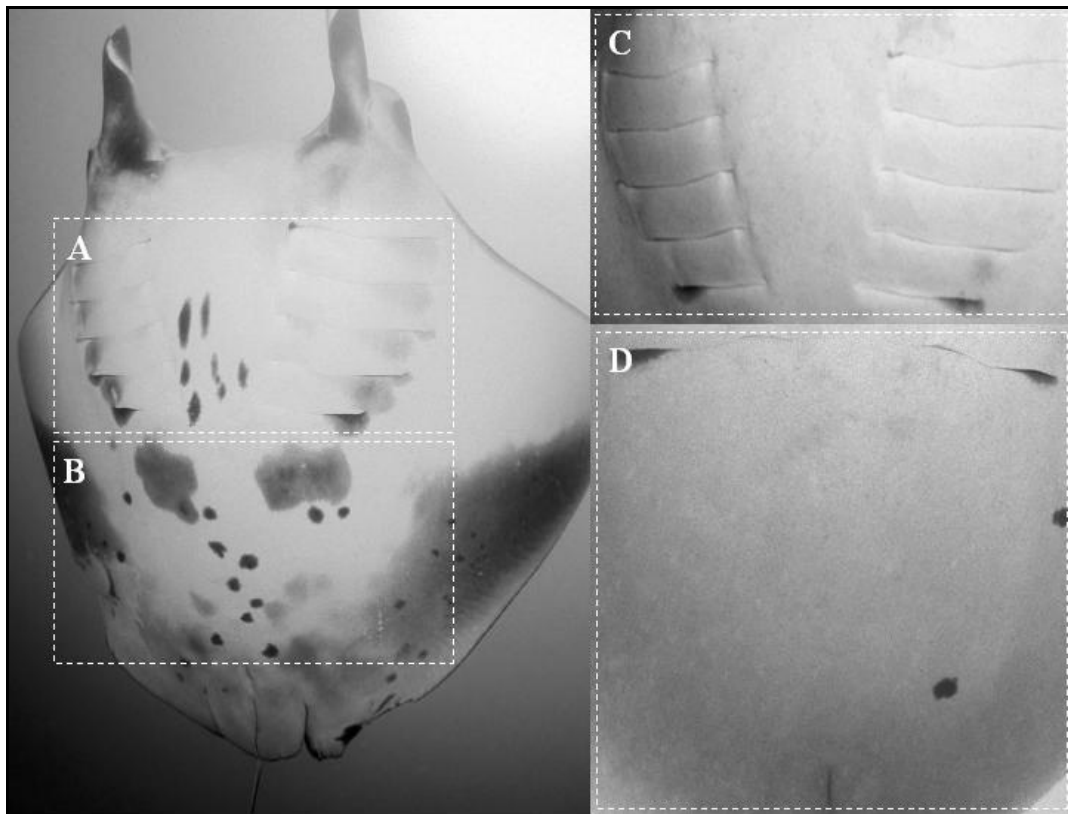
Figure 5.2 Natural markings on the ventral surface of *Manta birostris* variant A: (a) between the gill slits; (b) on the abdomen. Few or no natural markings on the ventral surface of *Manta birostris* variant A: (c) between the gill slits; (d) on the abdomen of two separate individuals.

Figure 5.3 Manta ray information and image database for the southern Mozambican region.
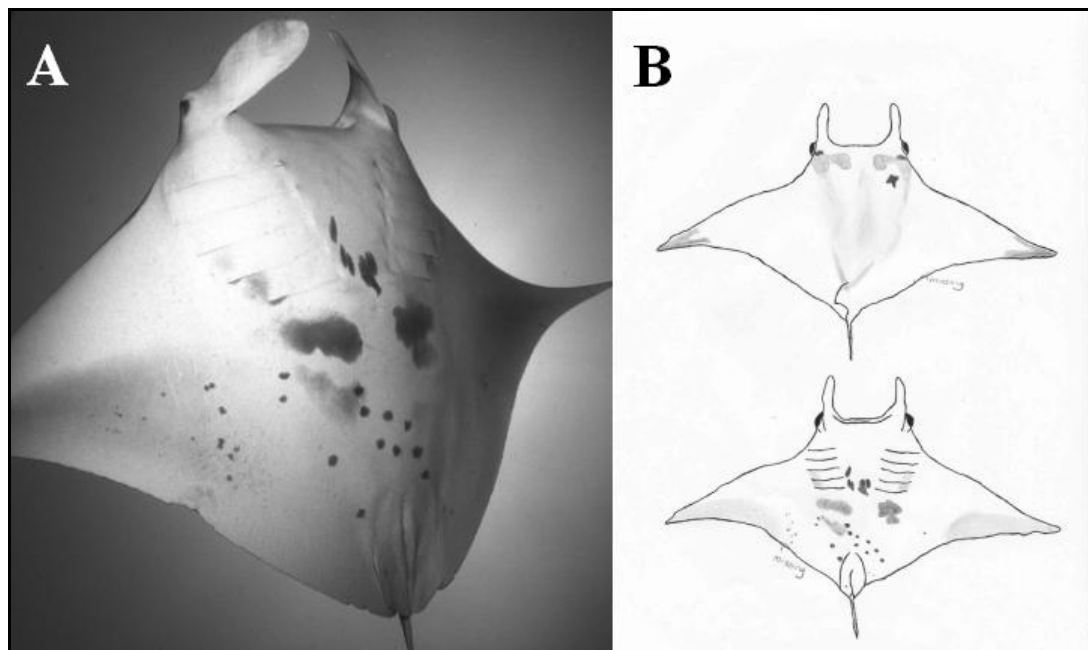
Figure 5.4 Female *Manta birostris* variant A: (a) identification image; (b) sketch.

Comparison of images

All photographs were divided into smaller more manageable groups for comparison to the photo and sketch databases on the basis on gender and occasionally distinctive scars, maturity or size. New images were cropped and the brightness or contrast of images were often manually adjusted to enhance the spot patterning. All photographs were sorted and matched by a single person, with random subsets of the master database and re-sighting events checked by two independent observers for accuracy.

All comparisons of photographs were done by eye. Although automated computer programs have been used for comparing spot patterns within various species of elasmobranchs (e.g. whale sharks and spotted raggedtooth sharks) (Arzoumanian et al. 2005; Van Tienhoven et al. 2007), the presence of many obvious identifying characteristics made identification by eye manageable. The study of a larger population or comparisons (cross-matching) of different populations would benefit from the use of a computer-based image-matching program.

Identifications and re-sightings

On each SCUBA dive, specific transect routes across study reefs were conducted. On both reefs the length of the transect was approximately 400 m. For Manta Reef the average transect time was 40 minutes, while for Giant's Castle the average transect time was approximately 20 minutes. Divers swam in a uni-directional fashion along a non-linear transect route that bounded all monitored cleaning stations. The total number of individuals seen along the reef transect during the dive was recorded. At a later stage, using photographic images or video footage, the total number of identifiable individuals per minute of the dive was also determined.

New individuals were accepted into the local database only when there was a minimum of one good image of the standardised ventral area of an individual. New identifications were plotted over time in a discovery curve to show the rate at which newly identified individuals were recruited into the population database. Re-sighting events were characterized by the positive identification of a previously known individual more than 24 hours after it was initially seen. Re-sighting events were tracked over time at both reefs as well as noted at other dive sites along the coastline.

Seasonality

To examine seasonal trends, both the total number of rays and the total number of photographically identifiable rays seen per minute of observational dive time (transect time) were calculated for each dive and then pooled by calendar month. Data were combined for the entire four-year sampling period and the means were compared across calendar months. For each of the months of November, December and January, there were four years of data combined; for May, October and February, three years; for July, August, September, March and April, two years and July was represented by a single year's data. The primary approach, referred to as sighting per unit effort (SPUE), offered the most realistic indication of manta ray abundance at the study sites for each month of the year as it reflected the total number of *M. birostris* var. A counted along the transect. While this approach conceivably allowed for the possibility of re-counts, manta rays were most often cleaning at one of the three cleaning stations during transect counting and did not often move from station to station. Using distinctive bite marks, spot patterns and the sex of sited individuals to identify between individuals encountered along the transect, an individual was rarely double counted if seen at a previous station. The latter approach, identifications per unit effort (IPUE), represented the minimum number of rays seen on the transect reef per dive without the possibility of recounts. These counts were a reflection of the photographic records from the dive and were counted at the end of each day. As commonly not all manta rays on the reefs each day could be positively identified, these data were analyzed separately in order to see if the total number of rays seen per dive and the total number of individual rays photographically identified during each dive differed significantly enough to warrant the use of both methods in future research endeavours.

Statistical analysis

Chi-square (χ^2) analysis was used to compare sex ratio data. Yates' correction factor was applied to all Chi-square tests where there was one degree of freedom. Seasonal SPUE and IPUE data were examined using the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks as data were non-normal and could not be normalized. An all-pairwise (Dunn's method) was used as the post-hoc test. Significance was accepted at $P < 0.05$. Analyses were conducted using Sigmastat (SigmaStat[®]

S.P.S.S.). Box plots were used to graphically represent non-normalised data, with the box itself representing the 25% and 75% confidence intervals (CI) and the whiskers representing the 5% and 95% CI. While the median values are reflected by these box plots, the mean is also displayed by a white line. Outliers are indicated by solid dots with the numbers directly over them indicating the number of sampling events (dive transects).

Population size

Sight-resight data from 2003 to 2006 was incorporated into four separate annual re-sighting periods based around the reproductive cycle of the *M. birostris* variant A in Mozambique (Chapter 6) rather than the calendar year. These annual re-sighting periods corresponded to the photographic survey's field seasons (e.g. Year 1, Year 2, etc.). As the assumption of population closure is violated when temporary emigration occurs (Otis et al. 1978), the open population Jolly-Seber model (Schwarz and Arnason 1996) was applied using the POPAN option in the program MARK version 4.3 (White and Burnham 1999) to estimate super population size from the identified individuals at the monitored aggregation site. Twelve models were fitted within the program to estimate manta ray abundance ($\hat{N}$) for each sex (g) for each cumulative year and for the entire sample period. Some models allowed for variability in time while others held time constant. Half of the models allowed for the effect of time on the probability of entry into the population (β). In the other half of the models, when β was kept constant and parameters were fixed to the value of 1 to account for extremely high confidence intervals (CI), which had caused them to be essentially inestimatable. Capture occasions also provided estimates of apparent survival (φ), capture probability (*p*), and probability of entry into the population (β) per year. Models were fitted using the logit link function for $\hat{\phi}$ and $\hat{p}$, the identity link function for $\hat{N}$, and the multinomial logit link function to constrain the set of $\hat{\beta}$ parameters to ≤ 1 (White and Burnham 1999). To correct for small sample sizes, the Akaike's Information Criterion (AIC$_c$) (Burnham and Anderson 2002) was employed to compare between the fitted models.

RESULTS

Effort and photo identification

In total, 15,930 minutes (358 dives) over a four-year period were spent underwater at the field sites. In the first year, 1665 minutes of effort were completed at the field sites, in the second, third and fourth seasons, 4635, 4410, and 5220 minutes were spent at the sites respectively. A total of 3,524 positive slides and 765 digital images of *M. birostris* variant A, including those captured from video, were taken at the two study reefs during the study period. Over 90% of the images taken of the standardised ventral area during the four-year study were of suitable quality for positive identification of individual manta rays.

Based on all examined images of *M. birostris* variant A, spot patterning in this population was determined to be highly variable in design (Fig. 5.1b-f,h) but not affected by sex or ontogeny, with similar spot patterning seen in all representative groups. Ventral patterning in the standardised area of all re-sighted individuals did not change during the study period (Fig. 5.5a,b). Spot patterning near to the margins of the pectoral fins was sometimes obscured or altered where individual rays had sustained injuries from sharks including scratches, scaring or missing flesh. With the exception of additional trauma to areas already containing bite marks or scars, all bite marks maintained their integrity throughout the study period as well (Fig. 5.5c,d). Even in cases where fresh scars or bite marks healed, there was still evidence of the original bite wound in re-sighted individuals.

Occurrence and re-sightings

A total of 852 observations of individual rays were made at the two study reefs during the four-year study period between 2003 and 2007. From these observations, 449 *M. birostris* variant A individuals were identified (Fig. 5.6). Sighting data from the two reefs were pooled as acoustic telemetry data (Chapter 7) and photographic data demonstrated that the same manta rays were using both study reefs (which were close in proximity to one another) as a part of their larger habitat. New individuals were identified in every sampled month except for September 2004, February, May and June of 2005, September 2006, and February 2007.

Despite numerous observations during the study period, *M. birostris* var. A was absent from the transect areas on the study reefs on 91 of the 354 dives completed during the study period. The mean number of individuals observed along the transect during dives was 4.81, however if examined only on days when at least one individual was present, then the mean number of individuals seen during surveys of the study reefs was 6.47 (Fig. 5.7).

Of the 449 individuals identified in this study, 182 (40.5%) were re-sighted on at least one occasion, with 403 re-sighting events made in total (Fig. 5.8). Of the identified individuals, 327 were sighted only within the initial year of identification. Eighty-seven and 27 individuals were seen in a total of two and three of the four years respectively, with only 8 individuals seen in all four years of the study period (Fig. 5.9). The mean number of re-sights for an identified ray in the observed populations was 2.21. The longest period between re-sighting events was 1252 days.
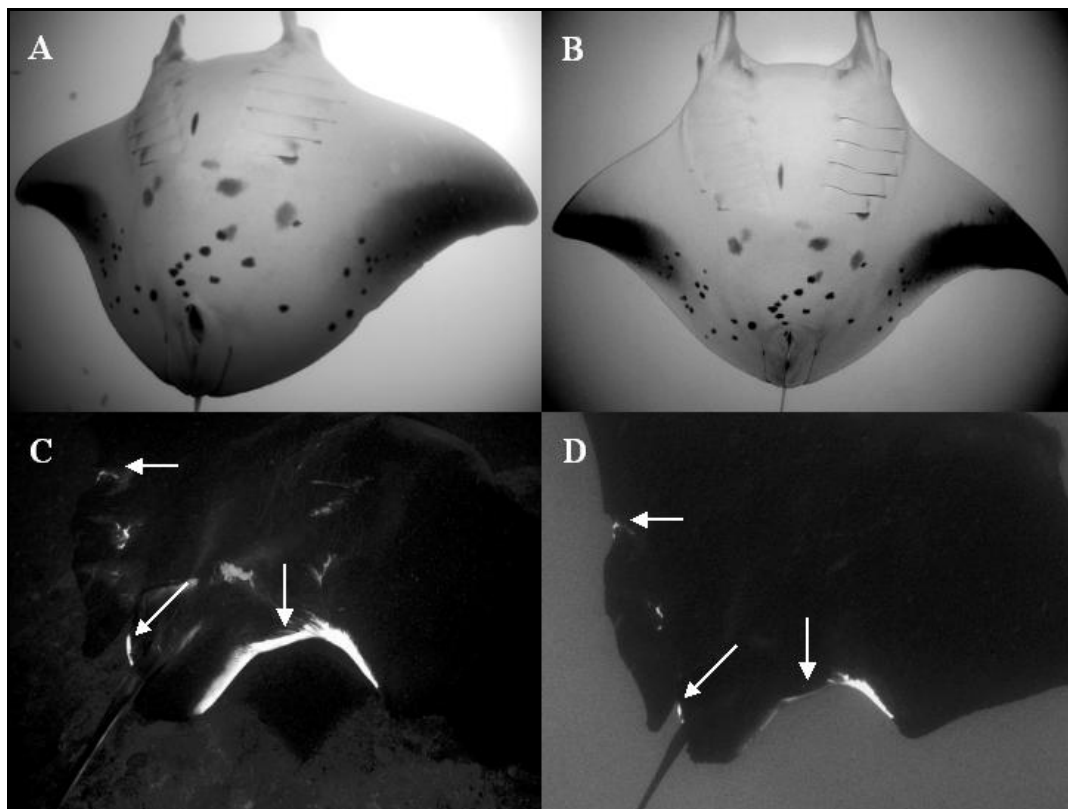
Figure 5.5 Example of unchanged spot patterning and bite marks in re-sighted individuals. Identification image of individual #081 in: (a) May 2003; (b) January 2007. Bite Mark of individual #018 in: (c) 2003; (d) 2006.
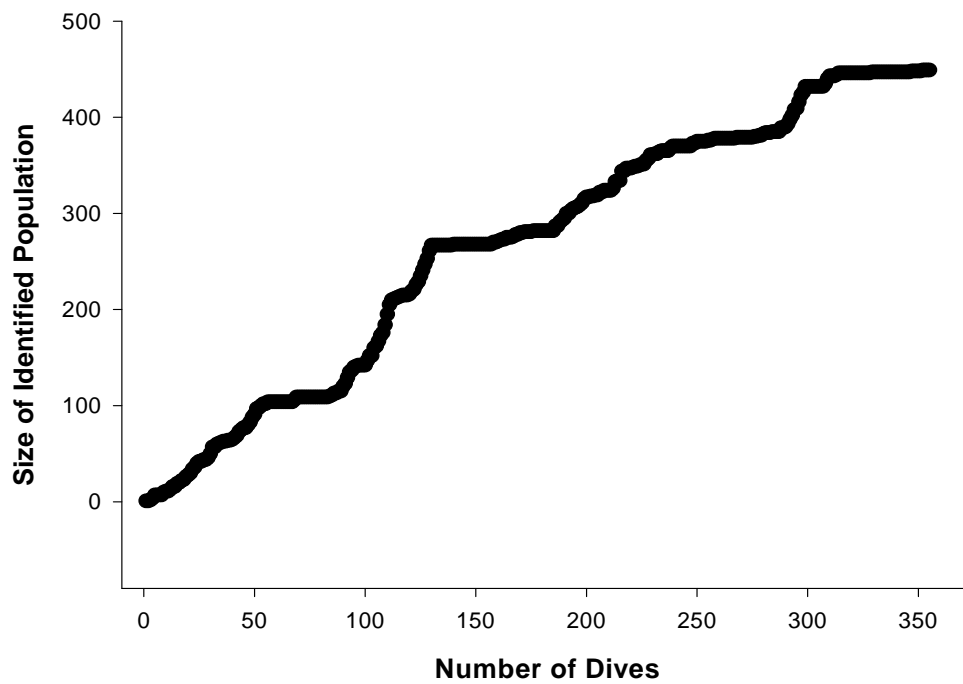
Figure 5.6 Discovery curve illustrating the rise in total number of identified individuals over the study period.
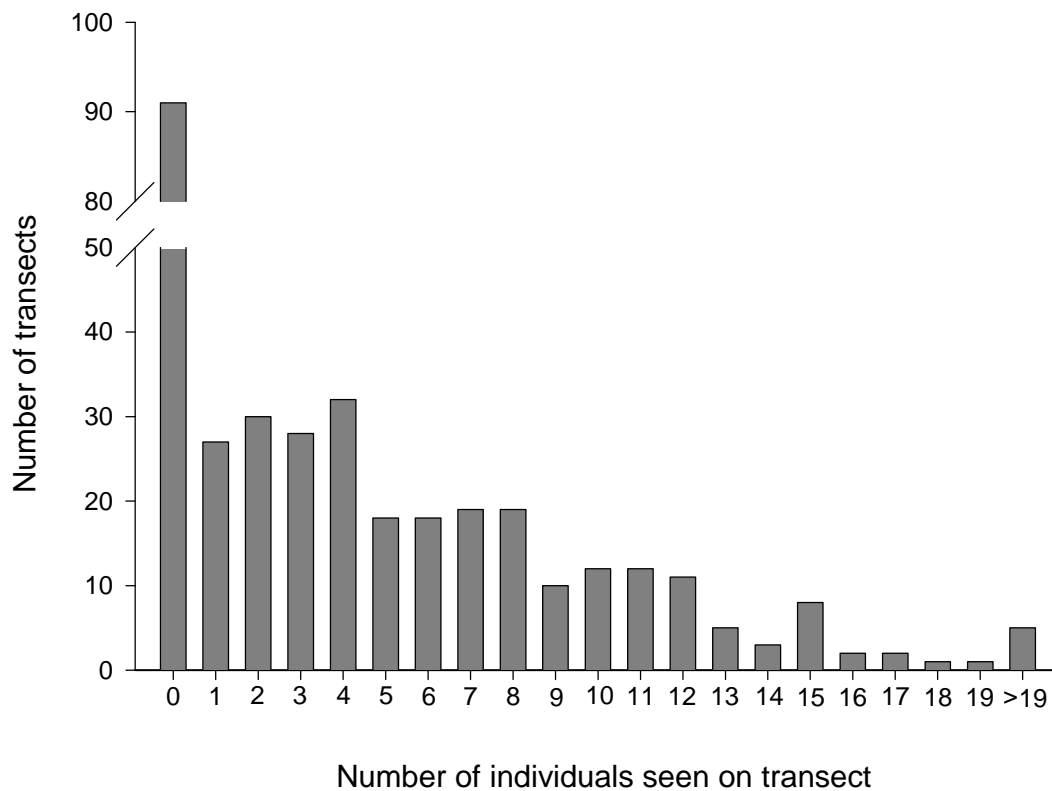
Figure 5.7 Total number of *Manta birostris* variant A recorded per dive transect.
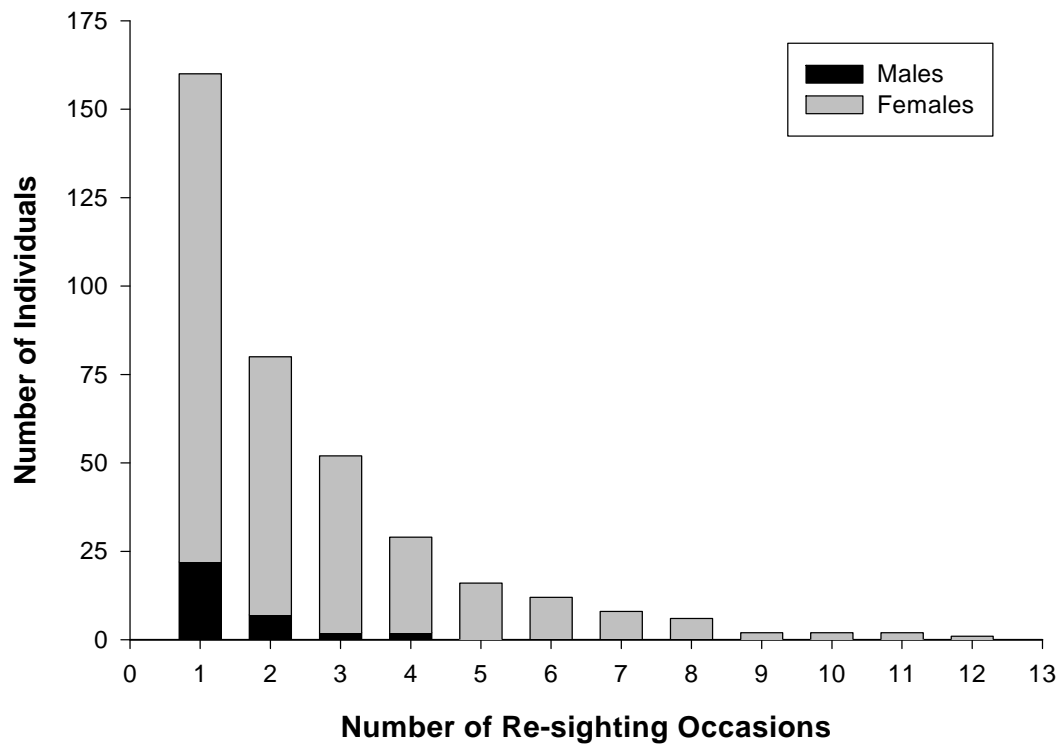
Figure 5.8 Total number of male and female individuals re-sighted during study period.

In years 1 and 2, the number of newly identified rays exceeded the re-sighting of previously identified individuals, but in year 3 and 4, the number of re-sights exceeded new identifications (Fig. 5.10). The ratio of re-sighted individuals to new individuals steadily increased in the first three years of the study but declined slightly in the fourth year.
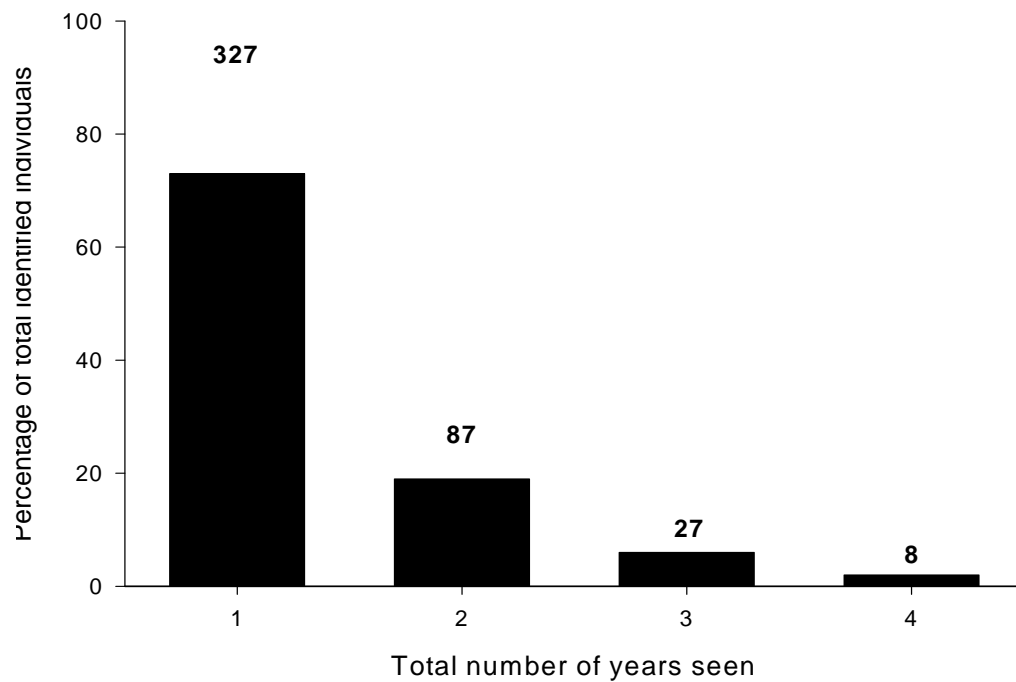
Figure 5.9 Total number of years each identified ray (n=449) was seen during the four-year study.
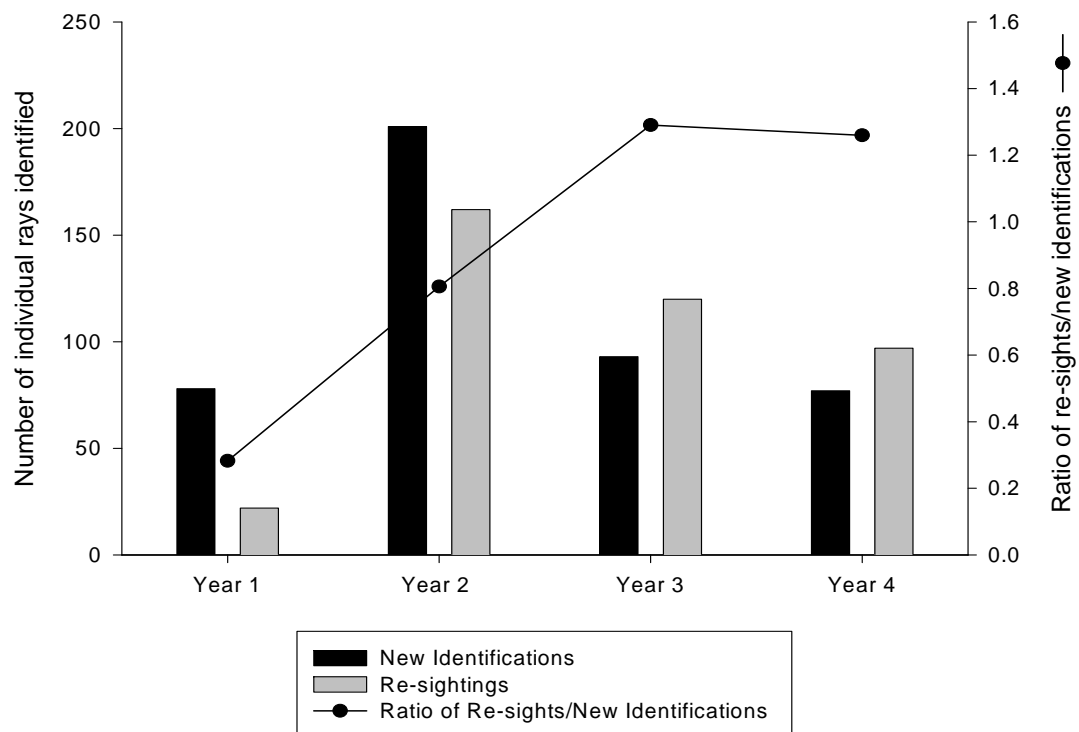
Figure 5.10 Total number of individuals identified and total number of re-sights in each of the years of the study and the ratio of re-sights/new identification plotted over the four years.

Sex ratios and size distribution

Of the total *M. birostris* variant A identified, 78% were female and 22% male, indicating a highly significant sex bias (χ^2 =140.31, df = 1, P < 0.0001). When examined by year, a significant difference in the sex ratio of sighted individuals was observed in all years (Fig. 5.11), with a bias towards females (ratios of 1:3.2, 1:5.4, 1:4.9, 1:10.7; chi-square for each year P < 0.0001).

Of the 99 males identified 89.9% were mature, while 3% and 7.1% were classified as sub-adult and immature respectively. Using pregnancy as an indicator of maturity, 17.1% of the females identified were classified as mature. However, with 42.9% of the identified females bearing visible reproductive scaring on their pectoral fins from mating (details in Chapter 6), it was considered likely that at least 49.7% of females were mature.
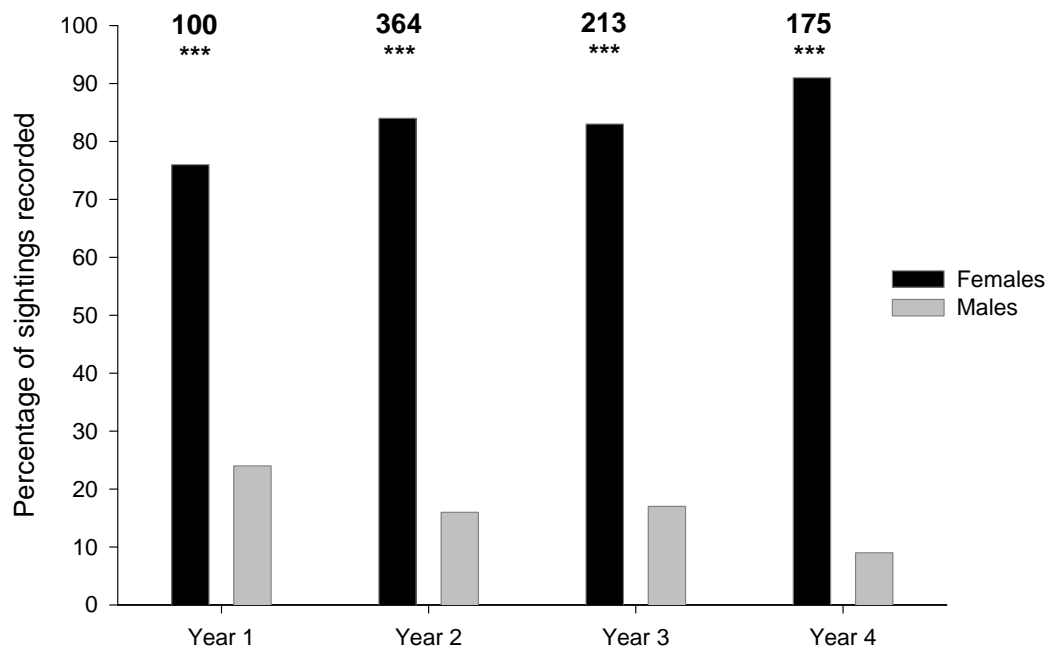
Figure 5.11 Number of male and female rays observed in each year. Numbers across the top indicate total number of individuals observed that year, *** indicates significance to the P < 0.001 level.

Of the 403 re-sighting events, 91.8% of the re-sights were of female rays and 8.2% were of males. There was a significant differences in the re-sightability of male and

female rays (χ^2 =7.66, df = 1, P = 0.006), with 45.7% of the total 350 identified female manta rays re-sighted on at least one occasion as opposed to only 22.2% (99) of the male population. The maximum number of re-sighting events for any individual between 2003 and 2007 was 4 times for males and 12 times for females, while the mean number of re-sighting events was 1.50 for male rays and 2.21 for female rays.

Only seven (1.6%) individuals in the identified population were less than 3 m DW, with most individuals ranging in size from 3.0-3.9 m DW to 4.0-4.9 m DW (22.5% and 75.1% respectively) (Fig. 5.12). Only 4 (0.9%) individuals, all females, were over 5 m DW. When analysed by sex, the vast majority of the females (85.7%) were between 4.0-4.9 m in disc width where in contrast, the majority of males (56.6%) were sized between 3.0-3.9 m DW.
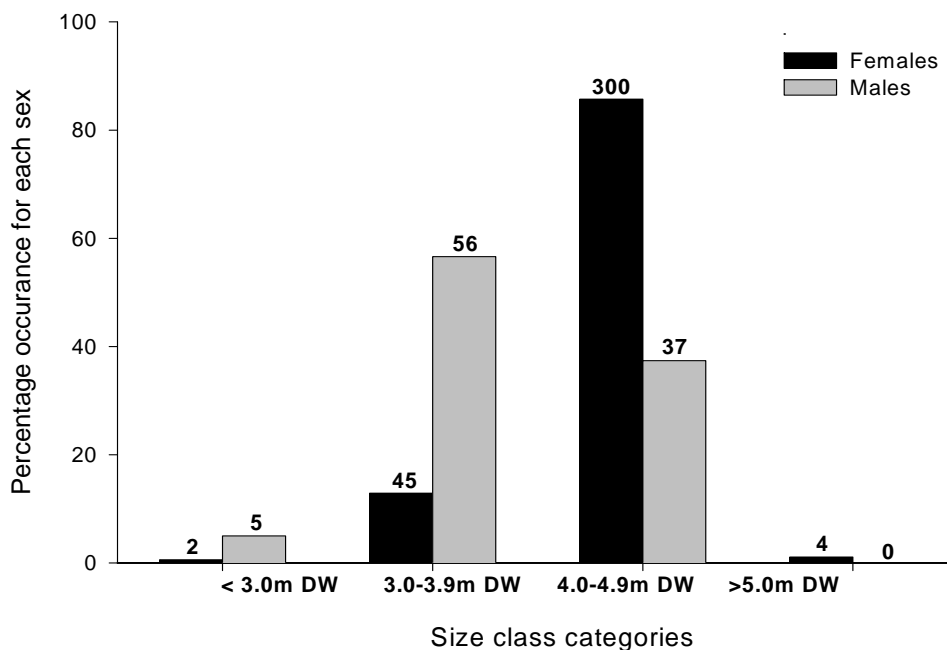
Figure 5.12 Size distribution of identified male and female manta rays. Numbers above the bars represent sample size.

Seasonality

Manta birostris variant A were present during all months of the calendar year (Fig. 5.13a). Dive transect effort was consistent over the study period as dive routes were very similar and the conditions and dive profiles did not commonly vary. The

maximum number of rays seen per dive during the study period was 30 individuals, with the months of November, December and January the only ones where an excess of 20 rays were seen during a single dive.

A Kruskal-Wallis one-way ANOVA on ranks demonstrated a significant difference among months in the median values of manta rays seen per minute of dive time (SPUE) (P < 0.001). The post-hoc all-pairwise comparison, however, only revealed significant differences between the months of May, August, November, December and January and the month of March (P < 0.05) (Fig. 5.13a) A Kruskal-Wallis one-way ANOVA on ranks also revealed a significant difference in the median values of identified individuals seen per minute of dive time (IPUE) (P < 0.001), with the post-hoc all-pairwise comparison this time showing significant differences between the months of November, December and January and the months of March and June (P < 0.05) (Fig. 5.13b).

A

B
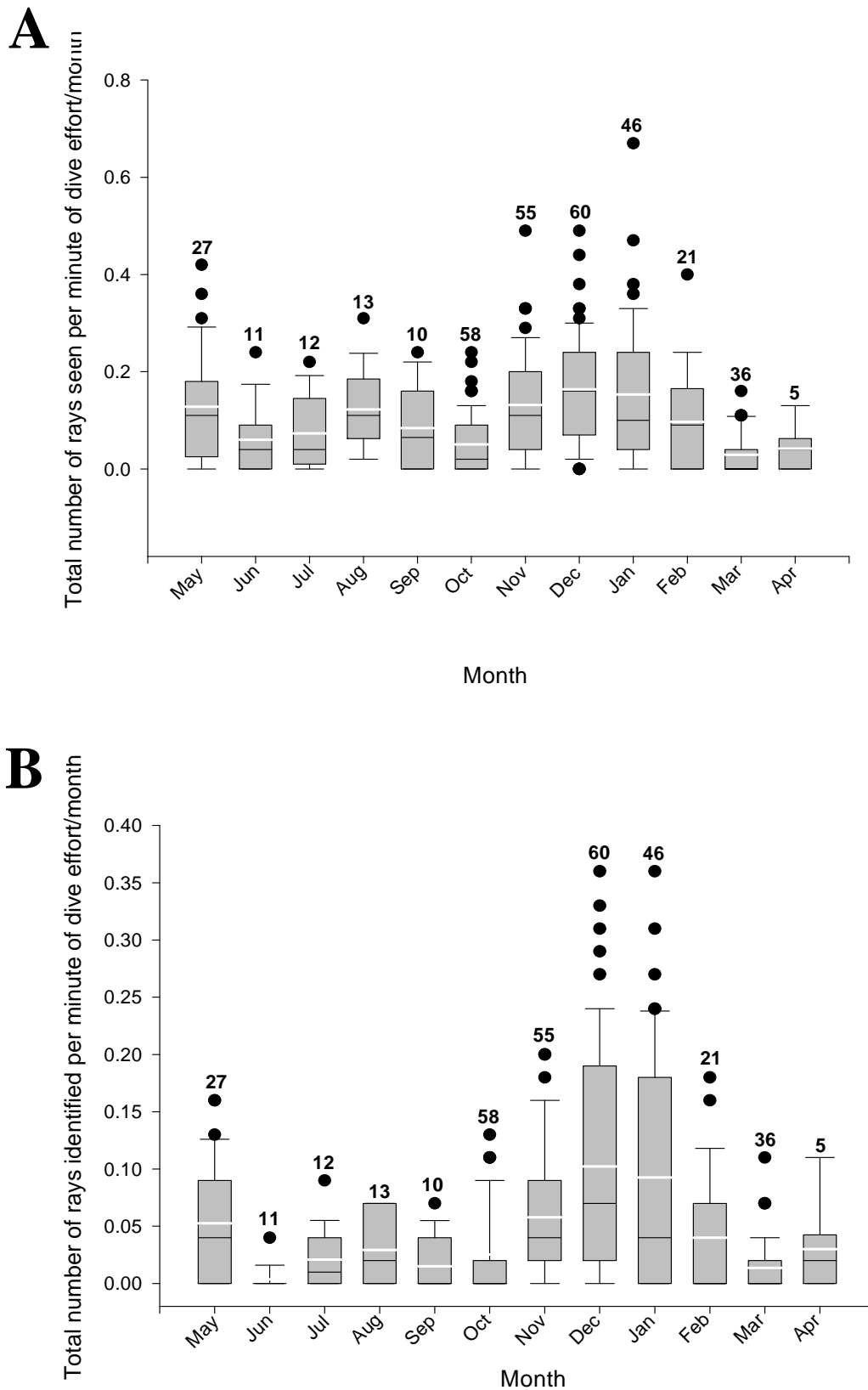
Figure 5.13 Inter-month comparisons: (a) SPUE median number of rays seen per minute of dive effort/month (all years); (b) IPUE median number of rays identified per minute of dive effort/ month (all years). Numbers in graph indicate the total number of dives for each month over the four-year study period. White lines indicate mean.

Population size and survivorship estimates

Of the 12 models fitted, the most parsimonious model was $\varphi(g)$ $p(g)$ $\beta(t)$ N(g) with an AIC$_c$ weight equal to 0.81. This particular model allowed for group differences (g) in apparent survival (φ) and capture probability (p) and allowed probability of entry (β) to vary with time. Four models were fitted that allowed survival probability to vary with time but as these models did not converge they were abandoned. All of the other seven fitted models converged although they were weighted inferior to the most parsimonious model, with only three additional models demonstrating information theoretic support (Table 5.1). These three models and the most parsimonious model were used for model average estimates of apparent survival (φ), recapture probability (p), probability of entry (β), yearly derived population estimates ($\hat{N}$) and derived super population estimates ($\hat{N}$ *) for both males and females.

For each of the four capture occasions, population estimates ($\hat{N}$) were calculated for males and females (Fig. 5.14). The super population estimate ($\hat{N}$ *) for female rays was 599.66 (SE = 33.09, 95% CI = 534.81-664.52) with 0.22% variation attributable to model variation and for male rays was 290.43 (SE = 60.66, 95% CI = 171.52-409.33) with 12.23% variation attributable to model variation, equalling a total of 890.09 individuals (95% CI = 706.33-1073.85). Standard errors were not large, suggesting a degree of confidence in estimates. However, given the extreme sex ratio of the observed population, this estimate may actually be more representative of the super population of female rays.

Female *M. birostris* var. A showed higher survival probabilities than males at 0.66 (SE =0.05, 95% CI = 0.55-0.76, % variation attributed to model variation 0.73%) compared to 0.42. (SE =0.11, 95% CI = 0.23-0.64, % variation attributed to model variation = 17.51%). Recapture probability was also higher for female rays at 0.49 (SE = 0.06, 95% CI = 0.38-0.60, % variation attributed to model variation 0.30%) than for male rays which was 0.37 (SE =0.12, 95% CI = 0.17-0.63, % variation attributed to model variation = 26.35%). Probability of entry did not vary with sex and was estimated to be 0.53, 0.10 and 0.14 for each of the three capture session with a standard errors of 0.05, 0.05, 0.03 (CI = 0.44-0.62, 0.04-0.25, 0.09-0.20) respectively. Probability of entry was high in the first capture session at 53% but dropped markedly in the second two capture sessions (Fig. 5.15).

Table 5.1 Comparison of models from the POPAN analysis used to estimate the abundance of *Manta birostris* variant A. AICc = Akaike Information Criterion for small samples; Delta AICc = difference in the AICc of a model from the minimum AICc model; AICc weight = Akaike weight used in model averaging; φ = apparent survival; *p* = capture probability; . = time constant; t = time varying by secondary sample; 1 = parameter fixed at the value of 1; g = sex; β = probability of entry; N = size of population.

Analysis	Model	AICc	Delta AICc	AICc Weight	Number of Parameters
POPAN	φ(g)*p*(g)β(t)N(g)	800.54	0.00	0.81341	7
POPAN	φ(g)*p*(.)β(t)N(g)	803.66	3.12	0.17090	8
POPAN	φ(.)*p*(g)β(t)N(g)	808.44	7.90	0.01565	8
POPAN	φ(.)*p*(.)β(t)N(g)	820.51	19.97	0.00004	7
POPAN	φ(g)*p*(.)β(1)N(g)	70634.01	69833.48	0.00000	5
POPAN	φ(g)*p*(.)β(1)N(g)	70634.09	69833.55	0.00000	6
POPAN	φ(.)*p*(.)β(1)N(g)	70656.15	69855.61	0.00000	4
POPAN	φ(.)*p*(g)β(1)N(g)	70657.61	69857.07	0.00000	5
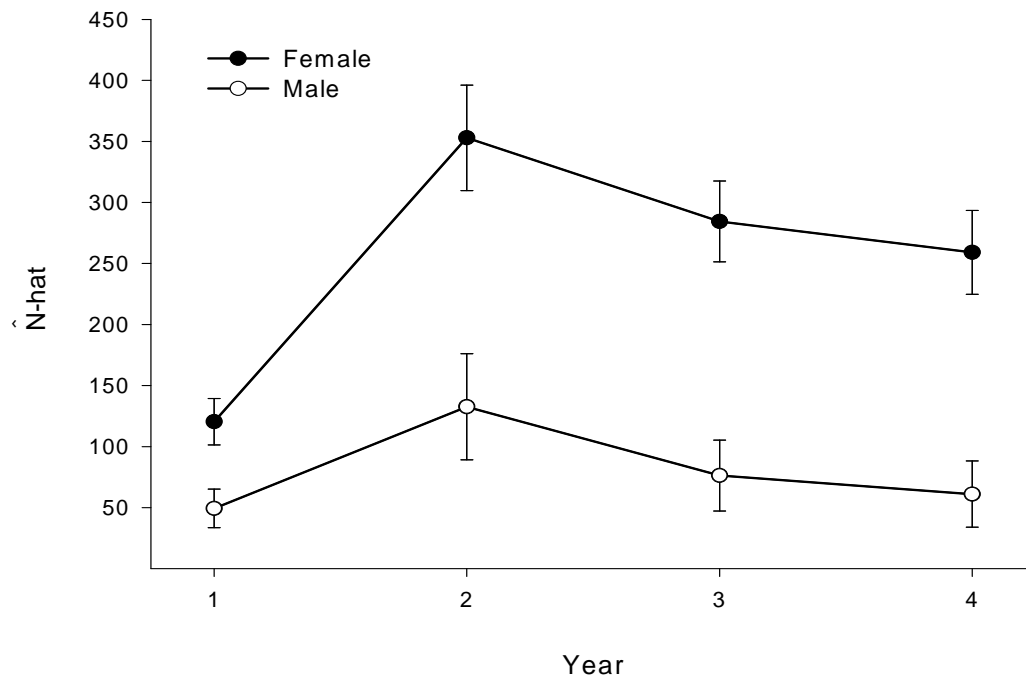
Figure 5.14 Projected population estimates ($\hat{N}$) for each year of the study for both male and female *Manta birostris* variant A.
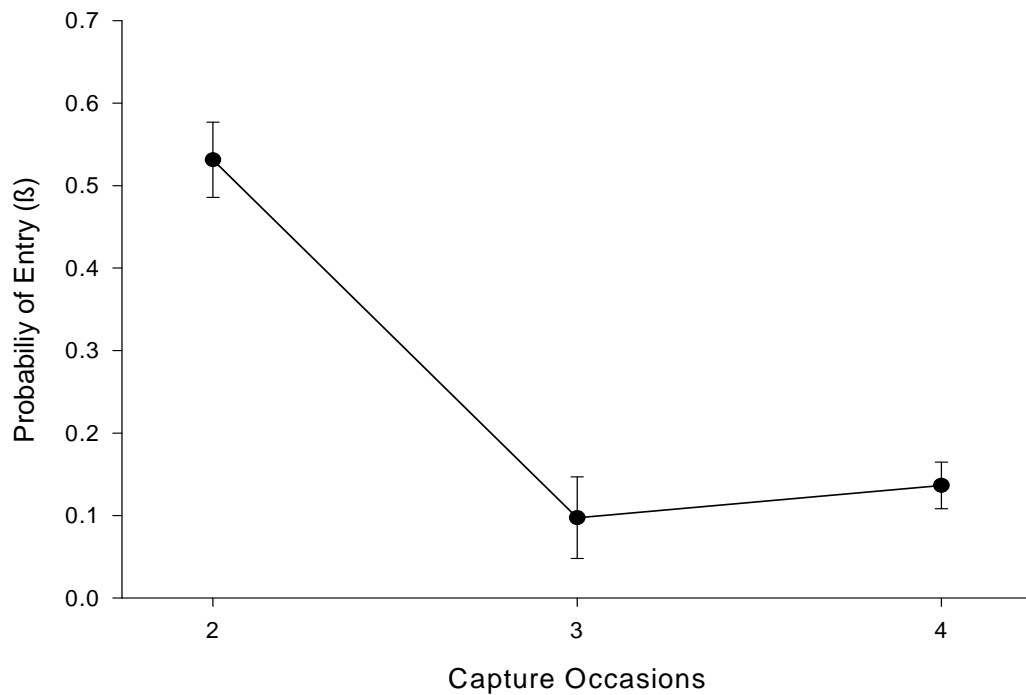
Figure 5.15 Probability of entry (β) into the population for both male and female rays during each of the three capture occasions.

DISCUSSION

Photographic identification

This study demonstrates that natural spot patterning as well as scars can be confidently used as unique identifiers of individual *M. birostris* variant A during discrete study periods at aggregation sites. Individual spot patterning would also be appropriate for the comparison of individuals in different populations to examine movement patterns between aggregation sites. The longer-term viability of these markings for individual recognition is likely, as these natural marks do not appear to change with age or alter dramatically with time. Other long-term photographic studies on manta rays have confirmed the long-term viability of ventral spot patterns (Homma 1999, Guy Stevens pers. comm., Robert Rubin pers. comm.) Similar findings have been reported in elasmobranchs like *Stegastoma fasciatum* (leopard sharks) or *Galeocerdo cuvier* (tiger sharks) (Last and Stevens 2004). Each individual in this study was able to be uniquely identified using natural markings in the standardised area on the ventral body surface (Fig. 5.2b), with no two rays having the same spot pattern. This result complements the findings of Corcoran and Gruber (1999), Meekan et al. (2006) and Van Tienhoven et al. (2007) that found that eagle rays, whale sharks, and grey nurse sharks could be unambiguously identified from natural markings alone. It is suggested that all researchers standardise this identification area on manta rays to facilitate global comparisons of individuals. Due to the obvious and unique nature of natural markings on *M. birostris* variant A it is unlikely that mis-identification would occur in photographic studies if photographic image quality was high and the area of interest was standardised. Additionally, like eagle rays, the photographic identification of manta rays presents a clear advantage over shark species in that only a single clear image of the standardised area on the ventral surface is needed for identification as opposed to of both sides of the dorsal fins, gill slits or flanks (Porcher 2005; Meekan et al. 2006; Bansemer and Bennett 2008). The results of this study also indicate the viability of natural markings to examine the structure, seasonality and behaviour of manta ray populations, as individuals were able to be photographically identified and 're-captured' over time allowing size, sex, and even maturity data to be reliably obtained.

Population composition

The majority of female rays in the observed population had larger disc widths than the majority of observed male rays (Fig. 5.12). These data may suggest sexual dimorphism in the maximum disc widths of *Manta birostris* variant A, with female rays achieving larger wingspans than male rays, an observation that was additionally made in Japan (Homma 1999). Sexual dimorphism in size is a phenomenon that is commonly seen in other shark and ray species particularly those in which the male reaches maturity at smaller sizes (Cortés 2000; Frisk et al. 2001), as is the case for *Manta* (Chapters 6, 9). Alternatively, observational data may suggest that larger male rays are segregating themselves off from the observed inshore population. However, as the monitored area is a breeding site for *M. birostris* var. A (Chapter 6), it is unlikely that a larger size class of mature males would be segregating themselves off from breeding females.

In this particular location there was also a strong sex bias in identified rays, with females out numbering males 3.5 to 1 over the study period. Re-sighting data show that females are not only more prevalent in the area but were re-sighted more commonly than males. Modelling results also showed that the observed population is skewed towards females, with female rays consistently showing higher population numbers each year than male rays as well as consistently higher rates of immigration into the population. Sexual biases occur at the population level in many species of elasmobranches in space and time (Springer 1967; Klimley 1987; Anderson and Pyle 2003). Whale sharks (*R. typus*) for instance show male biased ratios at particular aggregation sites (Meekan et al. 2006; Graham 2007), while nurse sharks (*G. cirratum)* have been shown to have a significant female bias at aggregation sites (Castro and Rosa 2005). Other species have shown pronounced, almost complete, sexual segregation outside of mating seasons (Lessa et al. 1986). A strong sex bias has not been formally reported previously for an identified population of *M. birostris* variant A (Homma 1999) and in Japan, twenty years of photographic cataloging of *M. birostris* variant A (n = 101) revealed significantly similar sex ratios of 1:1.1 (Itoh 2000) in the total identified population. Reports on *M. birostris* variant A landings in bather protection nets in South Africa have shown a slight bias towards females, with sex ratios of 1:1.25 (Young 2001), however this ratio is not nearly as pronounced as the one (1:3.54) reported in this study.

Population size and survivorship estimates

The super population estimate for the monitored aggregation sites was 890 individuals, almost double the number of the individuals identified during the four-year study period (449 individuals 50.45% of the projected super population). This estimation was supported by both the cumulative growth curve, which did not reach a plateau during the study period, and the annual ratio of new to re-sighted individuals both which suggest that the population using the study region was much larger than the 449 individuals identified. These estimates confirm suspicions that southern Mozambique is a large and important aggregation site for *M. birostris* variant A. While clearly serving as a reproductive area for *M. birostris* var. A (Chapter 6), the immediate area off Inhambane is also serves as an important feeding ground for this species in addition to hosting many inshore cleaning habitats.

With only a few studies worldwide using photography to identify individuals at aggregation sites, trends in abundance and distribution of this species remain largely unknown. The longest known photographic study of *M. birostris* variant A, spanning a period of three decades, has currently identified approximately 300 individuals in Japan (Homma 1999; Takashi Ito, pers. comm.). Most photographic catalogues of *M. birostris* variant A collected from other locations around the world have identified between 50-350 individuals (Clarke 2002; Marshall et al. 2006, Acker pers. comm.; Deakos pers. comm.). The exception appears to be the Maldives where over 1,000 individuals have been identified in the various atolls (Kitchen-Wheeler 2008; Stevens pers. comm.)

The most parsimonious model examined in this study supported group differences in both survivorship and recapture probability which in turn supports the observations that female mantas are both more prevalent in the population and are sighted with greater frequency over longer time periods. While the chosen model supported many of the field observations in the study, future modelling work may benefit from taking seasonal changes in individual visitation in to account as well as breaking calendar years in to smaller more discrete time periods. Also applying a robust model to this data set may ultimately be more appropriate and give a more comprehensive result. The robust modelling design involves sampling at two temporal scales, a long primary sampling period (typically modelled as an open population) and shorter secondary

sampling periods (typically modelled as closed populations) (White and Burnham 1999).

Seasonality

Although manta rays were present on the reefs throughout the calendar year in all four years of the study, there was a degree of seasonality in regard to their presence at these sites. Based on daily counts, which factored in dive effort, slightly more manta rays were present on the study reefs from November to January. Additionally, more manta rays were identified per minute of dive effort during these summer months than in the winter. These data correspond to trends in seasonal catches of manta rays in the bather protection nets in Kwazulu-Natal, South Africa, approximately 900 km farther south, where 48.6% of the total manta rays landed per year were caught between the months of November and February (Young 2001). As the reproductive season for *M. birostris* var. A roughly corresponds to this time frame (Chapter 6), it is suggested that the increase in activity in this region at this time may be related in to reproductive behaviour. Increased manta ray activity at this time of the year may also be linked to environmental conditions such as elevated water temperatures. *Manta birostris* variant A appears to prefer warm, tropical water. Both their worldwide distribution (Chapter 4) and anecdotal observations of rays failing to appear at coastal aggregation sites when the water temperature drops below 22° Celsius suggest that *M. birostris* var. A may seek out warmer water conditions. Dewar et al. (2008) reported a temperature range of 20-32° Celsius for cleaning stations in the Komodo Marine Park, Indonesia but noted that *M. birostris* var. A were encountered at cleaning stations when the water temperature was between 22-29° Celsius and may prefer water between 25-28° Celsius. The average daily surface temperatures in the summer months in southern Mozambique is several degrees warmer than in the winter. As such, temperature may also be influencing the seasonal abundance of *M. birostris* var. A in this region.

Site fidelity

Slightly over 40% of the identified individuals at the aggregation site were re-sighted on at least one occasion. Despite a relatively low daily effort, restricted mainly by dive time, some individuals were re-sighted multiple times over a several year period. As such, it may be reasonable to conclude that a proportion of the individuals in the observed population show site fidelity to this stretch of coastline as well as to critical

habitats like the study reefs, which host specific cleaning stations for manta rays. Site fidelity or philopatry is the tendency for an animal to stay in, re-visit or frequent particular regions or areas. Sharks and rays are often philopatric to their natal nursery grounds and aggregations sites that may include mating grounds, feeding sites, or cleaning stations (Morrissey and Gruber 1993; Klimley and Anderson 1996; Heupel and Hueter 2001; Sims et al. 2001; Feldheim et al. 2002b; Domeier and Nasby-Lucas 2006). However, even though some individuals were seen in all years of the study, it is uncertain whether the observed population of *M. birostris* variant A is permanently resident to the area.

Determining the migratory patterns of this population would be a logical continuation to this study. Closed populations or populations, like this one, where individuals exhibit site fidelity must be carefully managed as fishing impacts and other anthropogenic factors can have a more directed effect on the depletion of individuals in a specific region (Stevens 2000; Heupel et al. 2006). As target subsistence fishing for *M. birostris* variant A exists at various locations along the coastline in southern Mozambique, with opportunistic monitoring estimates of 20-50 individuals killed per annum (50 km area surrounding the study sites) (Marshall, unpublished data), conservation and management efforts to monitor and prevent the over exploitation of this population of manta rays should be a priority.

While population sizes of *M. birostris* variant A in different regions are likely to vary considerably, aggregation sites that have been monitored with similar photographic identification programs have typically yielded fewer individuals than the examined aggregation site (Homma et. al 1999; Ito 2000; Clark pers. comm., Deakos pers. comm., Dewar pers. comm.). Currently the population off Inhambane, Mozambique stands as one of the largest identified populations of *M. birostris* variant A in the world. Using the Mozambican population as a potential indicator of a large population size, it may be reasonable to conclude that population sizes of under a 1,000 individuals may exist in many regions. Cryptic mortality from associated human activities like boat strikes, net entanglements, and unsuccessful landings are difficult to estimate in large pelagic megafauna like manta rays, but do occur (Speed et al. 2008; Marshall unpublished data). Taking into consideration the known low level of reproductive fecundity of *M. birostris* variant A (Chapter 6), even if conservative

estimates of natural mortality are assumed, this species may not be able to sustain even low levels of fishing pressure. Intensive pressure from large target fisheries, as exists for manta rays in parts of Southeast Asia (Homma et al. 1999; Alava and Dolumbaló 2002; Dewar 2002; White et al. 2006), would certainly not be sustainable if indeed relatively small population sizes and high site fidelity are the norm (Stevens 2000; Marshall et al. 2006). Without concerted efforts to estimate the localised abundance of *M. birostris* variant A, assess population trends and pinpoint regional threats throughout its distribution (not just in areas supporting major tourism industries) there is little hope for the proper conservation of this species.

Chapter 6

The reproductive ecology of *Manta birostris* variant A in southern Mozambique
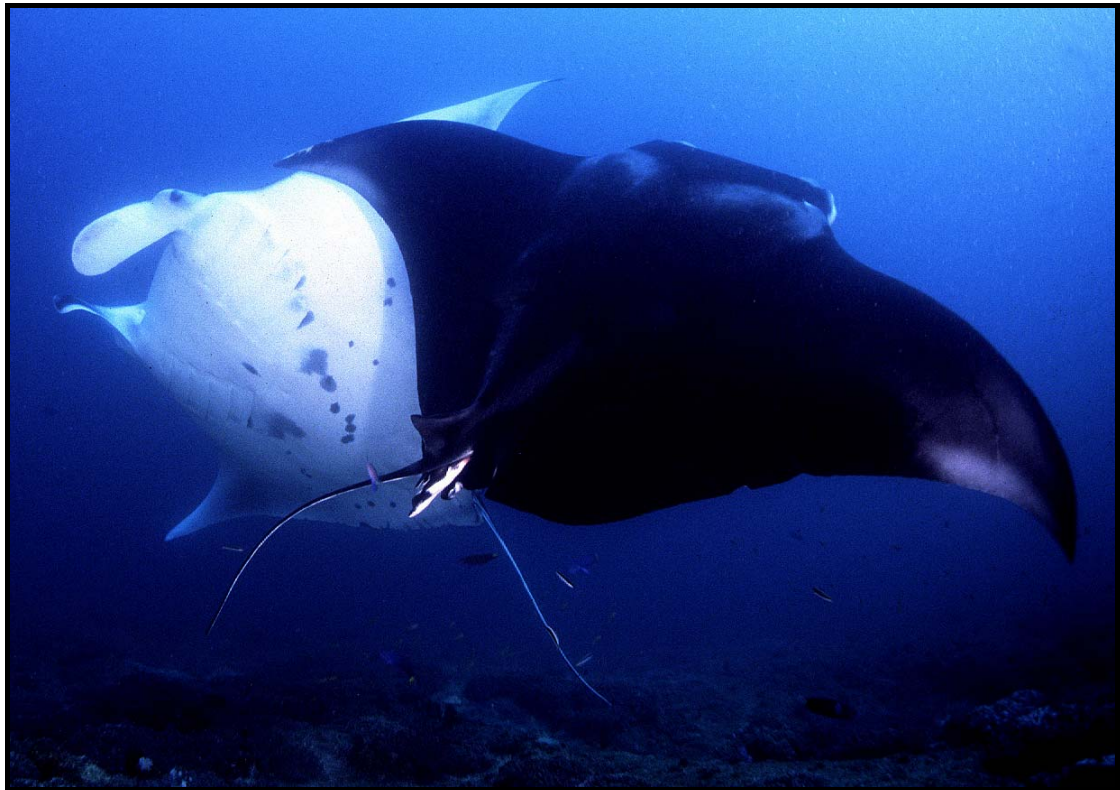
INTRODUCTION

While there exists a significant body of information on the reproductive biology of many elasmobranch species from anatomical examinations (reviewed in Worms 1977; Dodd 1983; Hamlett 1999; Carrier et al. 2004; Hamlett et al. 2005) there are still relatively few studies that examine the reproductive behaviour of wild sharks and rays (Carrier et al. 2004; Pratt and Carrier 2005). Additionally most studies that provide insights into the life history of elasmobranch species have extracted information from fisheries data or from captive specimens in aquaria (Walker 2005), as these data are difficult to acquire in the field using conventional techniques. But large or accessible fisheries do not exist for all species and many species are difficult to access in the wild as they are rare, elusive or have patchy distributions. Moreover, some species are large, charismatic or are protected by legislation. In order to acquire the basic life history data needed, research efforts on such species are often opportunistic or creative in design. Ultimately, however it is achieved, the success of conservation and management efforts depends strongly on the understanding of the life history parameters of a species (Pratt and Carrier 2005).

Manta rays, like other myliobatiform rays, exhibit definitive lipid histrotrophic development, a highly specialised form of aplacental viviparity where embryos are nourished in the uterus by uterine villi, which secrete a protein and lipid rich liquid called hisotrophe (Mitchell 1824; Wourms 1977; Dulvy and Reynolds 1997; Hamlett et al. 2005). The few observations made of pregnant manta rays revealed that females give birth to a single, relatively large pup (Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953, Anon 2007a). Some early reports suggested that manta rays have two functional uteri (Whitley 1936), but other and more recent findings suggest that only the left uterus, is functional (Coles 1916; White et al. 2006). Beyond these limited details, little is known about the reproductive ecology of manta rays.

Acquiring information on the reproductive ecology of large highly mobile species can be difficult to obtain and is presumably the reason for the paucity of data on mobulid rays (Notarbartolo-di-Sciara 1987b). *Manta birostris* variant A is seldom sighted unless surface feeding or being cleaned by small fishes on shallow reefs (Chapter 7). Little is known about their habits outside these events particularly those that are influenced by reproductive activities. While it is likely that food resources are the

driving force behind fine-scale movement patterns and may dictate the timing and location of known aggregations, it remains unclear when and why manta rays segregate themselves by sex or ontogeny, if individuals are philopatric to mating or birthing grounds, and how reproductive cycles may affect the movements and behaviour of individuals.

Long-term population monitoring can provide valuable life history and reproductive information, with minimally invasive photographic studies providing a wealth of information in certain cases. To date, information on the reproductive habits of *M. birostris* have only been inferred from opportunistic dissections, limited fisheries data, and opportunistic accounts of mating events (Lesueur 1824; Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953; Notarbartolo-di Sciara and Hillyer 1989; Yano et al. 1999; White et al. 2006). While these contributions are justifiably important, it remains imperative that long-term, quantitative research on this genus continue to ensure a more complete understanding of the reproductive habits of this species.

This study used minimally invasive photographic techniques to examine the reproductive ecology of *Manta birostris* variant A over a five-year period. By photographically documenting re-sighting events and observing the condition of individuals in the population over time, many reproductive parameters were estimated including size at maturity for both sexes, and the length of gestation period, reproductive periodicity and effective annual fecundity in female rays. Additionally seasonal patterns of mating and birthing were examined and trends in courtship and mating behaviour were described.

METHODS

Photographs, measurements and field assessments

A photographic survey of *Manta birostris* variant A in Mozambique was undertaken over a five-year period from May 2003 until March 2008 to examine reproductive parameters and behaviour. Unique ventral spot patterning in the standardised area was used to identify between individuals in the population (techniques detailed in Chapter 5). Total disc width (DW) estimates followed techniques in Chapter 5, with all

identified individuals placed in four size class bins < 3 m DW, 3.0 – 3.9 m DW, 4.0 – 4.9 m DW and > 5 m DW. More precise measurements of select individuals, particularly newborns, juvenile males and small pregnant females, were carefully estimated underwater with a measuring tape. Additionally, to supplement this information, exact measurements were obtained during dissections of individuals killed in fisheries or caught in bather protection nets in southern Africa.

Each individual in the population was sexed by the presence or absence of claspers (Fig. 6.1a,b). Male maturity was assessed by external observation and occasional manipulation of claspers following Walker (2005) and White (2006). Male rays were classified as (a) 'immature' if the claspers were small, un-calcified, and failed to reach beyond the posterior edge of the pelvic fin (Fig. 6.1c) or (b) 'mature' if the claspers were large, extended well beyond the posterior edge of the pelvic fins and were completely calcified (Fig. 6.1b). The claspers of male rays were observed throughout the study to examine the physical signs of the onset of maturity. Females were individually classified as mature where pregnancy was observed (Fig. 6.1d) or visible reproductive scaring on the pectoral fin tips from mating was present (Fig. 6.2a-c). Females under 2.5 m DW were classified as immature based on the preliminary assessment of maturity in dissected specimens (Chapter 3, 4). All other females were classified as unknown.

Mating scars were visible to observers diving on SCUBA and were also identified in photographic images of female rays. Reproductive scars appeared to be caused by abrasion of the teeth when male rays bite onto the pectoral fin to gain purchase during mating. *Manta birostris* variant A has 6 – 8 rows of small teeth with distinct cusps on the lower jaw (Chapter 4). Mating scars on the pectoral fin tips of female rays were initially observed at the onset of the study, however, scarring was only observed on the left pectoral fin tip. To test for a bias in the location of reproductive scars on the pectoral fin tips of female rays, reproductive scars were classified as either present or absent on the left and right pectoral fins of all identified individuals (Fig. 6.2a,b). Following Kajiura et al. (2000) reproductive scars were further classified as 'fresh mating wounds' if the scars were reddish in colour (Fig. 6.2c) or simply 'mating scars' if they were no longer fresh and were off-white to gray in colour (Fig. 6.2a).
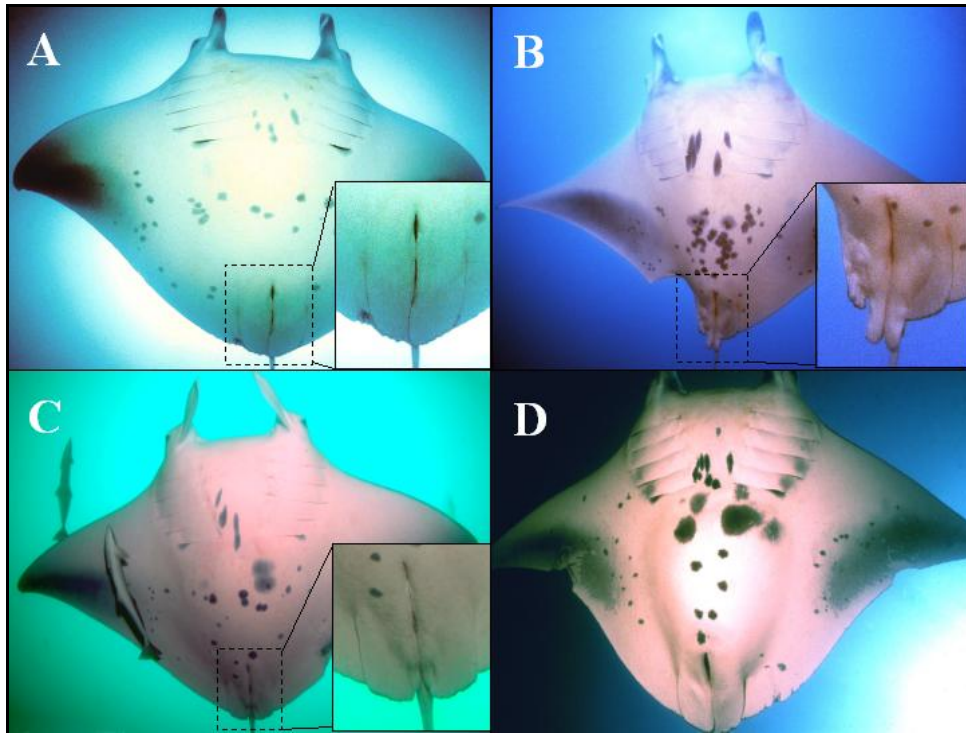
Figure 6.1 Determination of sex and maturity status: (a) female ray with boxed area showing pelvic fins; (b) male ray with boxed area showing pelvic fins and clapsers; (c) immature male ray with un-calcified claspers; (d) pregnant female showing distention of the abdominal region.
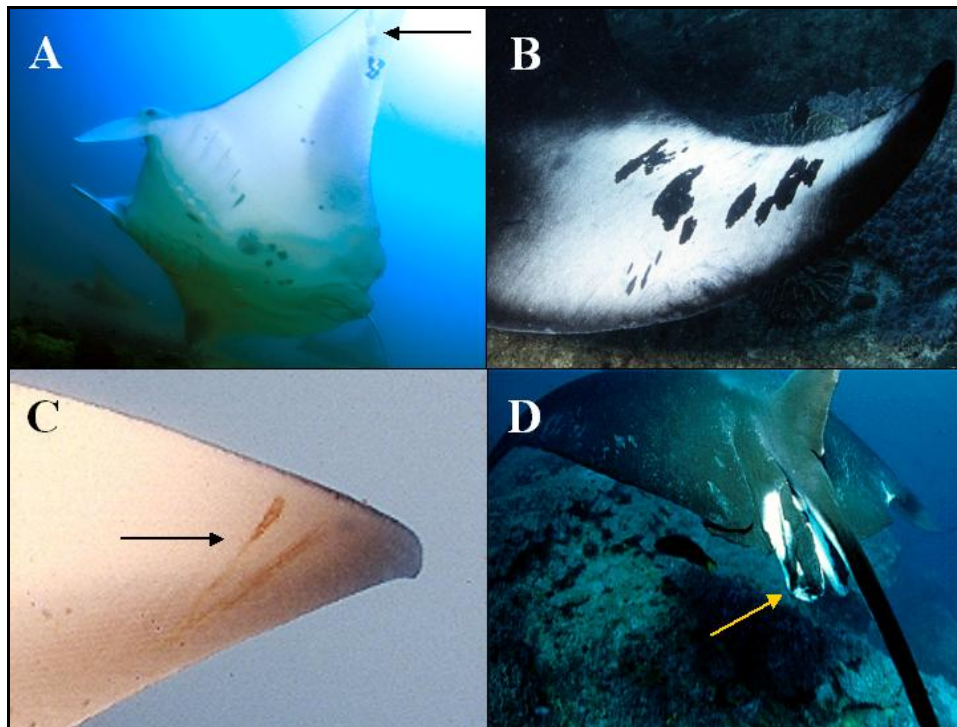
Figure 6.2 Reproductive scars on *Manta birostris* varient *A*: (a) reproductive scars on the left pectoral fin of female ray; (b) removal of overlaying white pigment (leaving black spots) on the dorsal surface of the left pectoral fin tip; (c) fresh reproductive scars on the ventral surface of the left pectoral fin; (d) reproductive scaring on the claspers of a male ray.

Early field observations also revealed that reproductive scarring occurs on the claspers of male rays. These mating scars appear to result from the friction of inserting a clasper into the female's cloaca and were identified by either the removal of black pigment from the dorsal surface of the clasper or the appearance of whitish scars at the tips of claspers (Fig. 6.2d). To examine for possible clasper preference, the claspers of 40 random mature males were analysed for reproductive scarring. Reproductive scarring on the claspers was recorded as being present on the left, the right, or both claspers or absent from both claspers.

Courtship behaviour, mating events and breaching behaviour were monitored throughout the study period. The length of mating events were timed using dive computers, watches or time counts on underwater video footage. Pregnancy in individual female rays was monitored through re-sighting events (i.e. from the time pregnancy could first be identified through to parturition). Parturition was determined to have occurred in the period of time between consecutive re-sightings of known pregnant females that when re-sighted were no longer pregnant. Gestation period and reproductive periodicity were examined in individuals where re-sighting events permitted monitoring of individuals throughout and between years.

RESULTS

Sexual segregation, size at maturity and sexual dimorphism in size

During the study period a total of 507 individual *Manta birostris* variant A were identified, 379 of which were female and 128 of which were male, indicating a highly significant sex bias in the observed population ($\chi^2 = 124.3$, df = 1, P < 0.0001).

Of the 128 male manta rays identified during the study period, 110 were mature and 18 were immature. All immature male individuals sighted during the study period were recorded between 2 November and 4 January in all years (Table 6.1). Of these 18 immature individuals, three individuals were classified as sub-adults at the time of identification and 15 individuals were classed as young of the year or juveniles. The sizes of the 18 immature males identified ranged from 1.5 to 3.2 m DW.

Thirteen of the identified immature individuals were young of the year or juveniles with disc widths of approximately 1.5 to 2.7 m and had claspers that did not reach the posterior edges of the pelvic fins were very small and lacked any calcification (Fig. 6.3a). Two males of approximately 2.8 – 3.0 m DW had claspers that were flush in length with the posterior edges of the pelvic fins (Fig. 6.3b). Three male 'sub-adults' measuring approximately 3.0 m DW had claspers that extended just beyond the pelvic fins but were only semi-rigid and not fully calcified (Fig. 6.3c).

The external surfaces of all non-mature claspers were smooth, exhibited counter-color shading (entirely black colouration on dorsal surface and white on ventral surface) and lacked clasper gland structure immediately anterior to the claspers (Fig. 6.4a,c). In contrast, the claspers of mature males were fully calcified, thicker in diameter than immature and sub-adult claspers and had a rough texture with white marks and scarring on the posterior half towards the tip (Fig. 6.4d). Additionally, in mature
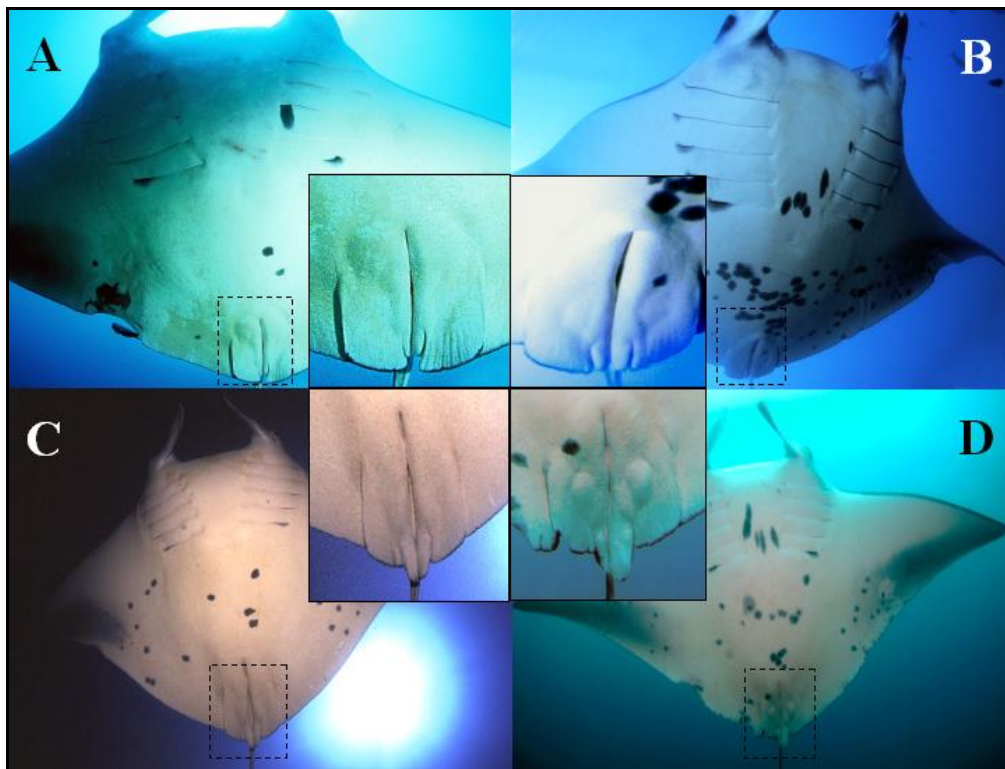
Figure 6.3 Progression of male clasper growth in *Manta birostris* variant A: (a) immature male (inset showing small un-calcified claspers); (b) immature male (inset showing claspers that have widened and now are flush with the pelvic fin margin); (c) sub-adult male (inset showing claspers which reach beyond the pelvic fin margin but are still not completely calcified and lack reproductive scarring); (d) mature male (inset showing the fully calcified claspers and clasper gland structure).

Table 6.1 Yearly sighting records of young of the year (YOY), juveniles, and sub-adult rays with notation on the presence of predatory bite marks.

ID #	Sex	Reproductive class	Date of Sighting	Re-sighting events	Predatory Scars
026	Male	Juvenile	02/12/2003	Not Seen	Yes
053	Male	YOY	30/12/2003	03/01/2004	No
316	Male	YOY	02/12/2003	Not Seen	No
246	Male	Juvenile	04/01/2005	Not Seen	Yes
329	Male	Juvenile	24/11/2005	Not Seen	Yes
330	Male	Sub-adult	24/11/2005	Not Seen	Yes
347	Female	Juvenile	28/11/2005	Not Seen	No
348	Male	Sub-adult	28/11/2005	Not Seen	No
356	Male	Juvenile	30/11/2005	Not Seen	Yes
417	Male	Sub-adult	20/11/2005	Not Seen	Yes
439	Male	Juvenile	09/11/2006	Not Seen	Yes
458	Male	Juvenile	21/12/2006	Not Seen	Yes
527	Male	Juvenile	06/10/2007	Not Seen	Yes
528	Male	Juvenile	06/10/2007	Not Seen	Yes
535	Male	Juvenile	07/10/2007	Not Seen	Yes
355	Female	Juvenile	30/11/2005	Not Seen	No
436	Female	Juvenile	02/11/2006	Not Seen	Yes
438	Female	Juvenile	07/11/2006	Not Seen	Yes
557	Male	YOY	16/10/2007	Not Seen	No
561	Male	Juvenile	06/11/2007	Not Seen	No
569	Male	Juvenile	29/02/2008	Not Seen	Yes
572	Male	Juvenile	29/02/2008	Not Seen	Yes

males the clasper glands, situated laterally to the cloacal opening, were visibly apparent from the ventral surface (Fig. 6.4b). All mature males with fully calcified claspers and scarring were approximately 3.0 m DW or bigger and comprised 89% of the total number of males in the observed population (Fig. 6.3d).

Of the specimens that were examined from fisheries and bather protection nets (n = 9), all males between 2.23 – 2.52 m DW were immature and the single mature male examined was 3.42 m DW.
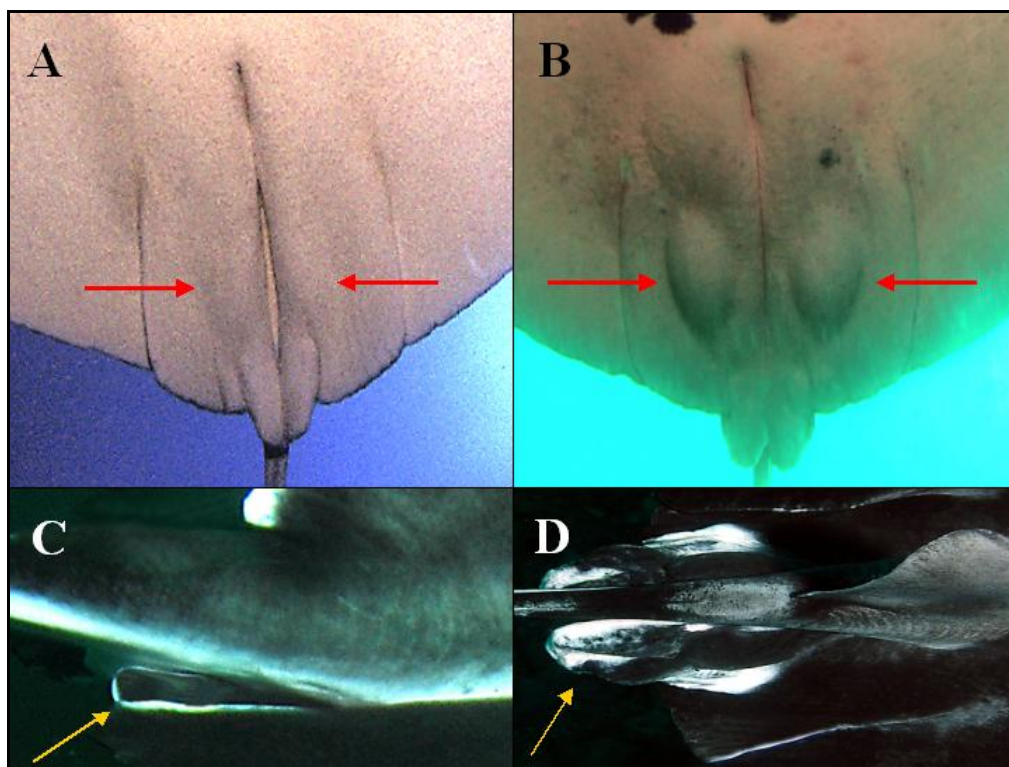
Figure 6.4 Reproductive maturity in male *Manta birostris* variant A: (a) sub-adult male without visible clasper gland structure; (b) mature male with distinctive clasper gland structure; (c) immature male with no reproductive scarring on the dorsal surface of the claspers; (d) mature male with calcified claspers and reproductive scarring towards the tip.

The disc widths of the female rays identified ranged from approximately 2.8 to 5.5 m, with the majority (85.7%) of female rays measuring between 4.0 – 4.9 m DW. While it was not possible to confirm immaturity in female rays, at least four presumably juvenile individuals less than 3 m DW were observed in the month of November in 2005 and 2006 (Table 6.1). Sixty-two female individuals (16.4%) were confirmed to be mature through pregnancy. These individuals ranged in size from approximately

3.9 to 5.3 m DW. Reproductive wing scars were present in 164 of the identified female rays (43.3%) ranging in size from approximately 4.2 to 5.0 m DW. If pectoral mating scars were accepted as an indication of maturity the percentage of mature females would rise from 16% to 48.8% in the sampled population. All dead female specimens examined where disc width could be measured were between 2.23 and 2.65 m DW and all were immature.

Breaching

On multiple occasions during the study period, *M. birostris* var. A were observed breaching out of the water. Unlike mobula species, which generally exit the water completely and often make entire revolutions, *M. birostris* var. A often did not appear to generate enough momentum to launch their entire bodies out of the water. Ordinarily breaching behaviour consisted of an individual ray propelling itself headfirst out of the water and landing forward on its ventral surface or backward on its dorsal surface. On occasion they were seen to breach entirely out of the water coming down with a loud slap (Fig. 6.5).

Figure 6.5 *Manta birostris* variant A breaching completely out of the water.

The behaviour building up to the breach was documented during a single breach seen from underwater. A lone female manta swimming mid-water changed course downwards towards the reef at high speed. Approximately one meter from the bottom the female ray changed direction again and headed directly towards the surface, propelling herself out of the water, before crashing down moments later with a large splash.

The sex of breaching rays could not be determined. While breaches were recorded in many of the calendar months, the vast majority (76.8% of breach events) occurred between October and January (Fig. 6.6). During those summer months, 90.6% of breaching activity took place on the same day or within 48 hours of noted courtship displays, mating behaviour, or upon sighting females with fresh pectoral wounds.

Courtship and mating behaviour

Numerous courtship displays were documented during the study, which took place exclusively in the summer months from October to January (Fig. 6.6). Mating events appeared in all years to take place within periods of a few days. These clustered mating bouts were punctuated over the summer months rather than occurring consistently during this period. During these brief mating periods the observations of courtship behaviour, breaching events, and fresh pectoral fin wounds increased.

Courtship displays appeared quite variable in length, but the most typical components included: (a) close pursuits of female rays; (b) faster than average swimming speeds; (c) multiple male participants performing movements in a line one after another 'in cannon'; (d) veering and swooping behaviour; and (e) somersaulting or looping behaviour (Fig. 6.7a-d). The same individuals engaging in these behaviours were often seen throughout and sometimes on consecutive dives, suggesting that courtship displays can extend for over two hours. The behaviour of other mature females on cleaning stations in the vicinity did not change in response to the surrounding activity. These females did not participate in courtship displays nor did they elicit responses from male suitors courting other females.

Mating behaviour was observed on five occasions during the study period, at locations less than a half a kilometre from shore, in depths of less than 40 m of water. Three of the observed mating events took place after similar courtship behaviours, with up to 13 male rays pursuing a single female. However, on two occasions, only a single male and female ray participated in courtship and mating behaviour and the drawn-out, pre-copulatory behaviours were not witnessed. During these events the single male initially approached the female from the rear. At the outset, the female ray made evasive movements to avoid his advances but ultimately after a period of approximately 2 – 4 minutes the male was able to approach her from the left side and grasp her left pectoral fin tip.

In the other three documented events, towards the end of the long courtship displays a single male from the participatory group would increase his speed and position himself immediately over the dorsal surface of the female ray with his pectoral fins beating in unison with hers. This often caused the female to 'rear up' and literally buck the male suitor off. This behaviour sometimes occurred several times over a period of a few minutes. During this period of time, the chain of following male rays was sometimes not visible. Ultimately, a male would approach from the left side of the female and would bite down on her pectoral fin tip with his mouth. In all observed mating events the male ray, while grasping the females pectoral fin in his mouth, would manoeuvre his body underneath hers so that the pair were situated belly to belly. During mating, the male continued to move his pectoral fins, presumably to maintain his position in the water column and keep his clasper inserted. Typically, despite the males efforts, the intertwined pair slowly sunk through the water column. During one of the five observations, the mating pair moved in a slow, corkscrew motion in the water column while engaged in copulation. In only a single event could the clasper used in mating be distinguished clearly. On this occasion, the male inserted the left clasper, which was positioned at a slightly acute angle to the male's body. In each of the five events clasper insertion lasted between 15 – 35 seconds. In four of the events a whitish fluid, presumably sperm, was seen streaming out from between the two rays. After copulation was completed, the male continued in four out of the five events to grasp onto the pectoral fin of the female for a period of approximately 5 – 20 seconds. On the single occasion that this did not occur, the male let go almost immediately,
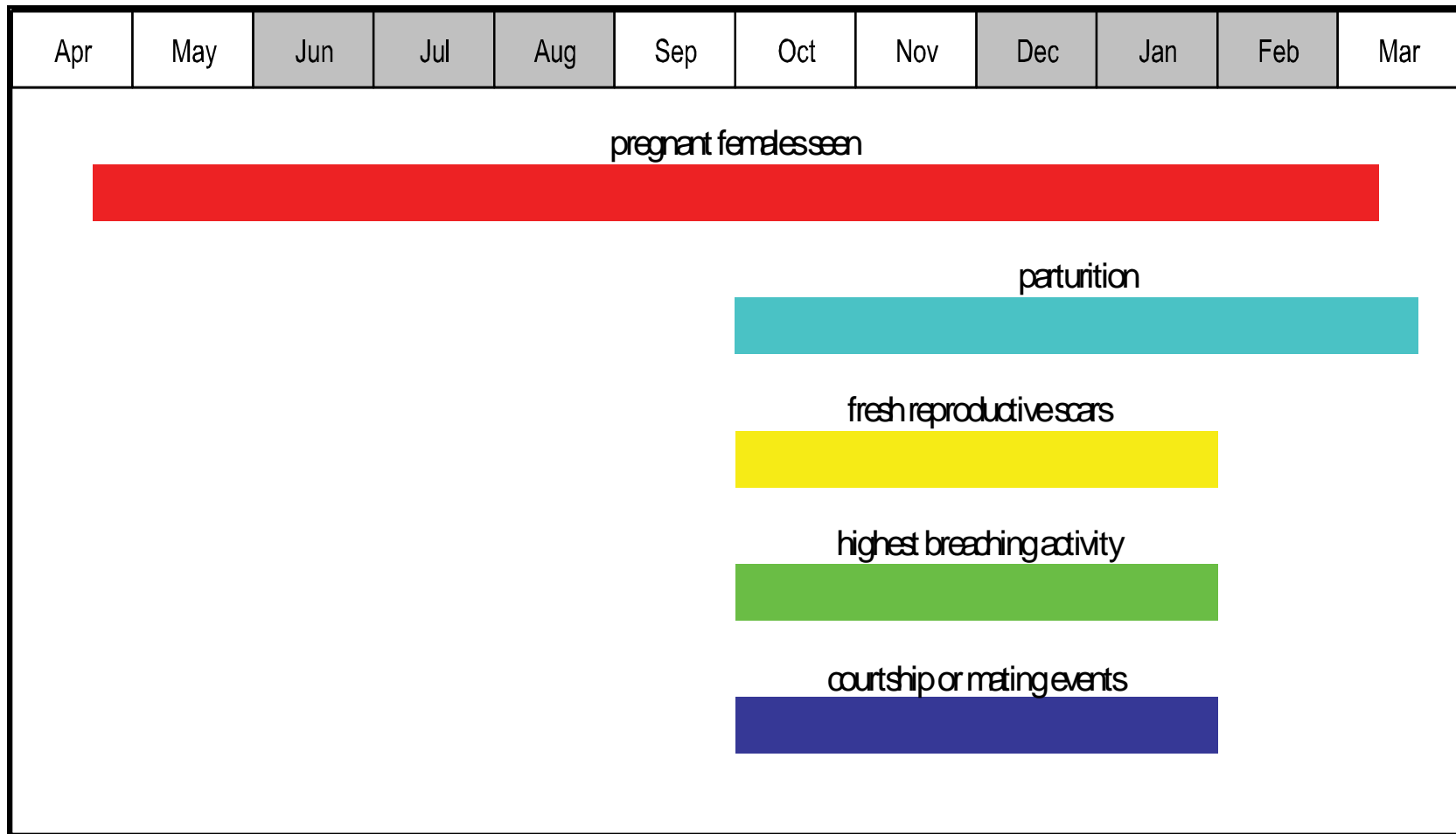
Figure 6.6 Seasonality of reproductive status and behaviour of *Manta birostris* variant A in southern Mozambique.

although it was noted that the fin might have been dislodged unintentionally due to the awkward angle of the male's grip. In all witnessed events, once the male let go of the female's pectoral fin no further interaction between the rays was observed.
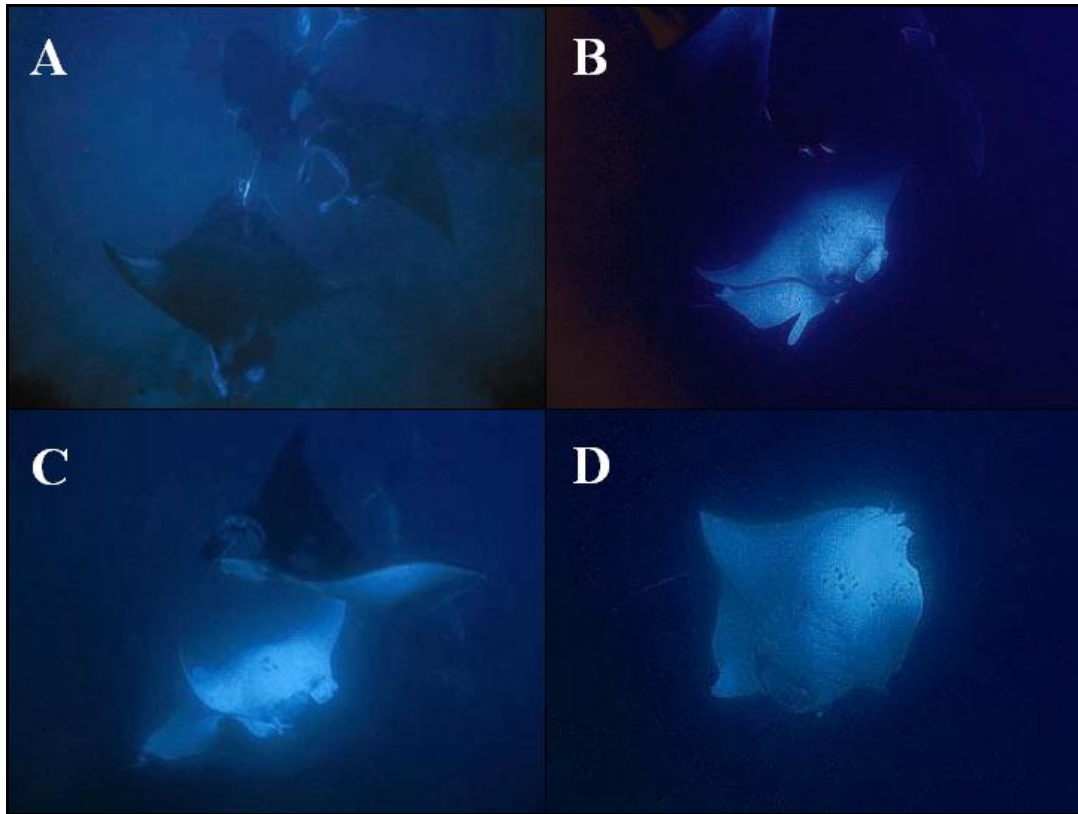
Figure 6.7 Courtship and mating behaviour in *Manta birostris* variant A: (a-d) seven males engaging in pre-courtship behavior with a female #169, who was eventually mated.

Reproductive scars and seasonality

Mating scars, resulting from the teeth of the male rays, were identified on the pectoral fin tips of 164 (43.3%) of the identified female rays. A highly significant bias in the selection of mating side was evident, with 164 of the 166 observed reproductive scars located on the left pectoral fin tip of female rays ($\chi^2 = 158.1$, df = 1, P < 0.0001) (Fig. 6.8, 6.9). While 98.8% of observed females had reproductive scars exclusively on the left pectoral fin, two females in the observed population had scarring on both pectoral fins.
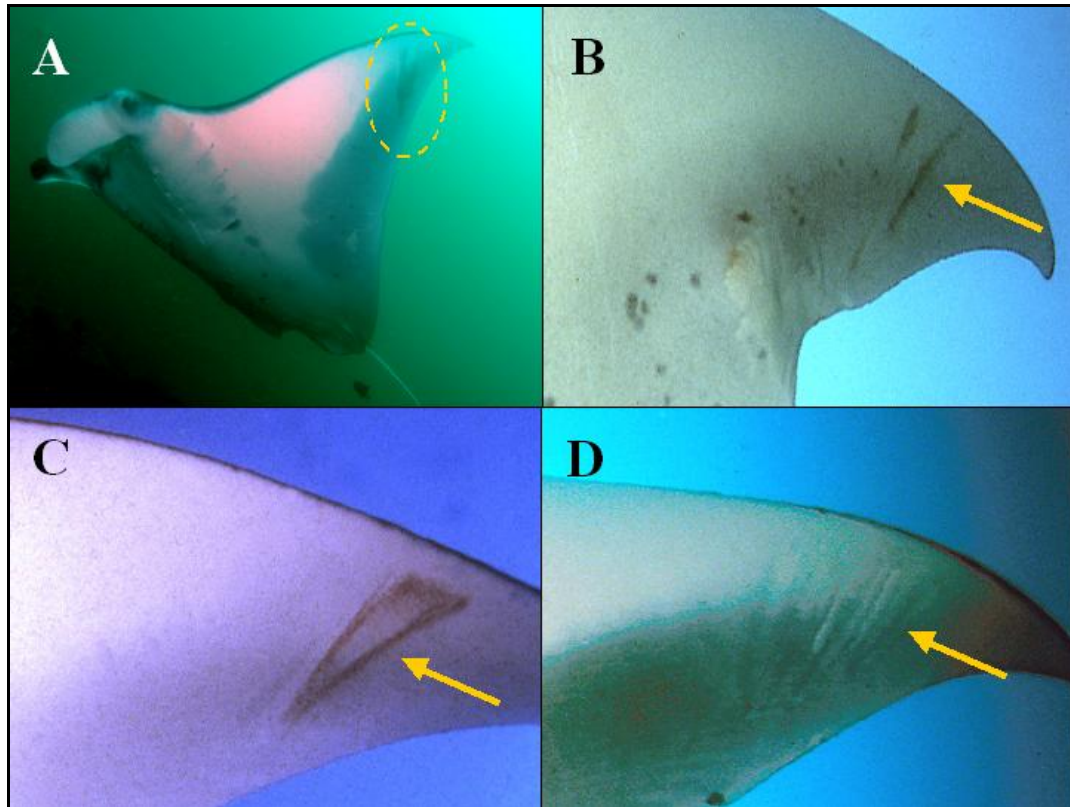
Figure 6.8 Fresh reproductive scars on the ventral surface of female *Manta birostris* variant A in southern Mozambique: (a) view of fresh scars on the underside of the left pectoral fin; (b) fresh scars in two conspicuous bands on the left pectoral fin; (c) fresh mating scars on the pectoral fin of a female ray that had recently given birth; (d) recent mating scars that have begun to fade.

Mating scars generally took the form of parallel bands across the pectoral fin tip (Fig. 6.8b,d, 6.9b). Some scars were small or faint (Fig. 6.8d, 6.9d) while others were more distinct (Fig. 6.8c, 6.9a). When fresh, the wounds often appeared reddish to brown in coloration (Fig. 6.8a-c). Both fresh wounds and less recent mating scars persisted in most of the identified female rays over the course of the study, often lasting years, even though the colour of the fresh wounds faded to white or various shades of gray (Fig. 6.2a). From re-sighting events it appeared that fresh mating wounds remain reddish for less than a week before beginning to fade (Fig. 6.8d). Mating wounds were evident in the months of October, November, December and January with the earliest recorded scars identified on 5 October and the latest scars recorded on 17 January. However, the majority (73.1%) of reproductive wounds were recorded in November and December.
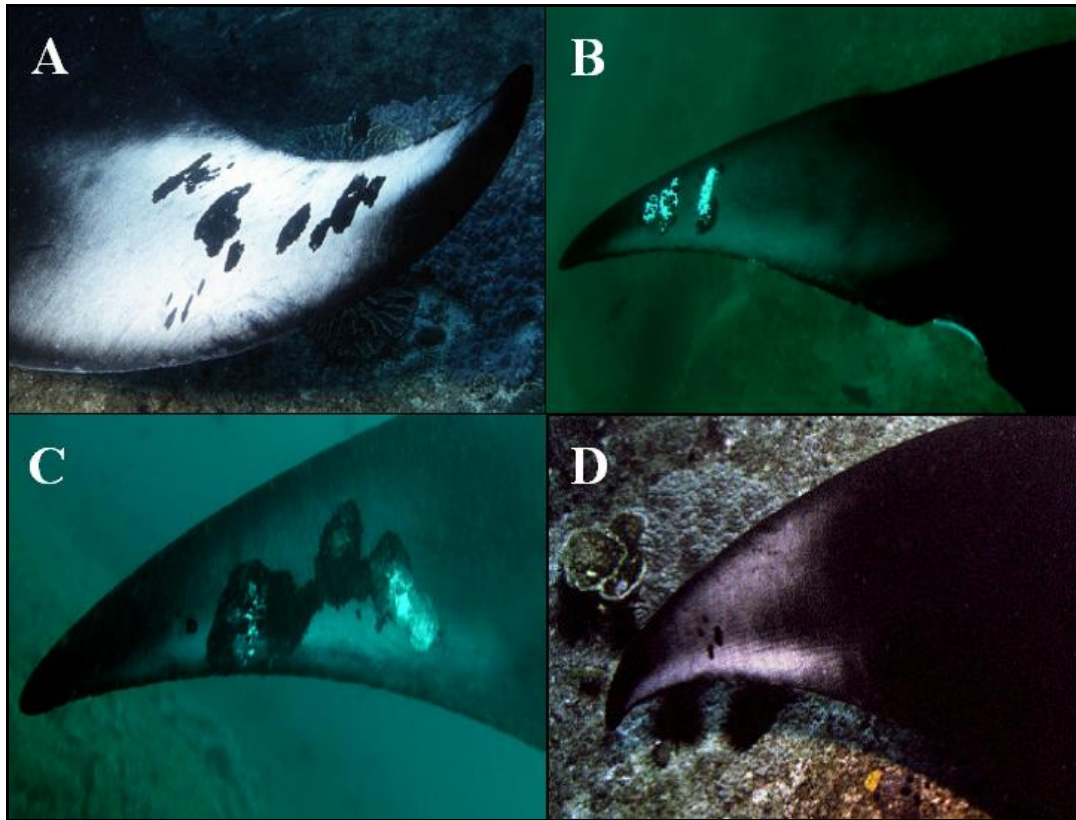
Figure 6.9 Reproductive scars on the dorsal surface of the left pectoral fin tip in female *Manta birostris* variant A: (a-d) variation in appearance of reproductive scars.

Reproductive scars were also visible on the claspers of male rays, which appeared to result from abrasion during penetration (Fig. 6.10). There was no significant difference in the frequency of reproductive scarring on the left and right claspers of male rays ($\chi^2 = 0.015$, df = 1, P = 0.9) and over 67.5% of males had scarring on both left and right claspers (Fig. 6.10b,c).

Figure 6.10 Reproductive scars on the claspers tips of male *Manta birostris* variant A: (a-c) mating scars on the left and right clapser tips.

Pregnancy, gestation period, and reproductive periodicity

In total, 69 pregnancies in 62 individuals were recorded from 2003 to 2008 (Table 6.2). Due to the laterally compressed shape of the manta ray body, mid-to-late term pregnancies were conspicuous from most angles (Fig. 6.11,6.12). The first distinctly pregnant females were observed from late April to July in most years, although pregnancies were difficult to distinguish without close inspection at this stage (Fig. 6.12). As the mating period took place over several months from October to January, pregnant females were likely at least six and a half month pregnant by the time the pregnancies could first be detected (Fig. 6.6). As they approached term, the distended abdominal area on both the dorsal and ventral surfaces increased in size (Fig. 6.11). In three individuals, the size and shape of their distended abdominal area was noticeably larger than other full-to-term females, and it is possible that these females were carrying two pups. Over the study period two pregnant manta rays were killed by local fishermen in Mozambique. One female was carrying a single near- term foetus while the other was carrying two small embryos.
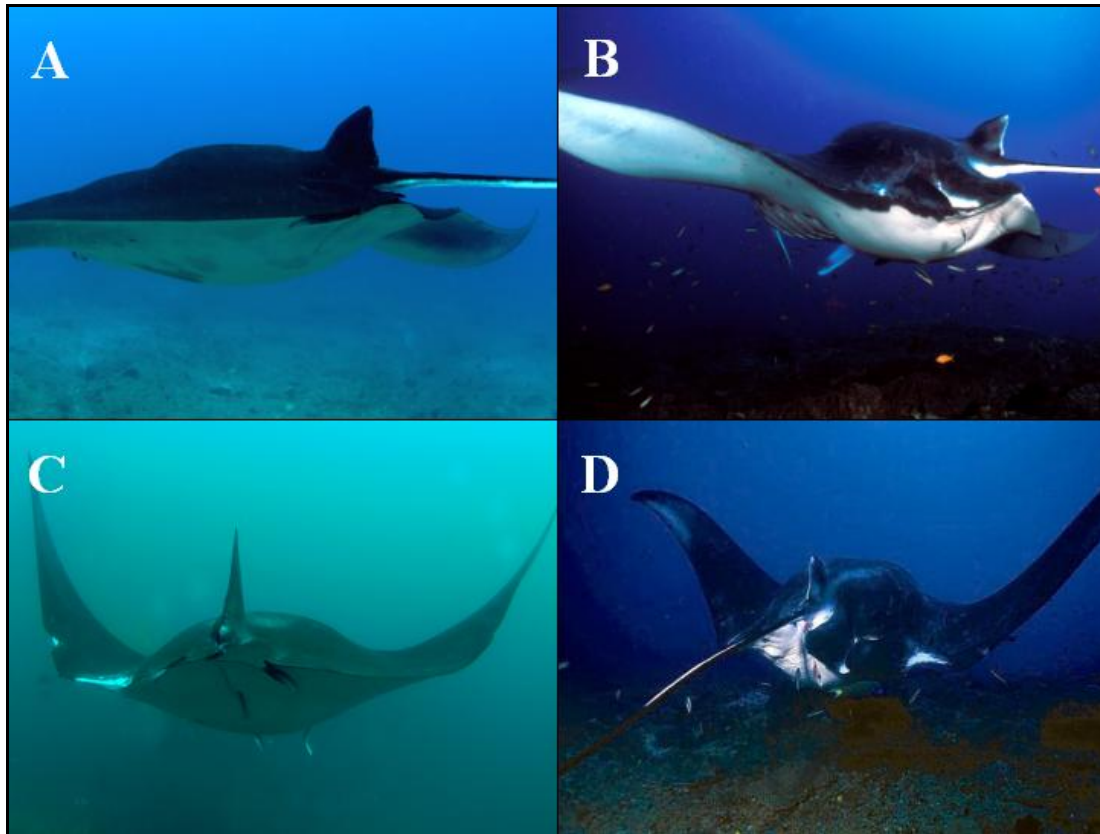
Figure 6.11 Lateral and rear views of full to term pregnant females: (a,c) average size of female when full to term (1 pup); (b,d) size of females suspected to be carrying 2 pups.

Pregnant individuals were recorded in every year but varied in frequency from year to year, with 12, 22, 26, two and six pregnant individuals recorded in each of the five years respectively. The last observed pregnant female in each year of the study was 13 January, 2004, 21 January, 2005, 29 January, 2006, 30 January, 2007, and 4 March, 2008. Of the total 62 pregnant individuals observed, five individuals were pregnant in consecutive years of the study although two of these female had unsuccessful mid-pregnancy abortions in the first of the consecutive years which were attributed to shark attacks (Chapter 8). In two of the remaining three females, the parturition dates for both pregnancies could be roughly estimated through re-sighting events to within a few weeks (example Fig. 6.13). In each of these females the first recorded parturition occurred in the first two weeks of November and the first two weeks of December 2004 respectively. The following year, parturition occurred slightly later, with both individuals giving birth between late December 2005 and late January 2006. Thus, the gestation period in *Manta birostris* variant A was estimated to be approximately 12

months or slightly more. One of these females was seen several times during the 2006 calendar year and was determined from these encounters to be not pregnant that season. A single female was observed to be pregnant in three of four years of the study (Table 6.2). Ten other female rays took at least a one-year resting interval between pregnancies, with one female taking at least two years off between pregnancies.
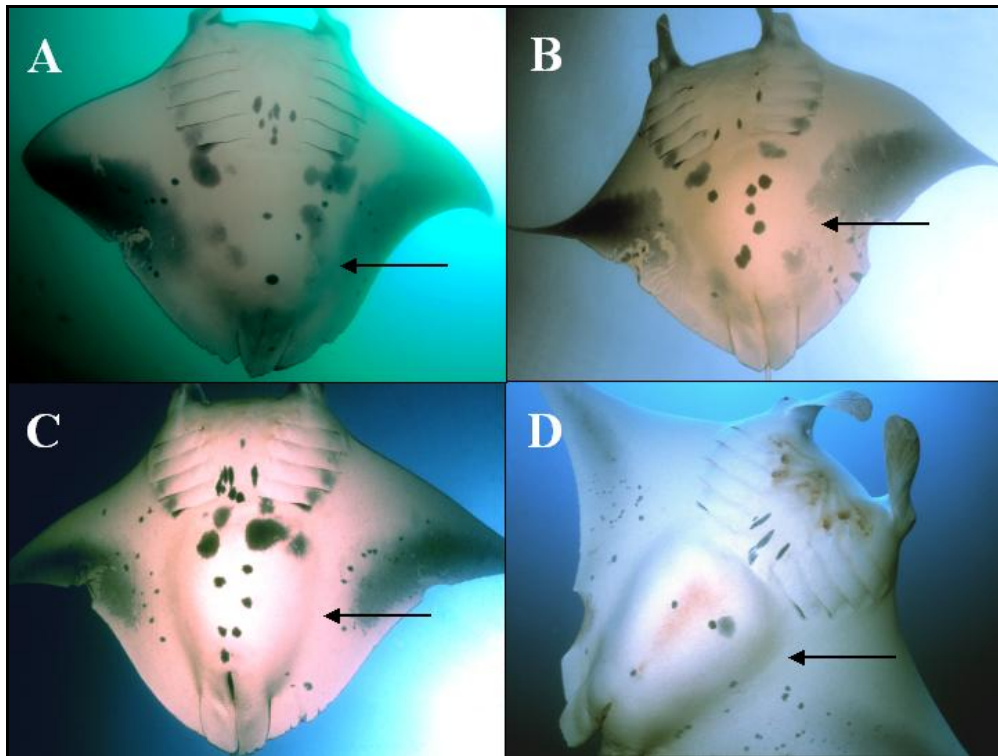
Figure 6.12 Stages of pregnancy in *Manta birostris* variant A: (a) pregnancy just visible at approx. 7 months; (b) pregnant female at approx. 9-10 months; (c) female full term approx. 12 months; (d) pregnant female days before parturition (potentially carrying two pups).
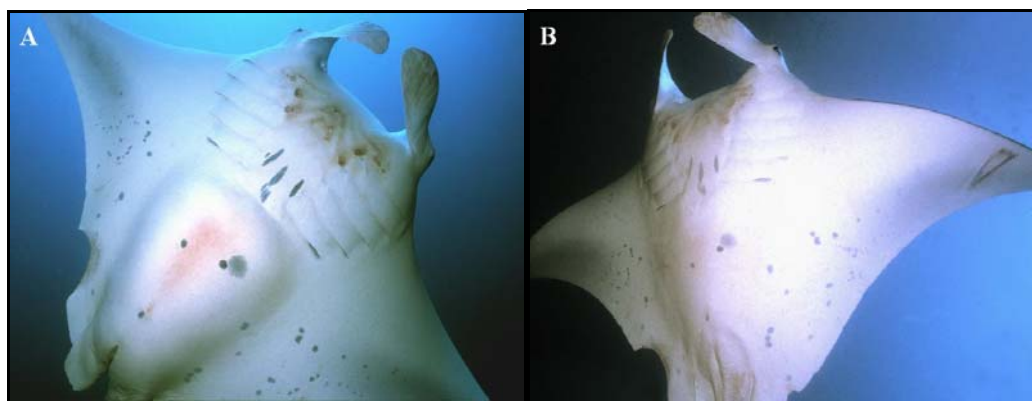
Figure 6.13 Pregnant *Manta birostris* variant A before and after parturition: (a) individual # 033 on 21 December, 2003: (b) individual #33 on 31 December, 2003. Note fresh reproductive wounds on the left pectoral fin tip.

Table 6.2 Reproductive periodicity of individual female manta rays with identified pregnancies. 'DD' (DD) indicates not enough re-sighting events to determine yearly pregnancy status, ---- indicates the individual had not yet been identified.

ID #	Year 1	Year 2	Year 3	Year 4	Year 5
006	DD	Not Pregnant	Pregnant	Not Seen	Not Seen
007	Pregnant	DD	Not Seen	Not Pregnant	Not Seen
015	Pregnant	DD	Not Seen	Not Seen	Not Seen
018	Not Pregnant	Pregnant	DD	DD	Not Seen
021	Not Pregnant	Pregnant	Not Pregnant	DD	Not Seen
023	Pregnant	Not Pregnant	DD	Not Seen	Not Seen
033	DD	Pregnant	Pregnant	Not Pregnant	Not Seen
042	Pregnant	Not Pregnant	Not Seen	Not Seen	Not Seen
045	Pregnant	Not Pregnant	DD	DD	Not Seen
050	Pregnant	Not Pregnant	DD	Not Seen	Not Seen
056	DD	DD	Pregnant	Not Seen	Not Seen
058	Pregnant	Not Seen	Not Seen	Not Seen	Not pregnant
065	Pregnant	Not Pregnant	Not Pregnant	DD	Not Seen
067	Pregnant	Not Seen	Not Seen	Not Seen	Not Seen
071	Pregnant	Not Seen	Not Seen	DD	Not Seen
076	-----	Pregnant	Not Seen	Not Seen	Not Seen
094	-----	Not Pregnant	Pregnant	DD	Pregnant
098	-----	Pregnant (aborted)	Pregnant	Not Seen	Not Seen
133	-----	Not Pregnant	Not Seen	Not Seen	Pregnant
142	-----	DD	Not Seen	Not Seen	Pregnant
146	-----	Pregnant	Not Seen	Not Seen	Not Seen
155	-----	Pregnant	DD	Not Seen	Not Seen
157	-----	Pregnant	Pregnant	Not Seen	Not Seen
167	-----	Pregnant	Not Seen	Not Seen	Not Seen
169	-----	Not Pregnant	DD	Pregnant	Not Seen
172	-----	DD	Pregnant	Not Seen	Not Seen
176	-----	Pregnant	Not Seen	Not Seen	Not Seen
179	-----	Pregnant	Not Seen	Not Pregnant	Not Seen
181	Pregnant	Not Pregnant	Not Seen	Not Seen	Not Seen

182	----	**Pregnant**	Not Seen	Not Seen	Not Seen
186	----	----	**Pregnant**	Not Seen	Not Seen
214	----	**Pregnant**	Not Seen	Not Seen	Not Seen
237	----	DD	**Pregnant**	DD	Not Seen
253	----	DD	**Pregnant**	**Not Pregnant**	Not Seen
257	----	**Pregnant**	Not Seen	Not Seen	Not Seen
268	----	**Pregnant**	Not Seen	Not Seen	Not Seen
269	----	DD	**Pregnant**	Not Seen	Not Seen
271	----	**Pregnant**	**Pregnant**	DD	**Pregnant**
280	----	**Pregnant**	Not Seen	Not Seen	Not Seen
281	----	**Pregnant**	Not Seen	DD	Not Seen
283	----	**Pregnant**	**Not Pregnant**	Not Seen	Not Seen
285	----	**Pregnant**	Not Seen	Not Seen	Not Seen
291	----	**Pregnant**	Not Seen	Not Seen	Not Seen
298	----	**Pregnant**	Not Seen	Not Seen	Not Seen
305	----	----	**Pregnant**	Not Seen	Not Seen
306	----	----	**Pregnant**	DD	Not Seen
307	----	**Pregnant**	**Pregnant (Aborted)**	Not Seen	Not Seen
314	**Pregnant**	Not Seen	Not Seen	Not Seen	Not Seen
322	----	----	**Pregnant**	DD	Not Seen
323	----	----	**Pregnant**	Not Seen	Not Seen
325	----	----	**Pregnant**	DD	Not Seen
327	----	----	**Pregnant**	Not Seen	Not Seen
336	----	----	**Pregnant**	Not Seen	Not Seen
339	----	----	**Pregnant**	Not Seen	Not Seen
341	----	----	**Pregnant**	Not Seen	Not Seen
360	----	----	**Pregnant**	Not Seen	Not Seen
361	----	----	**Pregnant**	Not Seen	Not Seen
374	----	----	**Pregnant**	Not Seen	Not Seen
388	----	----	**Pregnant**	Not Seen	Not Seen
500	----	----	----	----	**Pregnant**
508	----	----	----	**Pregnant**	Not Seen
570	----	----	----	----	**Pregnant**

Newborns and young of the year

The smallest identified individuals in the field for which size could be estimated ranged from 1.5 to 1.67 m DW. Based on the size of a near-term foetus from the region (Chapter 3), these individuals were believed to be newborn or young of the year. None of these small rays were accompanied by larger individuals at the time of observation. The umbilical scars of the smallest individuals observed were completely closed and no longer red in colour and fleshy in appearance as in the near-term foetus (Fig. 6.14, Chapter 3). Unlike larger size-class rays, these small individuals did not have visible ectoparasites loads nor did they have any scars from shark bites. Observations of slightly larger juveniles individuals revealed that by approximately 2 – 3 m DW the majority (79%) of observed individuals had acquired bite marks from sharks (Table 6.1). Of all the individuals in this smallest size class, only a single individual was sighted again four days after its initial identification.
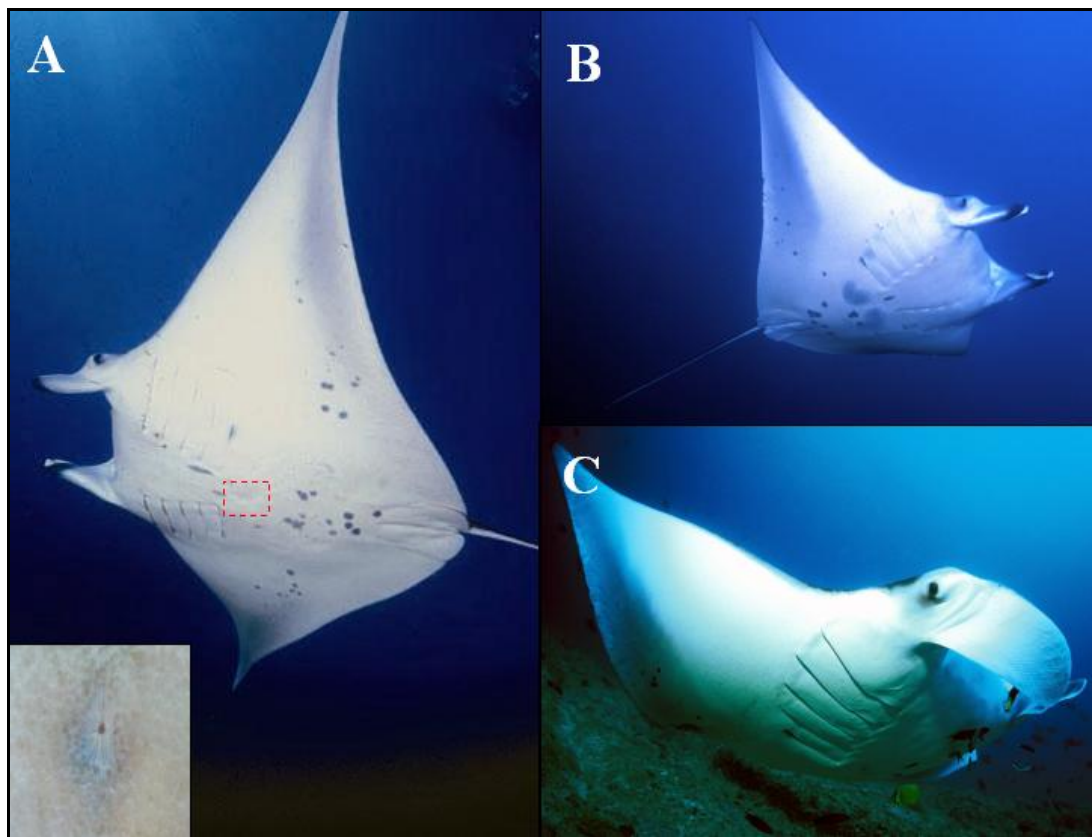
Figure 6.14 Juvenile *Manta birostris* variant A in southern Mozambique: (a) presumed new born manta; (b) young of the year; (c) juvenile ray.

DISCUSSION

The identifiable nature of the ventral spot patterns of *Manta birostris* variant A allowed hundreds of individuals to be identified and tracked through re-sighting events over a five year period. The application of this technique at a breeding and birthing ground enabled data to be successfully collected on the reproductive parameters of this species for the first time in the wild. In summary, male and female *M. birostris* var. A were seen throughout the year along the monitored coastline, however, females were consistently more abundant on inshore reefs than males. Sexually dimorphic in size, male rays reached maturity at approximately 3 m DW while female rays reached maturity at just under 4 m DW. Female rays exhibited approximately 12-13 month gestation periods, giving birth in the summer months most commonly to a single pup. Litter sizes of up to two pups occurred on occasion. *Manta birostris* variant A appears to have an annual ovulatory cycle and is capable of consecutive yearly pregnancies with mating appearing to occur directly after parturition. However, most commonly females display a bi-annual reproductive cycle, with at least one intervening year of non-pregnancy. Newborn pups, born at approximately 1.5 m DW, were not observed to interact with mature rays and may segregate themselves off from the adult population during the early stages of their lives.

Sexual segregation

The highly significant female bias in the identified population is likely a result of sexual segregation, however it cannot be ruled out that it may be a result of a skewed ratio of offspring (e.g. higher proportion of female offspring being born in the area). Sexual segregation by geographic location has been documented numerous times in elasmobranch species (Springer 1960; Pratt 1979; Klimley 1987), with possible explanations ranging from resource partitioning to habitat selection based on reproductive strategies (Springer 1967; Klimley 1987; Sims 2003; Ebert and Ebert 2005; Robbins 2007). Although sexual differences in the diets of manta rays in southern Mozambique have not been readily explored through stomach contents analysis, observations at established feeding grounds in the immediate area indicate that male and female rays are foraging in the same area on the same planktonic food resources. It has been widely proposed in animal behaviour studies that females may

migrate to, select for, or occupy areas that offer stable resources (e.g. food resources), that are appropriate for bearing young (i.e. in or close to nursery grounds), where water temperature is stable and warm and where predation or threats are low (Sih 1994; Main et al. 1996; Craig and Herman 2000; Sims 2003, Heithaus 2004). The study area in southern Mozambique was identified as both a breeding and birthing ground for *Manta birostris* variant *A*. The highly significant female bias in the identified population may therefore be connected with reproductive strategies.

Size at maturity

The majority of the male rays identified in the population were mature, with disc widths ranging from 3.0 – 3.9 m. Only 18 immature males were identified in this study, with an additional four individuals examined during opportunistic dissections. From these individuals size at maturity was estimated to occur at approximately 3.0 m DW. At approximately 2.8 m DW male individuals appear to begin their transition into maturity. It is unclear how long this transitional period lasts but it may be rapid, with only a few sub-adults ever observed. While not enough individuals of this size class were observed to give robust size at maturity estimates, the stages claspers undergo as males transition into maturity were well documented. Most noteworthy was the observation of conspicuous reproductive scarring on the tips of claspers, which may serve as a solid indicator of maturity. Additionally, the visible structure of the clasper glands on the ventral surface may also allow for a more confident assessment of maturity than using only the size of claspers in relation to pelvic fins, as sub-adult males possessed neither of those qualitative characteristics.

The majority of females in the identified population were presumably mature based on three factors: pregnancies, reproductive scars, and size. The majority of female rays fell into the 4.0 – 4.9 m DW size class. Individuals as small as approximately 3.9 m DW were observed to be pregnant and reproductive mating scars were observed in females as small as approximately 4.2 m DW. Thus, size at maturity for females in this population may be approximately 4 m DW.

Breaching behaviour

A relationship between breaching events and reproductive behaviour/fresh reproductive scars was found to occur, with 77% of observed breaching events

occurring in the summer months and 91% of those events occurring on the same day or within 48 hours of observed mating, courtship or fresh mating wounds. As breaching does not appear to be random and certainly appears to be seasonal, it is possible that breaching may serve as a form of intraspecific signalling between rays, particularly those engaging in reproductive activities. Surface cruising and breaching behaviour has been observed in connection with mating activities in *Mobula thurstoni* (Notarbartolo-di Sciara 1988). Breaching behaviour has also been linked to potential reproductive behaviour in white sharks and basking sharks (Pyle et al. 1996; Sims 2000). Cetacean breaching has also been well established in the literature as having links with communication and reproduction (Herman 1980; Whitehead 1985; Silber 1986). While it is cited in many popular articles that manta rays breach to remove parasites or remoras, during the five-year study period breaching was rarely seen to occur independent of reproductive or mass feeding activities. While removing parasites or remoras may be a by-product of breaching, it is proposed that breaching may serve primarily as a form of intraspecific communication, though the intended signal could not be established.

Breaching was observed outside the breeding season. On these occasions, particularly in February 2006, where 11 manta ray breaches were recorded in a four-day period (Marshall unpublished data), there were sightings of mass feeding aggregations along the coastline with up to 40 mantas feeding in the vicinity of the breaching events. Sound and vibrations can carry long distances in mediums like water and may be more efficient modes of communication than visual behaviours, particularly if a reduction in water clarity occurs as a result of a plankton bloom. Certainly more directed research on this topic is warranted.

Courtship and mating behaviour

The only published description of copulation in manta rays was by Yano at al. (1999) from two opportunistic events witnessed at the Ogasawra Islands, Japan. However, the individuals taking part in the observed behaviours were *Manta birostris* variant B. The two mating events, which were observed in a single day, involved different males but the same female. This remains the only time that an identified female manta ray has been documented to mate twice in the same day or season. No published information exists for *Manta birostris* variant A, however copulation has been

recorded in two consecutive years in the Charaumi Aquarium, Japan (Ushida, pers. comm.) and was noted to be similar to the published account of *M. birostris* var. B. Observations of mature females in southern Mozambique suggest that females may advertise their willingness to mate through behavioural or physiological cues, as not all females participated in courtship or mating events at the same time or even the same season. Carrier et al. (1994) proposed that parturition may act as a stimulus or readiness cue for copulation in elasmobranchs. While *M. birostris* variant A did not always exhibit an annual reproductive cycle, consecutive pregnancies did occur, with mating appearing to following closely after parturition (Fig. 6.13), a common strategy in smaller viviparous elasmobranches (Carrier et al. 1994; Lutton et al. 2005). In captivity, it was noted that males exhibited the same following behaviour of pregnant females as seen in the field in Mozambique with mating taking place within 12 hours of parturition (Ushida, pers. comm.).

From their observations in Japan, Yano et al. (1999) identified five key mating steps: chasing, biting, copulating, post-copulation holding and separating. Observations of courtship and mating behaviour of *M. birostris* var. A in Mozambique agree with these five steps but suggest that there may be many different behaviours taking place in step one and thus subdivision is appropriate. The first step should include the following or chasing behaviour described by Yano et al. (1999) and the new step should encompass the complex series of interactive turning and flipping behaviours performed by both the female ray and her male suitors. The chasing behaviour in the first step is conducted at higher than average speeds (Yano 1999). The close following by males in chain formation may be an olfaction-mediated behaviour as is seen in a plethora of animal species (Shorey 1976; Tristram 2003; Hurst 2005) and which has been implicated in the reproductive behaviour of other elasmobranchs (Johnson and Nelson 1978; Pratt and Carrier 2004). During the second stage, participating members of the mating trains completed a series of turns, dips, and flips. The behaviours in this second stage appear complex and additional research is needed to explore the stereotypical nature of the movements. There may be a relationship between the length of these primary courtship stages and the number of males involved in the courtship of the female ray. When one male was involved in mating events, only a few minutes of courtship behaviour were observed. Alternatively, when long chains of male suitors were competing to mate with the female ray, longer courtship displays

took place. The length of these courtship stages may fluctuate to afford the female with the necessary time to choose a mate or may be lengthened by extended competition between males for positioning in the mating chain.

Lateralisation

Lateralised behaviour patterns are those in which an individual, group, or population shows a consistent preference for the use of a hand, a side, or a direction of movement (Clapham et al. 1995; Bisazza et al. 1998). Once thought to exist exclusively in humans, *Homo sapiens*, a suite of recent studies has demonstrated that behavioural asymmetries or lateral bias occur in a number of species, including lower vertebrates (Bisazza et al. 1998; Rogers and Andrew 2002; Vallortigara and Rogers 2005). Lateralisation has not been previously described in elasmobranch behaviour although pre-courtship behaviour, copulatory biting and mating scars have been documented in several batoid species (Nordell 1994; Kajiura et al. 1996; Bisazza et al. 1998; Pratt and Carrier 2005; Vallortigara and Rogers 2005).

The statistically significant bias evident in step three of the mating sequence (the grasping of the female's pectoral fin by the male ray) suggests strong lateralisation in the approach behaviour of male *M. birostris* var. A. For *Manta birostris* variant B Yano et al. (1999) described both males concurrently approaching from the left side of the female and grasping her left pectoral fin to facilitate clasper insertion (Appendix 1). Mating events observed in Mozambican waters also documented male rays approaching from the left side of the female and biting the left pectoral of the female during mating. Data collected on reproductive scars from this population confirmed that visible abrasions from male teeth occur almost solely on the left pectoral fin of female rays, suggesting that mating may almost exclusively take place with the male on the left hand side of the female. The present study provides compelling evidence for lateralised reproductive behaviour at the population level in Mozambique. Furthermore, these data are consistent with reports and photographs of *Manta birostris* variant A from other aggregation sites, suggesting that this behaviour may occur at the species level worldwide (Marshall unpublished data).

Reproductive scars

Manta rays, which are planktivores, may have retained their teeth for the sole purpose of mating (Motta and Wilga 2001). Many batoid species including stingrays (Dasyatidae), stingarees (Urolophidae) and skates (Rajidae) exhibit sexual dental dimorphism (Bigelow and Schroeder 1953; McEchran 1977; McCourt and Kerstitch 1980; Taniuchi and Shimizu 1993; Young 1993; Nordell 1994; Kajiura and Tricas 1996, Motta and Wilga 2001). The more pronounced cusped tooth morphology in males often causes abrasion wounds and scarring on the tips of the pectoral fins and posterior margins of the female's disc (Tricas 1980; Kajiura and Tricas 1996; Kajiura et al. 2000). Long parallel mating scars were readily visible on the pectoral fin tips of many female *M. birostris* var. A during the study. Two primary functions have been proposed for the biting behaviour in male rays. The first is that biting affords the male with the necessary leverage to mate with the female and a means to maneuver into an appropriate mating position (Tricas 1980; Kajiura and Tricas 1996; Kajiura et al. 2000). Biting may additionally be a pre-copulatory release mechanism, with bites serving to induce female receptiveness (Springer 1960; Nordell 1994). Reproductive biting in the Mozambican manta ray population was consistently observed only directly before and during mating when a male was attempting to position himself for clasper insertion and while maintaining a grip on the female during mating. Biting behaviour was not witnessed during the elaborate courtship displays nor did these reproductive scars occur on any other part of the body than the pectoral fin tips.

Fresh mating wounds were seen in Mozambique from October to January during discrete periods of a few days to a week during these summer months. Fresh reproductive wounds maintained their reddish appearance for less than a week. Kajiura et al. (2000) suggest that fresh mating wounds provide a useful, non-invasive method to determine the occurrence of mating activity even when the mating activity itself is not observed. Analysis of the fresh pectoral wounds in Mozambique indicated that mating events occurred seasonally at this location, between October and January but suggests that mating activity may be bound to particular days, perhaps weeklong periods, during these summer months.

Pregnancy, gestation period and parturition

Manta rays, like all mobulid rays, give birth to live young (Coles 1916; Worms 1981; Notarbartolo-di Sciara 1988; White at al. 2006). Gestation period has only been previously reported for one captive female manta ray that gave birth in two consecutive years in the Churaumi Aquarium, Okinawa, Japan. The reported gestation periods of this ray was 374 days and 368 (Anon 2007a, Ushida, pers. comm.). The current study in Mozambique agrees with the Japanese aquarium data and suggests that gestation period in this wild population of *M. birsotris* var. A may also be approximately one year.

From the same captive female at the Churaumi Aquarium it was noted that, at least in captivity, *M. birsotris* var. A can produce an offspring every year (Ushida, pers. comm.). The Mozambican field study confirms that female *M. birsotris* var. A are physically capable of producing offspring in consecutive years and therefore must have an annual ovulation cycle. However, it is equally evident that the majority of re-sighted females in this study (where pregnancies could be determined) did not bear offspring in consecutive years, taking at least one and occasionally a two year resting period between pregnancies. In one case, a single individual was observed to be pregnant in three of the five years of the study.

The reproductive periodicity of some species of mobula is also every two or more years (Notarbartolo-di-Sciara 1988). A few of obvious explanations exist for bi or triennial reproductive cycles. Producing large offspring is energetically costly and females may require time to recuperate energy stores between pregnancies (Carrier et al. 2004). Another explanation lies in the timing of the breeding and birthing season, which overlap one another in the summer months. Gestation periods were estimated at one year or slightly over one year. Female rays that mated early in the reproductive season would bear their pups early in the following season, leaving them ample time to mate successfully after giving birth. However, individuals that mated late in the season the previous year would give birth late in the season the following year, potentially leading them to miss the mating activity that year. Lastly, mating events may not always be successful, with females failing to conceive. Several female individuals in the identified population that were either observed mating or with fresh mating scars were determined not to be pregnant the following year.

While it is unknown where parturition occurs in southern Mozambique, it is likely from re-sighting events that it takes place relatively close to the study sights. Heavily pregnant females, re-sighted a few days later, were observed to have given birth within that short stretch of time. Mating and parturition in other mobulid species takes place in the shallower portion of a population's range (Notarbartolo-di-Sciara 1987a), as is the case for many elasmobranch species (Springer 1960; Carrier et al. 1994). With only a few young of the year observed at inshore reefs in the summer months the existence of birthing or nursery habitats remains unclear.

Embryos, newborn pups, and young of the year

Uniparity is a common pattern in other mobulid rays (Notarbartolo-di-Sciara 1987a,b) and manta rays also appear to most commonly give birth to a single offspring (Lamont 1824; Lesueur 1824; Coles 1916; Beebe and Tee-Van 1941; Bigelow and Schroeder 1953; Notarbartolo-di-Sciara 1988, Marshall et al. 2007). However, an opportunistic dissection of a pregnant ray (killed by local fishermen in Mozambique) carrying two pups, as well as observations of the size and shape of the distended abdominal regions in near-term females, suggests that more than one pup may be born on occasion.

Two *Manta birostris* variant A pups born in captivity at the Churaumi Aquarium in Japan were 1.92 m DW (68.5 kg) and 1.82 m DW at birth (Anon 2007a, Ushida pers. comm.), considerably larger than the smallest free swimming individuals measured in the field in Mozambique. It is possible that this discrepancy is due to natural variation in the size of pups at birth but captivity may also have been a factor. The first ray born at this aquarium ultimately did not survive. Staff at the aquarium reported aggressive behaviour by the paternal male ray toward the pup in the enclosure after it was born. It is not known if the harassment lead to the newborn ray's death (Anon 2007b, Ushida pers. comm.). No interaction between presumed newborns and larger, mature male manta rays were observed in Mozambique. Field observations in Mozambique suggest that manta rays are similar to all other documented elasmobranch species in that they do not exhibit obvious parental care, but it does not confirm that aggressive behaviour by mature males toward younger juvenile rays does not occur in the wild.

Small immature manta rays were only sighted in the summer months in Mozambique, from October through to February. The three smallest rays observed during the study,

individually identified by their ventral markings, were not re-sighted more than four days after first identification, despite two of these individuals being first identified early in the study during December 2003. The lack of parasite loads or bite marks may explain their absence from cleaning stations, although their absence at popular feeding areas cannot be so readily explained. These observations suggests that newborn pups and young of the year may segregate themselves from the adult population. Ontogenetic habitat partitioning has been well documented in elamobranchs, with sharks and rays either using specific nursery grounds or expanding their home range as they grow in size (Springer 1967; Bass 1978; Simpfendorfer and Milward 1993). Nursery grounds are geographically discrete areas where pregnant females deliver their young. These areas afford vulnerable neonates a degree of protection from predation (Springer 1967) as well as provide them with a shallow, sheltered environment where food is abundant and they can rapidly increase in size (Branstetter, 1990; Castro, 1993). Tagging studies have demonstrated that juveniles often exhibit strong site fidelity to nursery grounds and may, at this stage of their lives, exhibit narrow ranges of movement (McKibben and Nelson, 1986; Castro, 1993; Morrissey and Gruber, 1993). While the use of nursery grounds by newborns or juvenile rays in Mozambique cannot be confirmed, segregation of the smaller size classes seems likely as there was a highly significant bias for larger, mature individuals in the identified population. Alternatively, young of the year or juvenile rays may have high mortality rates. The majority of juvenile rays that were observed during the study exhibited evidence of encounters with sharks, such as bite mark scars, at less than 3 m DW (Table 6.1 and Chapter 8). At this size, it is unclear whether many individuals would survive a serious shark attack.

Conclusion

While not optimal for all species, the use of non-intrusive photographic sight-resight techniques proved both practical and valuable in helping to establish many unknown aspects of the reproductive ecology of *Manta birostris* variant A. These techniques were particularly useful in a population where re-sighting events allowed for the observation of individuals throughout and between years. As such, reproductive parameters such as size at maturity, gestation period and reproductive periodicity were estimated for *M. birostris* var. A for the first time in the wild.

Similar to most elasmobranchs, it is not likely that populations of *M. birostris* var. A could sustain heavy fishing pressure (Musick 1999). As aspects of their reproductive ecology are revealed, such as their small litter size, long gestation period, and variable annual to triennial reproductive periodicity, manta rays appear even less equipped to withstand fishing pressure than most elasmobranchs species. Fishing has been implicated as one of the most significant factors driving variation in fish communities (Worm et al. 2005). The recovery of this species from unsustainable fishing would be a particularly slow process and localised extinctions remain a possibility in areas where they are heavily overexploited (Casey and Myers 1998).

Chapter 7

Habitat use and cleaning behaviour of *Manta* *birostris* variant A in southern Mozambique

INTRODUCTION

Cleaning behaviour in reef fishes has been studied in detail both in the field and experimentally (Feder 1966; Poulin and Grutter 1996; Wicksten 1998; Losey et al. 1999; Côté 2000). During cleaning interactions, individuals seeking to be cleaned, henceforth referred to as 'clients', visit a cleaning station or areas with cleaner 'hosts'. Clients will often make repeated passes over a particular area of reef or hover motionless while cleaners remove everything from ectoparasites and algal build-up to scales, tissue and mucus from the client's body (Feder 1966; Losey 1972; Foster 1985; Poulin and Grutter 1996). Typically clients will advertise their willingness to cooperate in the cleaning process by striking a stereotypical posture (Losey 1972). Although ectoparasites often form a large part of the material ingested by cleaner fish, parasite removal may not be the sole benefit derived by a client, as the actions of the host may also promote wound healing and the prevention of infection (Foster 1985). This symbiotic cleaning behaviour, which often results in the presumed mutual benefit of both client and host, occurs between a wide range of invertebrates, teleost and chondrichthyan fishes and marine reptiles (Feder 1966; Keys 1982; Losey 1987; Hart et al. 1990; Losey et al. 1994; Poulin and Grutter 1996; Sazima et al. 2004) Over one hundred species of marine teleosts in nineteen different families are reported to engage in cleaning interactions in the wild (Côté 2000).

Despite their tendency to carry heavy ectoparasite loads, scant attention has been paid to the role of sharks and rays as clients in cleaning interactions (Cressey and Lachner 1970). More recent anecdotal observations or studies on a limited number of elasmobranch species, such as bull sharks (*Carcharhinus leucas*), lemon sharks (*Negaprion brevirostris*), Caribbean reef sharks (*Carcharhinus perezi*), nurse sharks (*Ginglymostoma cirratum*), scalloped hammerhead sharks (*Sphyrna lewini*), white tip reef sharks (*Triaenodon obesus*), thresher sharks (*Alopias vulpinis*) and stingray species (*Dasyatis americana* & *Dasyatis macrophthalma*), have revealed that sharks and rays often participate as clients in cleaning interactions with various reef fishes (Keys 1982; Snelson 1990; Sazima and Moura 2000).

Manta rays are widely observed by SCUBA divers visiting inshore reefs to be cleaned by small host cleaner fish (Homma 1999; Rubin 2002; Clarke et al. 2008; Dewar et al. 2008; Kitchen-Wheeler 2008). The areas that are commonly frequented by manta rays are often referred to as 'hot spots' or 'aggregation sites', and these rays will visit particular reefs to be cleaned with such regularity that they often create predictable dive sites for eco-tourism industries (Anderson 2002; Clarke 2005). In some locations, these 'cleaning stations' are active year-round, while in other locations the presence of manta rays at inshore reefs is seasonal or erratic (Dewar et al. 2008, Townsend pers. comm., Stevens pers. comm.). Details of the cleaning behaviour between fish hosts and manta ray clients have yet to be reported in the literature in detail (Mitchill 1824; Homma 1999) and the frequency with which manta rays visit designated cleaning stations has only been reported for one aggregation site in Indonesia (Dewar et al. 2008).

This study examined the habitat use, with respect to cleaning behaviour, of a semi-resident population of *Manta birostris* variant A in southern Mozambique in addition to the behavioural interactions between the rays and cleaner fishes. Major aims included estimates of the frequency with which *M. birostris* var. A visits commonly used cleaning stations on inshore reefs and the total time individuals spend at the cleaning stations per day. These estimates may offer new insights into the importance of these sites to the daily and seasonal activity of these rays. The diversity and behaviour of cleaner fish species associating with *M. birostris* var. A at reefs in southern Mozambique was also examined. Investigations into the cleaners' ability to cooperatively utilise manta rays as a food resource was also examined to gain insight into host-client interactions and how host fishes partition the habitat/resources presented by a visiting manta ray client.

METHODS

Study period, location, and study reefs

A population of *M. birostris* var. A off the coast of Inhambane, Mozambique was monitored over a four and a half year period from May 2003 to November 2007. The primary field sites for this study were Manta Reef and Giant's Castle. At these two

study reefs, five major cleaning stations (areas where mantas were commonly observed being cleaned Fig. 7.1) were identified in 2003. Manta reef was found to support three major cleaning stations for manta rays, while Giant's Castle supported two.

Detailed maps constructed for each site outlined the shape and depth contours and prominent structures of each reef as well as prominent reef fauna. Reef maps also indicated the locations of acoustic receivers, deployed to record the presence of acoustically tagged rays, and manta ray cleaning stations.

Descriptions of sites

Primary Site: Manta Reef

The primary study site, Manta Reef (MR) sits in 16-26 m of water (Fig. 7.2), with all three cleaning stations located in approximately 20 m depth. The current on this reef typically ran from north to south. The acoustic receiver was placed on the western side of the reef along a sloping sandy area, situated less than 200 m from all identified cleaning stations. The base of the chain was anchored in 28 m depth with the actual receiver suspended in 20 m of water by a small buoy.

The most northerly cleaning station had sheer walls on three of its sides and was filled with large schools of snapper, grunts, triggerfish and big-eye crescent fish. The substrate, largely rocky reef, was encrusted with sponges and had patches of hard corals and sea urchins. *Manta birostris* variant A typically approached the northerly cleaning station (NCS) from the north making large wide loops in the water column. When being cleaned at this location individual rays typically hovered 1 – 3 m above the reef (Fig. 7.1).

The middle cleaning station (MCS) was located on the reef plateau and was split in two by a large sand gully. The rocky area on either side of the gully was predominantly covered by soft coral (*Sinularia sp.*). Anthias, triggerfish, butterflyfish and wrasse species were the most prevalent fish types in this area. *Manta birostris* var. A typically cleaned on both sides of the sand gully, approaching MCS from either the northerly or southerly stations. Individuals swam in slow tight loops 0.5 – 1.5 m above the soft coral patches at this station (Fig. 7.3).

140

The most southerly station (SCS) was bordered on one of its sides by a large sheer drop off into a crescent-shaped, sandy bowl. *Manta birostris* variant A usually approached SCS from the south, or if already present on the reef they approached via NCS and MCS. This southerly cleaning station was similar to MCS in that it was covered by soft coral (*Sinularia sp.*) and had swarms of anthias, butterflyfish, and wrasse species directly above it. *Manta birostris* variant A cleaned approximately 0.25 – 1 m above the soft coral at this station (Fig. 7.4).
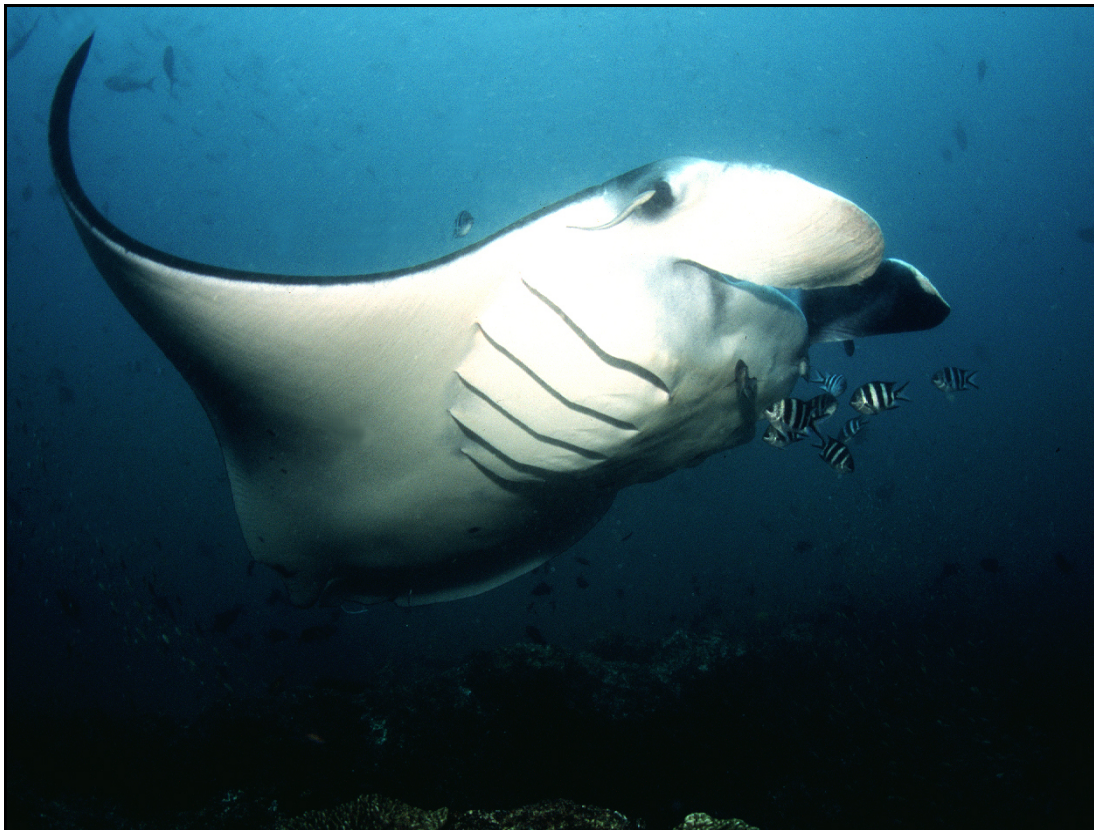
Figure 7.1 *Manta birostris* variant A being cleaned by *Abudefduf natalensis* on the most northerly station on Manta Reef.

Figure. 7.2 Map of Manta Reef with topographical detail, position of acoustic receiver with dashed areas indicating the three cleaning stations (NCS = northern cleaning station, MCS = middle cleaning station, SCS = southern cleaning station) for *Manta birostris* variant A.
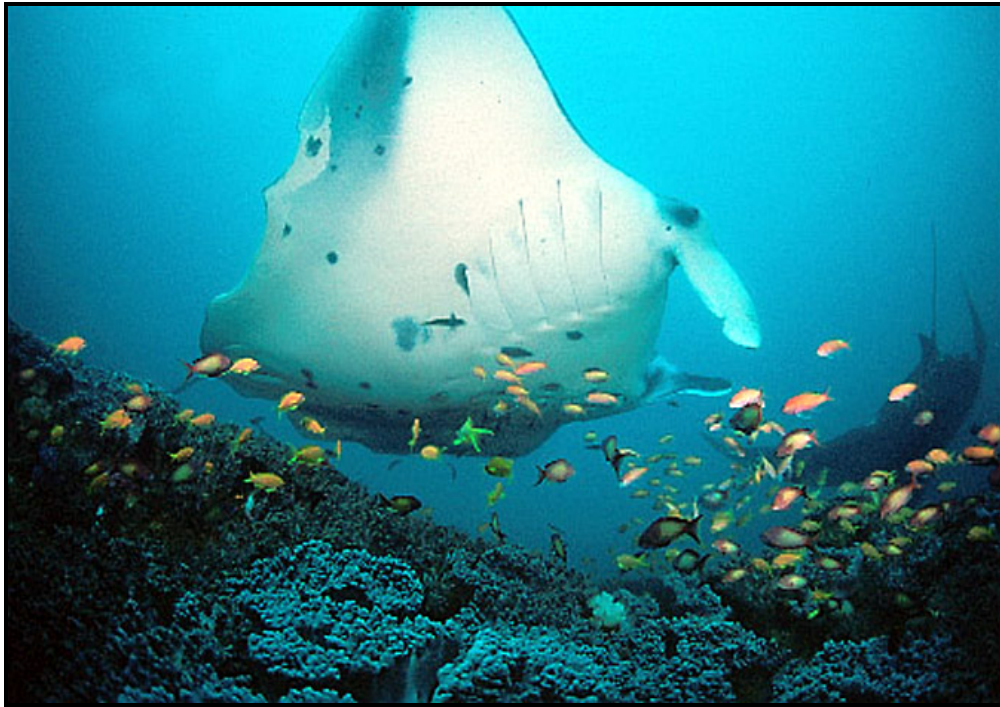
Figure 7.3 *Manta birostris* variant A being cleaned on the middle cleaning station on Manta Reef.

Figure 7.4 *Manta birostris* variant A cleaning over a patch of *Sinularia sp.* on the most southerly cleaning station on Manta Reef.

<u>Secondary Site: Giant's Castle</u>

The secondary site for this study, Giant's Castle, was in approximately 25 – 29 m of water. This reef was long and narrow with a large, steep dropoff along the entire length of the most southern side and a more gradual tapering slope along the northern end. Both cleaning stations (ECS and WCS) were located in 25 – 28 m of water and were situated along rocky reef ledges (Fig. 7.5). These stations hosted large numbers of reef fishes including triggerfish, travelly, butterflyfish, moorish idols, and grouper. *Manta birostris* variant *A* commonly cleaned close to the reef at these stations, hovering approximately 1 m above the substrate (Fig. 7.6). In general, individual rays approached both stations from the far south-western side of the reef. The current most often ran in the opposite direction from the north-east. The acoustic receiver on this reef was placed on the northern side of the reef and was situated less than 250 m from either cleaning station. The base of the listening station was anchored at 31 m with the VR2 receiver suspended beneath a sub-surface float at approximately 25 m depth.

Cleaner (host) fishes

Study dives on the reefs were made between the hours of 7:00 am and 3:00 pm with underwater transects used to count and identify individual *M. birostris* var. A at the various cleaning stations (Chapter 5). Cleaning behaviour data were collected on a subset of dives. A single diver with an underwater slate made observations onsite. Observations of the cleaning activity were taken at least five minutes after the initial sighting of a manta ray at a cleaning station to allow the manta ray and cleaner fish to acclimatise to the diver's presence. A photographic record (still and video images) was often made by a second diver to check for accuracy of data collection.

The behaviour of individual rays immediately before and during cleaning events was examined on 40 separate occasions. Based on observations from a pilot study, manta ray behaviour at cleaning stations was divided into four distinct categories: (1) reduction in swimming speed, (2) hovering above the reef, (3) adoption of specific body postures, (4) multiple passes made during a cleaning session. For manta rays, time spent at cleaning

stations was comprised of time that was actually spent being cleaned by host fish and time that was spent swimming in a circular fashion between passes over the cleaning station. For ten of the individual rays examined, ten passes during a cleaning bout were recorded and broken down into cleaning time (seconds) and circling time (seconds) to determine the relative time that individual rays spent being cleaned when present on cleaning reefs.

Cleaner fish species were identified and observed during cleaning interactions with *M. birostris* var. A over a four and a half year study period in order to construct a host cleaner fish species list for this region of southern Mozambique. To determine the relative involvement of the various host cleaner fishes in cleaning interactions with *M. birostris* var. A, direct counts were made during cleaning sessions. Twelve replicate experimental observations were conducted for four of the monitored cleaning stations, with six replicates conducted at the fifth, most northerly station at Manta Reef.

Figure 7.5 *Manta birostris* variant A being cleaned by *Chaetodon kleinii* at the most easterly cleaning station at Giant's Castle reef.
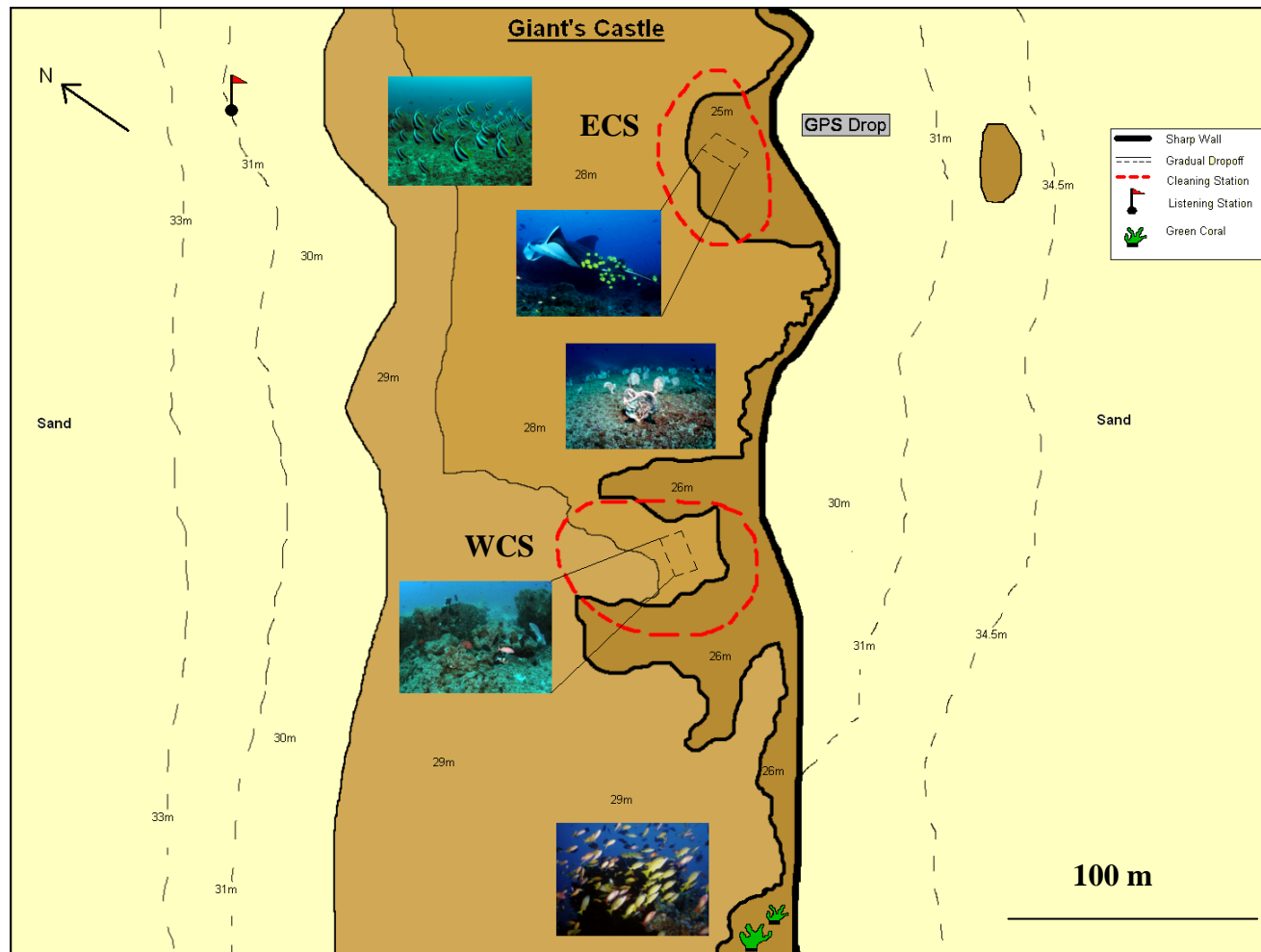
Figure 7.6 Map of Giant's Castle reef with topographical detail, position of acoustic receiver with dashed areas indicating the two cleaning stations (ECS = eastern cleaning station, WCS = western cleaning station) for manta rays.

During the pilot study, it appeared that different cleaner fish species focused their efforts on different regions of the manta ray body. To test the hypothesis that cleaner fish were partitioning the body of manta ray clients, cleaning activity in relation to body region was investigated. Five distinct body surface regions were defined which comprised (1) the head, (2) the supra-branchial region (3) the ventral gill slit area, (4) the trailing edge of the pectoral fins (including the dorsal fin and pelvic fins) and (5) the tail (Fig. 7.7). These areas were selected based on observations of regional cleaning intensity in a pilot study. Remaining sections of the body were grouped into a sixth composite group collectively referred to as 'other'. Twenty separate cleaning observations were conducted at the five cleaning stations. In these observations, the region targeted by each cleaner fish during an encounter was recorded.

Ectoparasites

Observations and samples of ectoparasites were obtained opportunistically in the field. The dorsal and ventral surfaces of 15 different rays were closely inspected for ectoparasites while they hovered above the reef being cleaned. The same body region chart used in the cleaning behaviour observations (Fig. 7.7) was used when describing ectoparasite location. Parasites were collected from live rays with the aid of a small v-shaped scraping tool fashioned to dislodge ectoparasites from the skin's surface and trap them in a small, fine-mesh bag attached to the tool's trailing edge. Parasites were also collected from inside the mouth, buccal cavity and the gill arches of four *M. birostris* var. A killed by fishermen in Mozambique. All samples were stored in 70% ethanol prior to identification and photography, using a binocular dissection microscope (Wild Type M3Z, Heerburg, Switzerland) and attached camera (Coolpix 995, canon, Japan). Gut contents analysis to inspect for ingested ectoparasites was not performed on cleaner fishes, as the collection of these fishes may have compromised the viability of the cleaning stations and would have raised strong opposition with both local Mozambicans as well as local eco-tourism operations in the area.
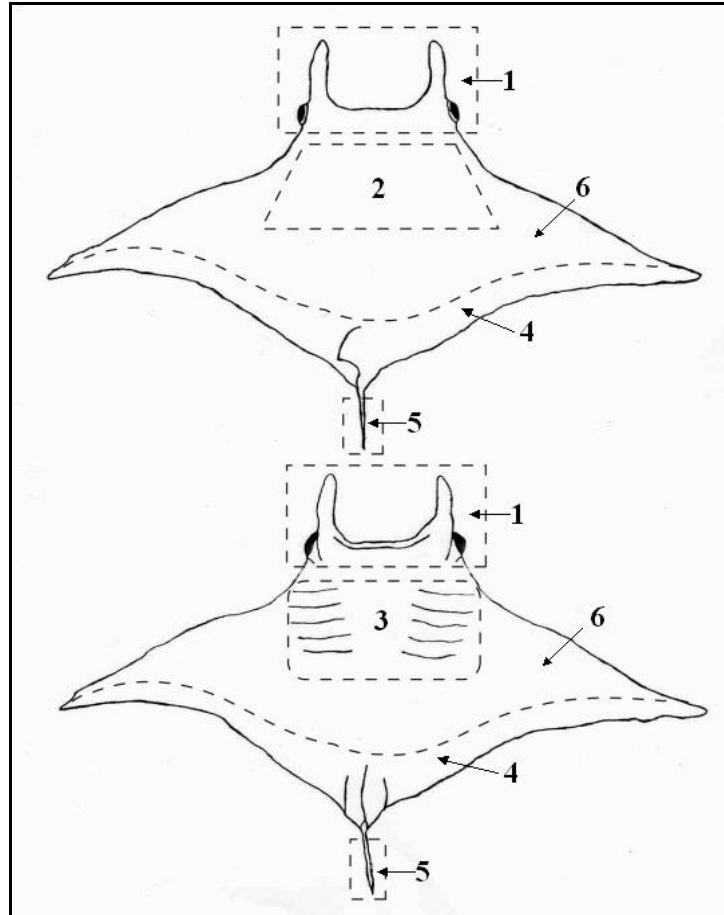
Figure 7.7 Designated regions on the dorsal and ventral surfaces of the manta ray body used to examine both the occurrence of ectoparasites and the areas of the manta ray body targeted by cleaner fish.

Acoustic telemetry

Acoustic receivers (VR2, Vemco, Canada) were moored to the seafloor, with an anchor chain and a sub-surface bouy (Fig. 7.8a), at both Manta Reef and Giant's Castle in July 2005. Field-testing determined that each receiver had clear, 360-degree reception up to 300m. Receivers were placed on the slopes of the reefs away from the main cleaning stations to avoid collision or entanglement by manta rays. All receivers were situated in approximately 30 m depth with direct line-of-sight coverage of cleaning stations. Seven deployments of these receivers were made between July 2005 and September 2007. When receivers were retrieved for data download they were re-deployed immediately if weather permitted.
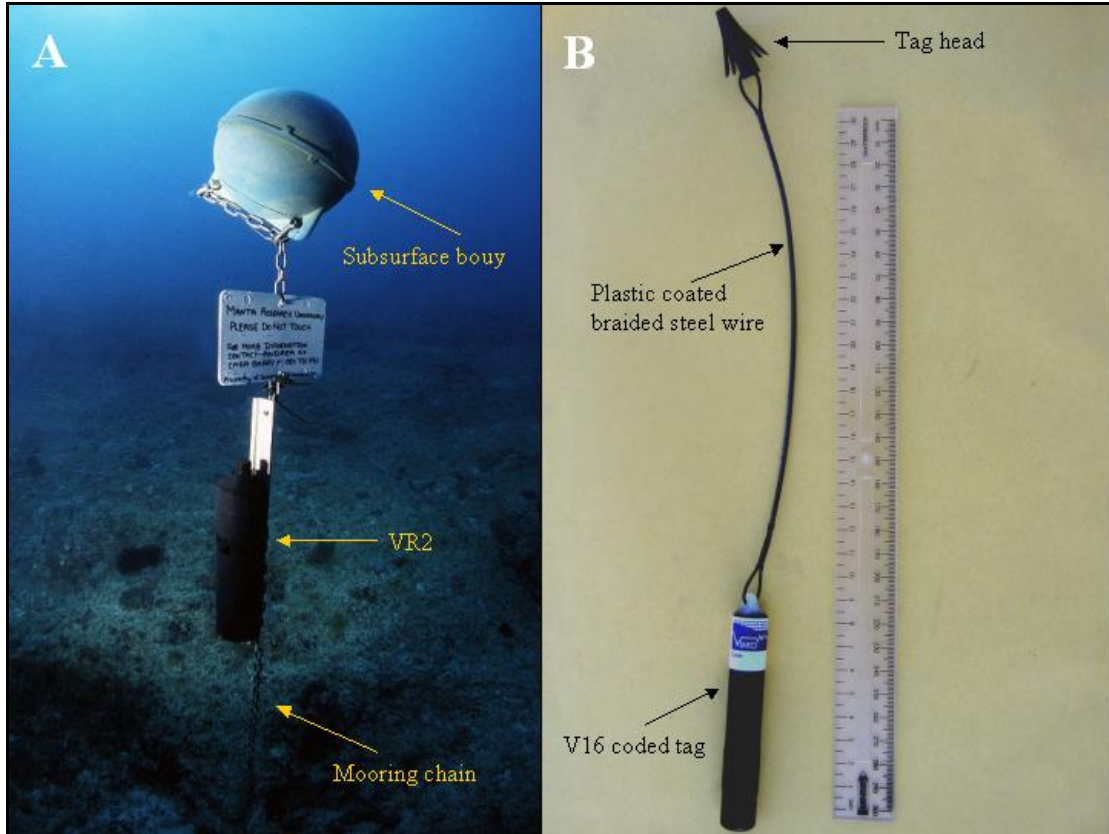
Figure 7.8 Acoustic telemetry equipment: (a) receiver attached to semi-permanent station on reef; (b) coded tags attached to *Manta birostris* variant A with plastic tag head.

Fourteen acoustic tags (V-16, Vemco, Canada), approximately 90 mm in length and 15 mm in diameter attached to a plastic anchor head by approximately 250 mm of plastic-coated fishing wire strengthened by black plastic shrink wrap (Fig. 7. 8b) were deployed on to identified individuals. Tags were initially inserted with a Hawaiian sling-type spear. In 2006 a small speargun was modified for tagging and was used thereafter (Fig. 7.9a). All manta rays were tagged in the posterior half of their body in the dorsal musculature of the pectoral fin slightly lateral to the body cavity (Fig. 7.9b). This particular placement helped to prevent the splitting of the pectoral fin margin from drag or the loss of tags due to shark-inflicted injuries that were common in this region of the body (Chapter 8). The acoustic tags were programmed to transmit at 20-69 second intervals and had a battery life of approximately 1255 days.
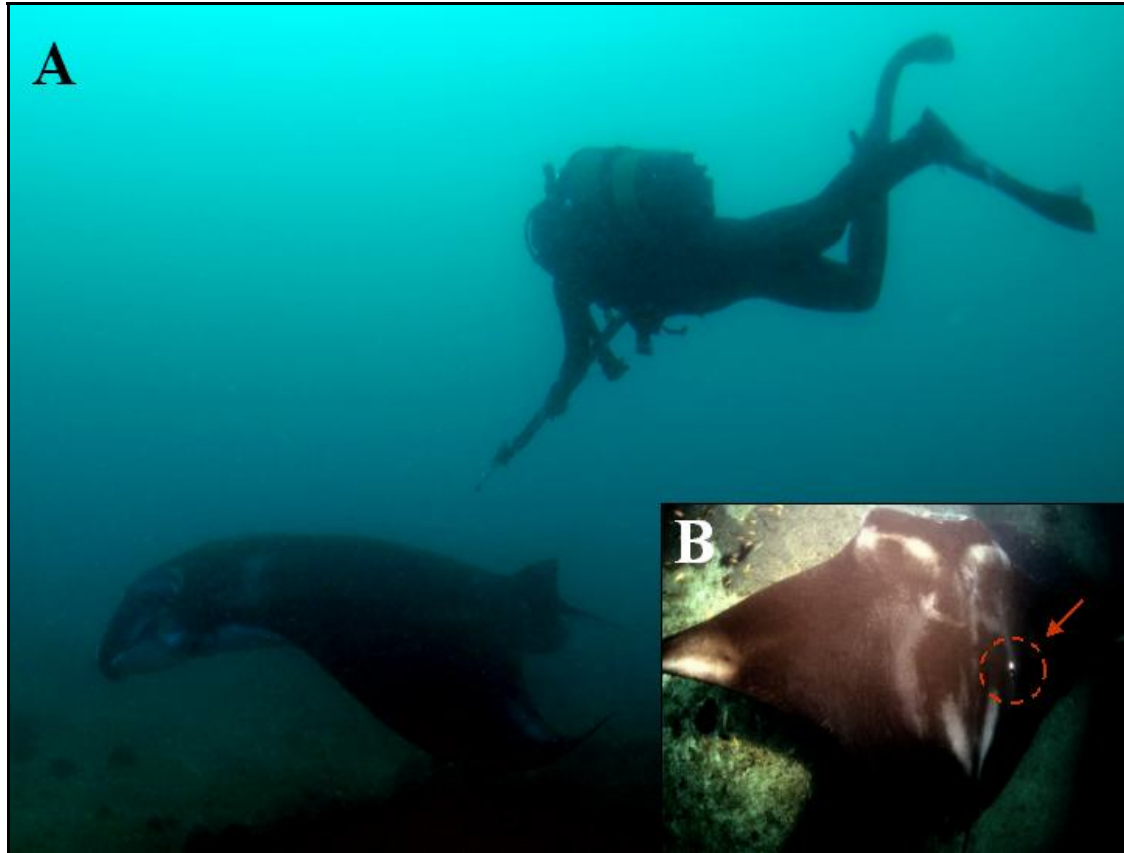
Figure 7.9 Tagging in the field (a) Diver approaching manta from the dorsal surface to insert tag with a modified speargun (b) manta ray returning to station after tag has been deployed, tag is firmly anchored in the dorsal musculature of the pectoral fin.

Throughout the VR2 deployment periods acoustic receivers allowed for the continuous surveillance of the two monitored cleaning reefs. When tagged rays were present on the reef the receiver would log the presence of the individual. To be considered a cleaning event, an acoustically tagged individual needed to be present on the reef for a minimum of three minutes based on observations of actual cleaning events. Individuals that passed within range of one of the acoustic receivers for shorter periods of time were regarded as 'in transit' and were not considered to be actively involved in cleaning activities.

Seasonality and sightings per unit effort

To examine temporal trends in cleaning activity on the study reefs, the total number of rays seen per minute of observational dive time (transect time) were calculated for each dive and pooled by calendar month (SPUE, Chapter 5). Data were combined for the

entire four and half year study, with mean numbers of rays compared across calendar months. Practical issues resulted in variable sampling periods in each year. For the month of November five years of data was collected. For each of the months of January, February, October, and December four years of data were collected. For March, May and September three years of data were collected. For the months of April, July, and August only two years of data were collected. The month of June is only represented by a single year's data.

Data analyses

Chi-square (χ^2) analysis was used to compare sex ratio data. Yates' correction factor was applied to all Chi-square tests where there was one degree of freedom. SPUE data were examined using the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks as data were non-normal and could not be normalised. An all-pairwise (Dunn's method) was used as the post-hoc test. Significance was accepted at $P < 0.05$. Analyses were conducted using Sigmastat (SigmaStat® S.P.S.S.). Box plots were used to graphically represent the median value with the box representing the 25% and 75% confidence intervals (CI) and whiskers representing the 5% and 95% CI. The mean value was also displayed with a white line. Outliers were indicated by solid dots with the numbers directly over them indicating the number of sampling events (dive transects).

Ordination of the mean number of fish species per cleaning interaction was conducted using multivariate statistical software (PRIMER v5) (Clarke and Gorley 2001; Clarke and Warwick 2001). Six cleaner fish groups were compared, five containing individual cleaner fish species, *Chaetodon kleinii*, *Abudefduf natalensis*, *Pomacanthus rhomboids*, *Odonus niger*, *Thalassoma lunare* and a single group containing two cleaner wrasse species, *Labroides bicolor* and *Labroides dimidiatus* (as sample sizes for both were small, they were difficult to differentiate from a distance and they were observed cleaning the same body regions of *M. birostris* var. A). Cleaner fish composition data for each interaction examined were randomly allocated into groups of five and the mean abundance calculated for each of the six groups. Pooling data has been advocated in several studies to overcome the biases of individual samples (Platell and Potter 2001;

White et al. 2004). Prior to ordination, the data were square root transformed and similarity matrices were constructed using the Bray-Curtis similarity coefficient (Clarke and Warwick 2001). Multi dimensional scaling (MDS) was performed on the resulting similarity matrices to provide a visual representation of the differences between the observed cleaner fish compositions within the five designated body regions. One-way analysis of similarity (ANOSIM) was then used to determine if cleaner fish composition during cleaning interactions with *M. birostris* var. A was significantly influenced by the region of the manta ray's body. Similarity percentages (SIMPER) were then used to identify the fish species which characterised the cleaner fish composition at each regional body location in addition to which fish groups were responsible for the dissimilarities between regional body locations (Clarke and Warwick 2001; Linke et al. 2001).

RESULTS

Manta rays at cleaning stations

Manta birostris variant A was observed being cleaned at the reefs during every month of the year, with 1,879 separate manta ray encounters observed at the monitored cleaning stations over 436 dives to the study reefs. The largest total number of manta rays seen per dive on either of the two monitored reefs was 30 individuals, while the mean number of individuals seen cleaning per dive was 4.31 ± 4.8 rays. Manta rays were not always present on dives to cleaning stations. During the four and a half year study period, *M. birostris* var. A was absent from cleaning stations on 130 (30%) of 436 dives.

An ANOVA on ranked SPUE data revealed a significant difference in the median values of sighted individuals among calendar months (P < 0.001). An all-pairwise comparison showed significant differences in manta ray sightings between December and March, September and October (P < 0.05). While the difference was not large, this analysis suggested that there were slightly more *M. birostris* var. A present and cleaning on inshore reefs in the summer months, particularly the month of December (Fig. 7.10).
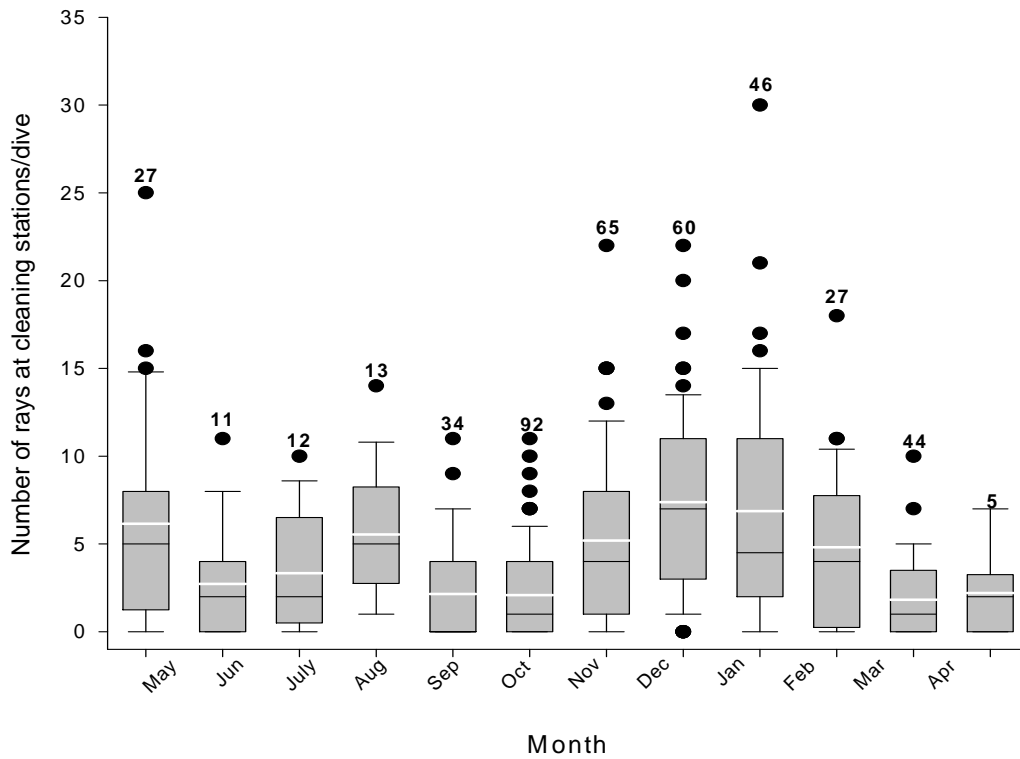
Figure 7.10 Total number of *Manta birostris* variant A identified on cleaning stations per minute of dive transect time per month (monthly data pooled for all years of study).

Many of the individuals observed at monitored cleaning stations exhibited injuries attributed to shark attacks (see Chapter 8 for more detail) and blemishes or welts on their upper gill sit area (Fig. 7.11). The persistent reddish welts on the ventral body surface around the anterior gill slits were not abrasions, with symptoms lasting for years in some individuals, and were presumed to be skin infections of unknown origins. The welts looked very similar in all individuals (Fig. 7.11a,b), although some appeared more aggravated than others. These welts always occurred in the same region, medially and anterior to the two most anterior gill slits. Although samples were not obtained, observations of identified individuals revealed that these welts were present in 45.9% of the observed population of *M. birostris* variant A. The incidence of these welts was not significantly different between females (49.9%) and males (30.9%) ($\chi^2 = 3.49$, df = 1, P = 0.062).
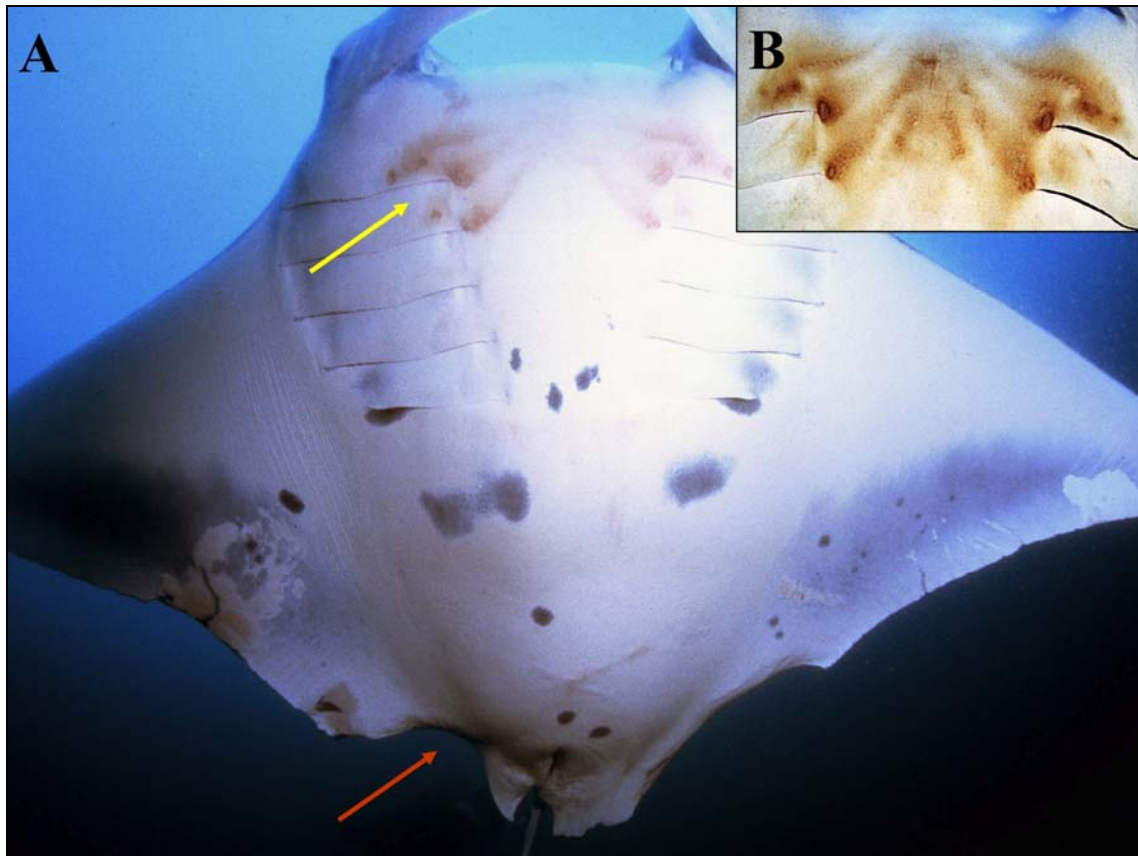
Figure 7.11 *Manta birostris* variant A: (a) bite wounds from sharks along the trailing edge of the pectoral fins and welts on the gill slits area; (b) persistent welts on the gill slits of a different individual.

Behavioural observations of *M. birostris* var. A during cleaning events revealed that individuals always reduced their swimming speed when approaching cleaning stations. Additionally most individuals (90%) hovered above stations during cleaning events (90% of the time) and displayed angled posturing and gill slit flaring (80%) (Fig. 7.2). Most individuals also made consecutive passes over the cleaning stations (90%) by slowly circling around and re-approaching from the initial entry point. The behaviours of manta rays remained consistent irrespective of the number of individuals present at the cleaning station.

Cleaner fishes

Seven species of reef fish were observed cleaning *M. birostris* var. A (Fig. 7.12); Klein's butterfly fish (*Chaetodon kleinii*), sergeant major damselfish (*Abudefduf natalensis),*

rhomboid angelfish (*Pomacanthus rhomboids*)*,* common blue triggerfish (*Odonus niger),* moon wrasse (*Thalassoma lunare)* and two species of cleaner wrasse (*Labroides dimidiatus & Labroides bicolor*).
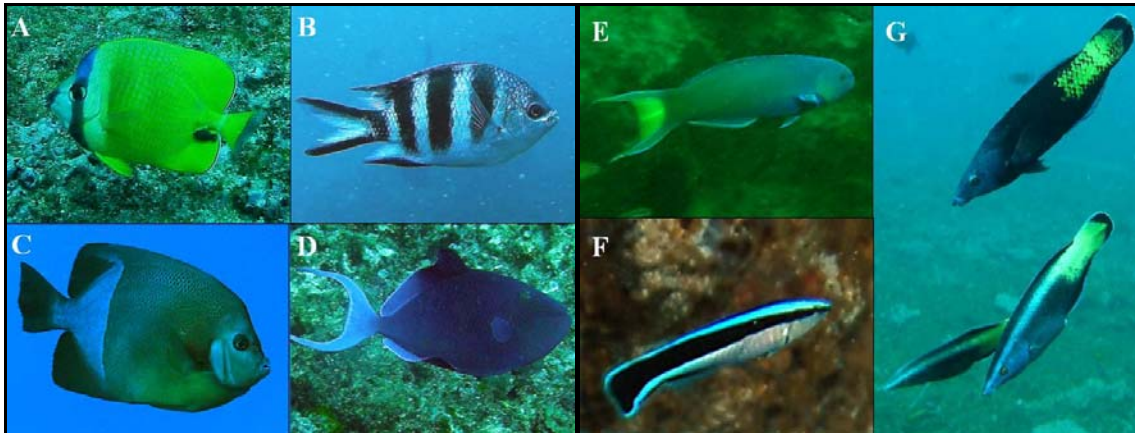
Figure 7.12 Seven species of cleaning fish identified in Mozambique including: *Chaetodon kleinii*, *Abudefduf natalensis*, *Pomacanthus rhomboids*, *Odonus niger*, *Thalassoma lunare*, *Labroides dimidiatus* and *Labroides bicolor.*

Chaetodon kleinii, *A. natalensis*, *P. rhomboids*, *T. lunare, L. bicolor*, and *L. dimidiatus* were regularly observed removing parasites, algal build-up, or necrotic tissue from the body surface of manta rays. However, *O. niger* seldom interacted with *M. birostris* var. A, despite its prolific numbers on the reefs. *Odonus niger* was only observed to approach a manta ray if it was already being cleaned by another host fish species at a cleaning station. Unlike the other species of cleaner fishes that focused on the body of the ray, *O. niger* exclusively took bites from the tail of *M. birostris* var. A (Fig. 7.13a), often biting at the tips of damaged tails that were covered in an unidentifiable whitish buildup (Fig. 7.13b). This interaction usually incited a negative reaction from *M. birostris* var. A, with the ray jerking away or swatting the triggerfish with its tail.
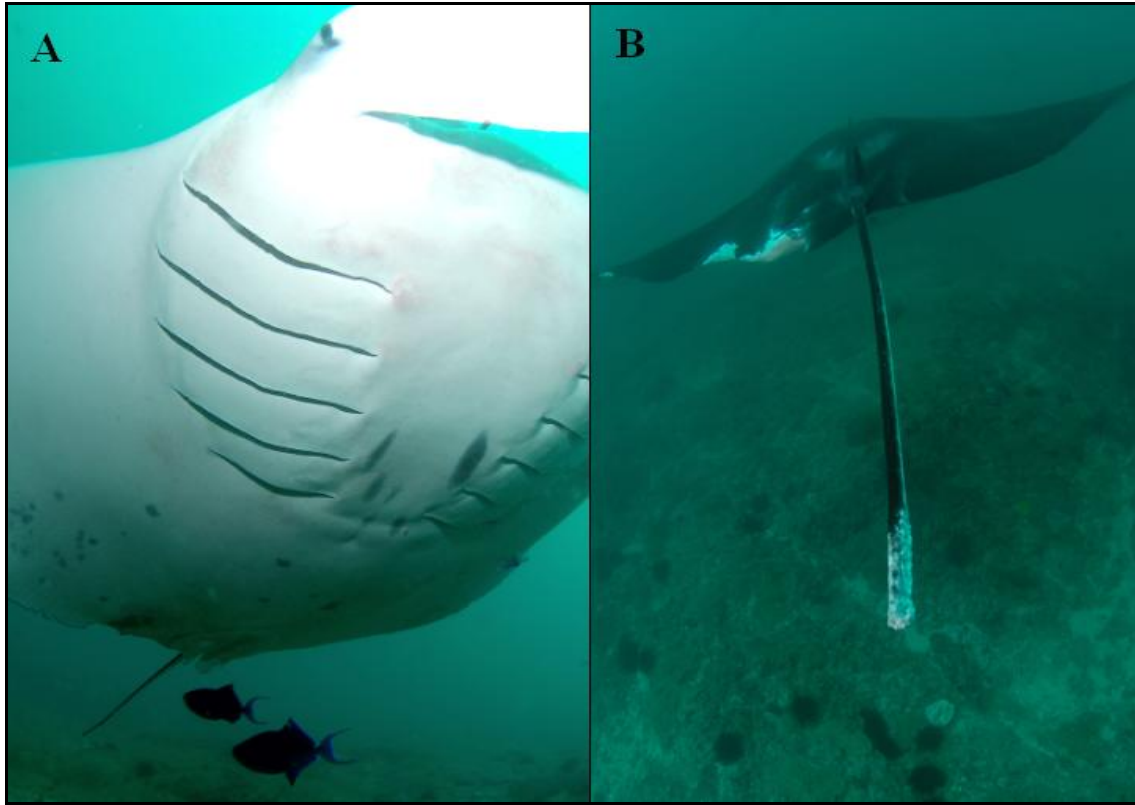
Figure 7.13 (a) *Odonus niger* with *Manta birostris* variant A on cleaning station (b) whitish build-up on the tip of the tail of *Manta birostris* variant A.

The species and density of cleaner fishes present at the five cleaning stations varied considerably, particularly between NCS and all of the other monitored cleaning stations. As a result, the number and species of host fishes cleaning manta rays varied between these different sites (Fig. 7.14). At NCS, the most abundant cleaner of *M. birostris* var. A was *A. natalensis* followed by *P. rhomboids* however these two fish species were absent from the other four monitored cleaning stations. *Chaetodon kleinii* was the most abundant cleaner fish at all other monitored cleaning stations and was seen in highest abundance at ECS and WCS on Giant's Castle reef.
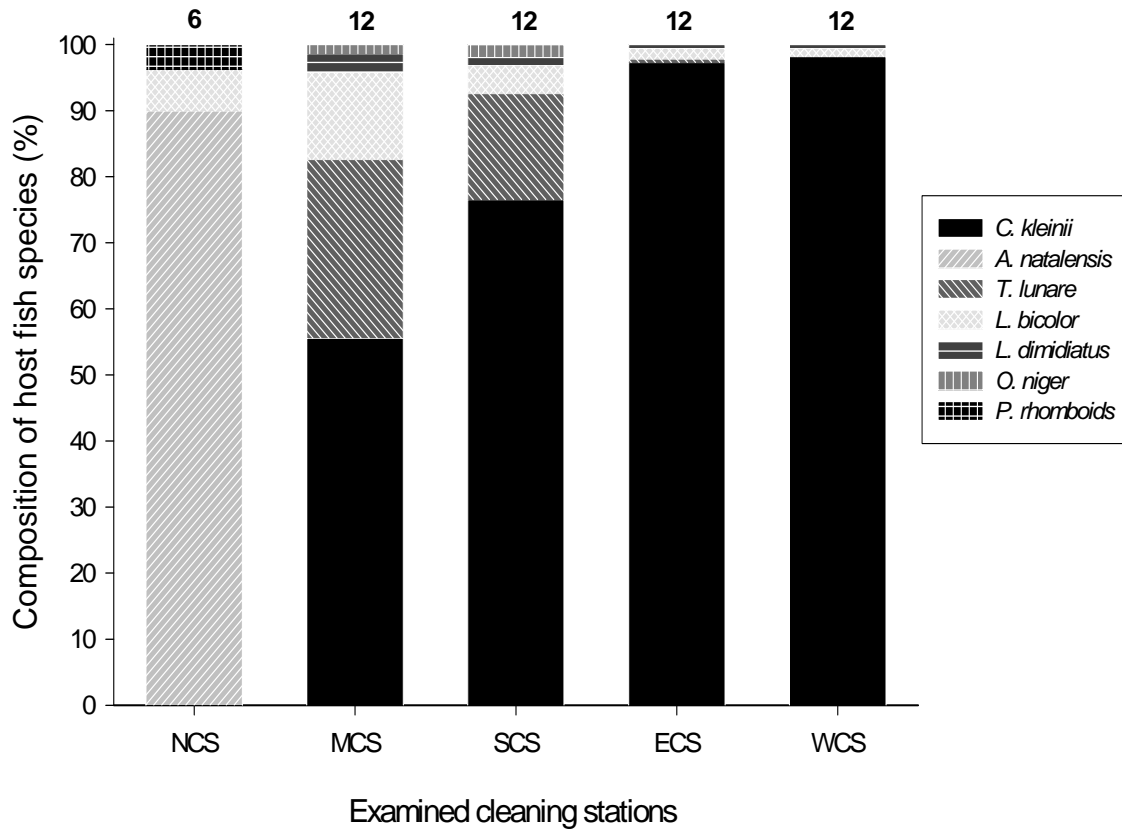
Figure 7.14 Composition of fish species cleaning *Manta birostris* variant A at five examined locations: Site 1: northern station of Manta Reef (NCS); Site 2: middle station at Manta Reef (MCS); Site 3: southerly station at Manta Reef (SCS); Site 4: eastern station at Giant's Castle reef (ECS); Site 5: western station at Giant's Castle reef (WCS).

One-way ANOSIM tests demonstrated that there was a significant difference in the composition of cleaner fish species in the five designated body regions (R-statistic = 0.87, P = 0.001) (MDS ordination plot Fig. 7.15). Pairwise comparisons between each of the five body regions showed that the composition of cleaner fish species differed significantly (P < 0.05). SIMPER revealed that for most regions, a single host species was responsible for characterising the overall cleaner fish composition. *Thalassoma lunare* typified the composition of cleaner fish on the dorsal supra branchial region (region 2) of the manta ray's body, while *P. rhomboids*, *C. kleinii* and *O. niger* typified the species composition on the ventral gill slits area (region 3), along the trailing edge of the pectoral fins (region 4), and on the tail (region 5), respectively.
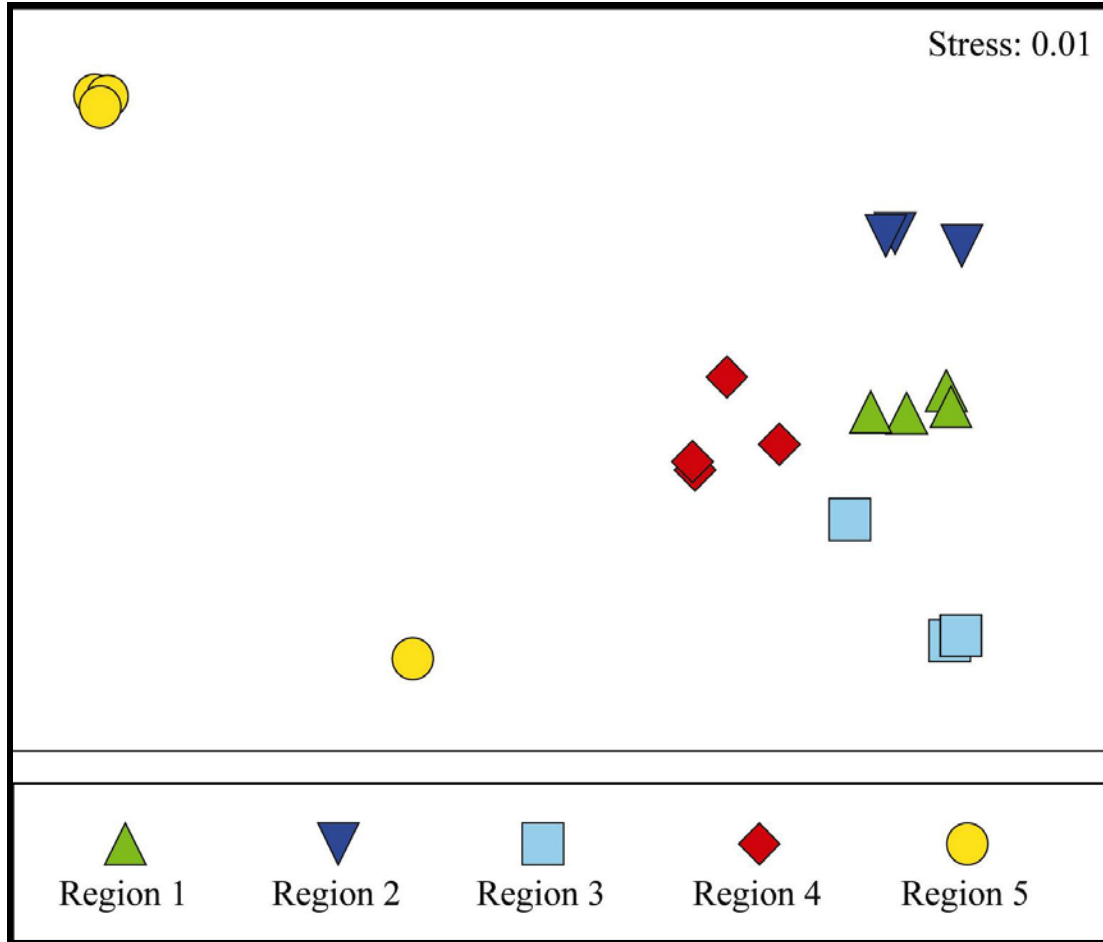
Figure 7.15 MDS plot showing composition of cleaner fish species at the five different body regions of *Manta birostris* variant A.

Only around the face and head (region 1) did three groups of host cleaner fishes typify the cleaner fish composition. Of the four species in these three groups, the host fish that most characterized the cleaner fish composition in region 1 was *T. lunare* followed by *A. natalensis* and cleaner wrasse (*L. bicolor* and *L. dimidiatus*) (Table 7.1). *Chaetodon kleinii* and *A. natalensis* were each responsible for contributing the greatest interspecies differences in most body regions, however other species were also responsible for major and minor interspecies differences in cleaner fish composition in particular regions (Table 7.1).

Table 7.1 Host fish species detected by SIMPER as those most responsible for typifying the cleaning composition in the five designated body regions of *M. birostris* variant A (see species arranged along diagonal cells in bold) and distinguishing each pair of those regions in order of importance (see species arranged along vertical cells). The region at which distinguishing species were most abundant is also provided (see superscripts).

	Region 1	Region 2	Region 3	Region 4	Region 5
Region 1	***Thalassoma lunare*** ***Abudefduf natalensis*** ***Labroides* spp.**				
Region 2	*Abudefduf natalensis* [R1] *Labroides* spp. [R1] *Thalassoma lunare* [R2]	***Thalassoma lunare***			
Region 3	*Pomacanthus rhomboids* [R3] *Abudefduf natalensis* [R1] *Thalassoma lunare* [R1] *Labroides* spp. [R1] *Chaetodon kleinii* [R3]	*Thalassoma lunare* [R2] *Pomacanthus rhomboids* [R3] *Chaetodon kleinii* [R3] *Abudefduf natalensis* [R3]	***Pomacanthus rhomboids***		
Region 4	*Chaetodon kleinii* [R4] *Abudefduf natalensis* [R1] *Labroides* spp. [R1] *Thalassoma lunare* [R1]	*Chaetodon kleinii* [R4] *Thalassoma lunare* [R2] *Labroides* spp. [R4]	*Chaetodon kleinii* [R4] *Pomacanthus rhomboids* [R3] *Thalassoma lunare* [R4] *Labroides* spp. [R4] *Abudefduf natalensis* [R3]	***Chaetodon kleinii***	
Region 5	*Abudefduf natalensis* [R1] *Thalassoma lunare* [R1] *Labroides* spp. [R1] *Odonus niger* [R5] *Chaetodon kleinii* [R1]	*Thalassoma lunare* [R2] *Odonus niger* [R5]	*Pomacanthus rhomboids* [R3] *Odonus niger* [R5] *Chaetodon kleinii* [R3] *Abudefduf natalensis* [R3]	*Chaetodon kleinii* [R4] *Odonus niger* [R5] *Labroides* spp. [R4] *Thalassoma lunare* [R4]	***Odonus niger***

On several occasions during the study period, a single host cleaner fish species would attend to *M. birostris* var. A at a cleaning station. Observations made on these occasions suggested that host fishes may expand their cleaning efforts to include regions of the body that were not commonly targeted (Fig. 7.16). For example, *C. kleinii*, if multiple species of cleaners are present, will almost exclusively target both the trailing edge of the pectoral fins (Fig. 7.16d) and bite wounds (Fig. 7.17). However if *C. kleinii* was cleaning *M. birostris* var. A alone, individuals would often attend to other regions of the body including inside the mouth (Fig. 7.16a). The same applied for other host cleaner species like *T. lunare*, which when cleaning in the presence of *C. kleinii* and other wrasse species would almost exclusively forage on the dorsal surface of *M. birostris* var. A picking off caligid copepods (Fig. 7.16b), but in the absence of other cleaner species, was observed to clean both inside the buccal cavity and along the pectoral fin margin (Fig. 7.18b).
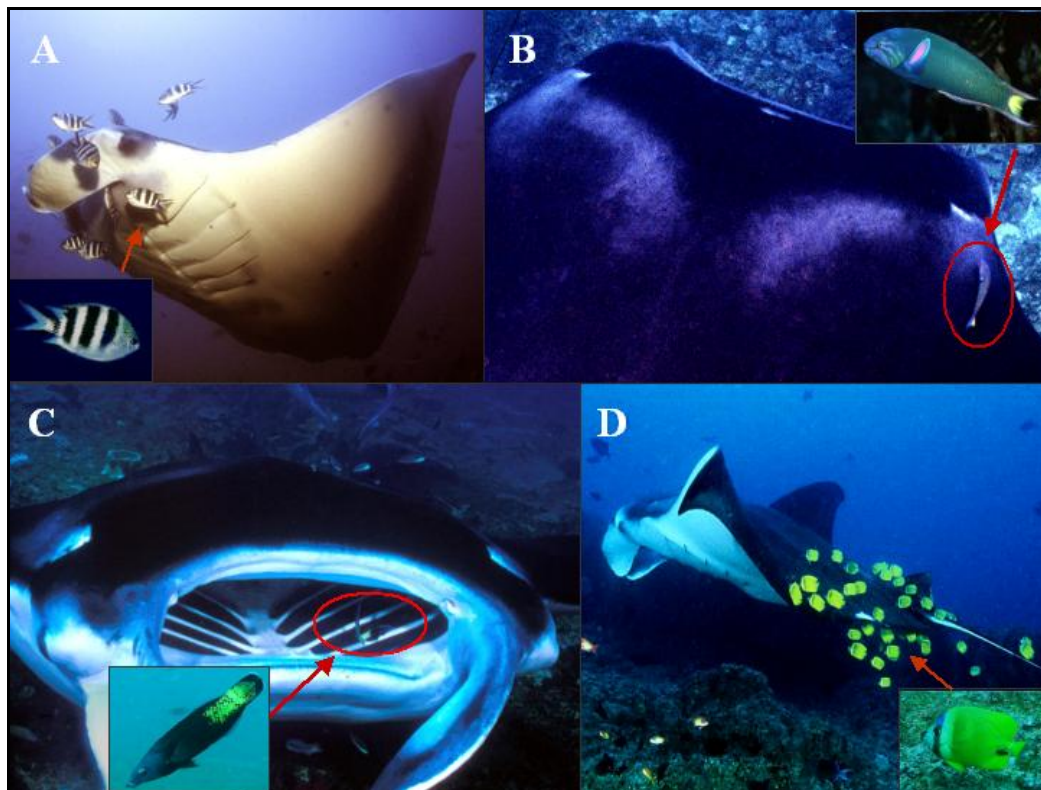
Figure 7.16 Regions of specialisation for cleaner fish (a) head region, sergeant major damselfish; (b) supra branchial region, moon wrasse; (c) inside the buccal cavity, wrasse species like *Labroides bicolor*; (d) trailing edge of the pectoral fins, butterfly fish.
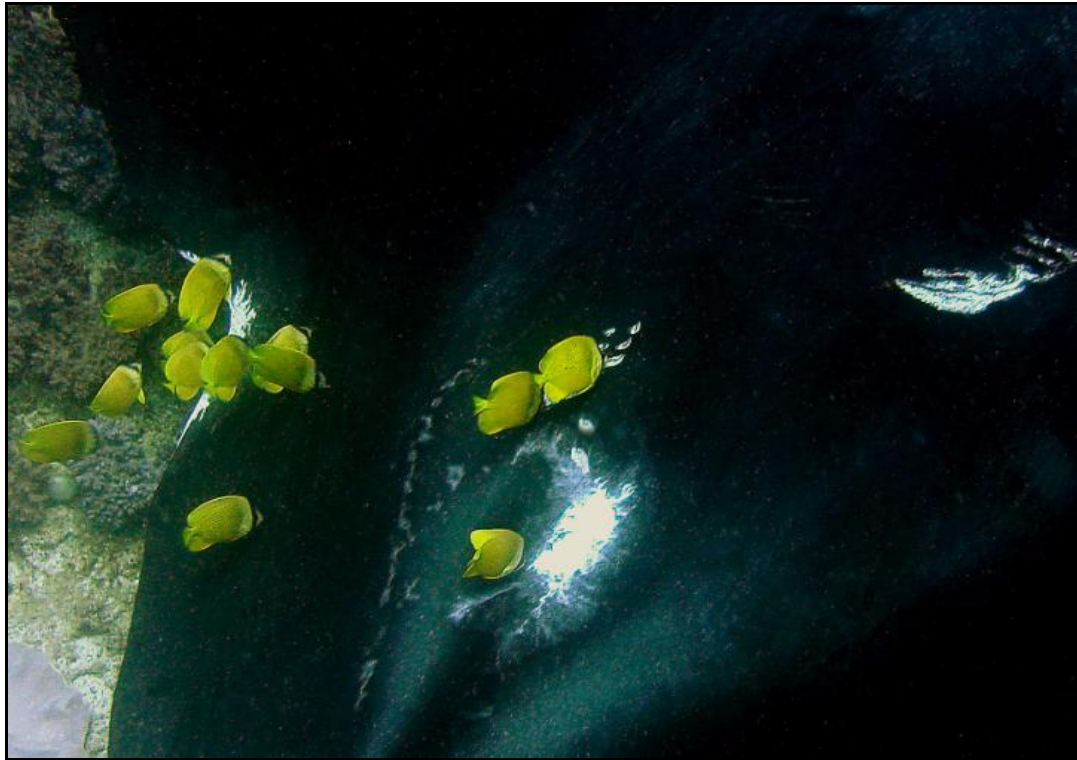
Figure 7.17 *Chaetodon kleinii* cleaning shark bite wounds on the dorsal surface of *Manta birostris* variant A.
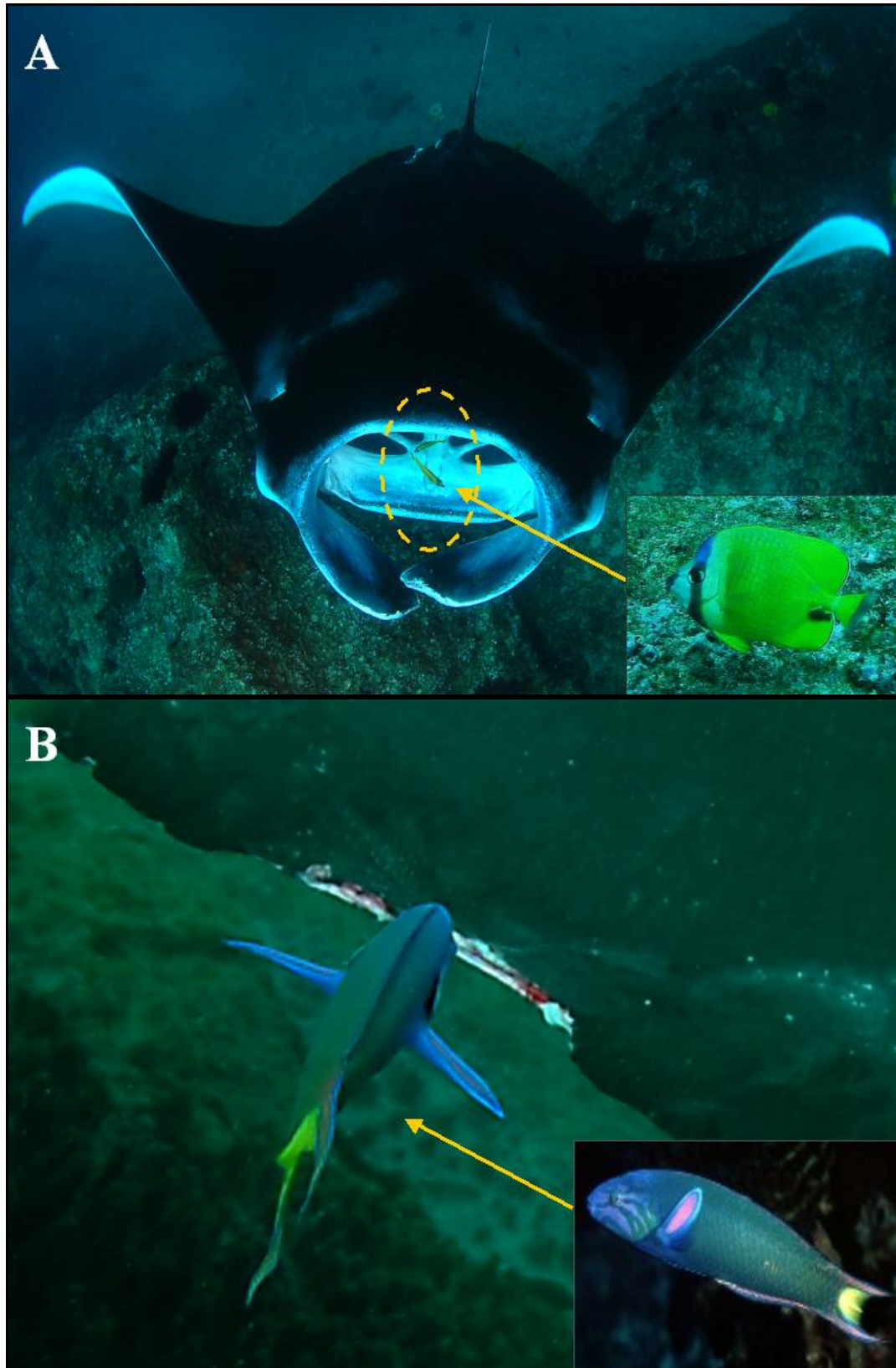
Figure 7.18 Region expansion by host fishes (a) *Chaetodon kleinii* cleaning the inside the mouth of *Manta birostris* variant A in the absence of other cleaner fish species (b) *Thalassoma lunare* cleaning a small fresh bite wound along the trailing edge of the pectoral fins of *Manta birostris* variant A.

Parasites

All manta rays (n = 15) that were systematically examined were infected by ectoparasites. However, the distribution of ectoparasites was not uniform across the different regions of the manta ray's body (χ^2 = 14.23, df = 1, P = 0.014). Parasites occurred on the dorsal supra-branchial and ventral gill slit regions of 93% and 60% of the individuals examined respectively. Ectoparasites were also found around the mouth in 40% of rays and around the ventral pelvic fin region in 26.7% of rays. While it was not possible to determine the absolute number of parasites, visual estimations of abundance suggested that the greatest density of ectoparasites occurred in the dorsal supra-branchial region.

Ectoparasite samples were collected from the skin surface of individual *M. birostris* var. A while cleaning on the reef. Eight samples were taken from the dorsal surface and four samples were taken from the ventral surface. Only a single type of ectoparasite was identified, a caligid copepod (*Lepeophtheirus* sp.), measuring between 2 – 8 mm. Up to 30 individual copepods per scrape were collected from the supra branchial region (Fig. 7.19a). When present, caligid copepods were also numerous around the most anterior gill slits on the ventral surface (Fig. 7.19b) where they often clustered around areas of apparent infection (Fig. 7.19b insert). The four dead *M. birostris* var. A specimens examined on the beach lacked ectoparasites on the external body surface. However, up to 17 parasitic ganthid isopod larvae per ray (Fig. 7.19c inset) were found attached to the dermal wall, inside the buccal cavity (Fig. 7.19c). These parasites measured between 4 – 6 mm long and were engorged with blood.

Passive acoustic receivers

Between 2005 and 2007 each VR2 receiver was deployed a total of seven times for periods of one to three months. The average time of deployment was 55 days (1320 hours). The shortest deployment of a listening station was 28 days (672 hours), while the longest deployment was 82 days (1968 hours). One of the listening stations was
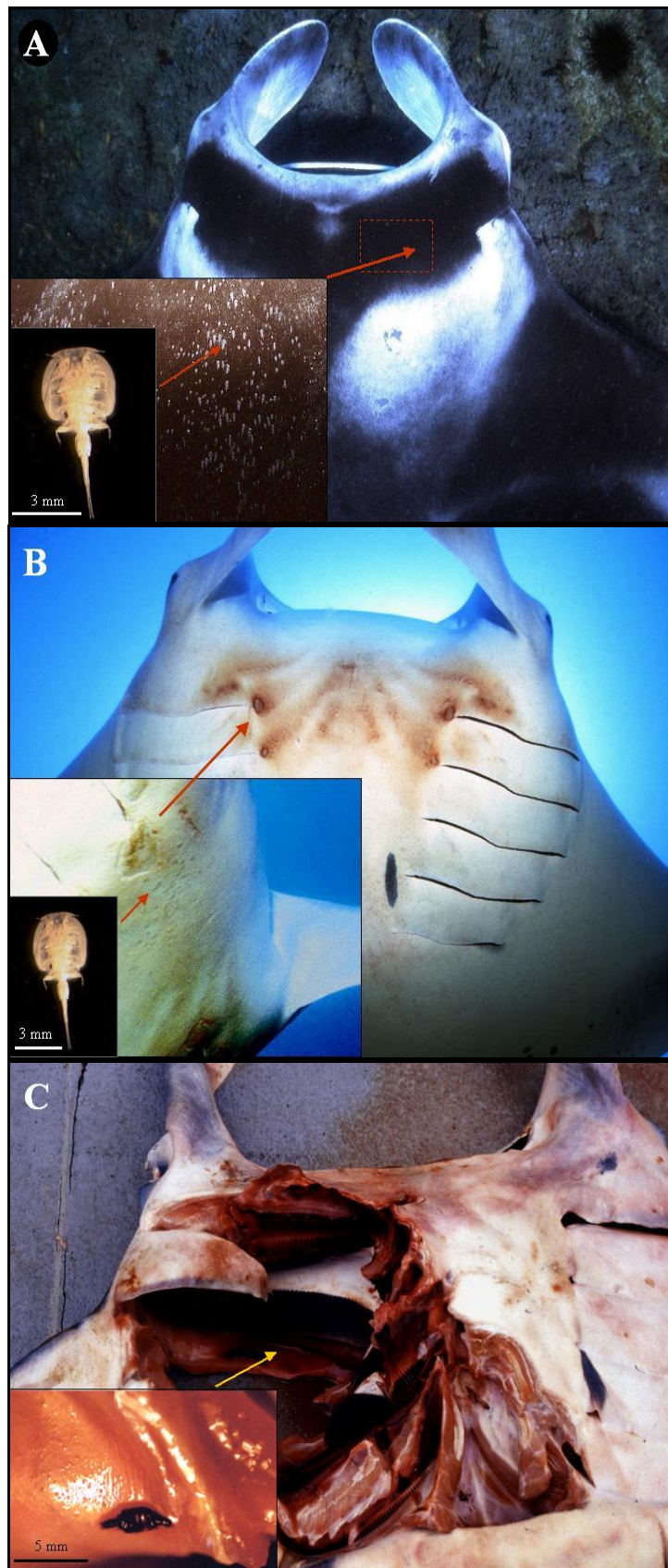
Figure 7.19 Distribution of parasites on *Manta birostris* variant A: (a) caligid copepods on the supra branchial region on the dorsal surface; (b) caligid copepods around infections on the gill slit region on the ventral surface, (c) gnathid isopods attached to the branchial cavity.

lost due to cyclonic conditions and the data for that period were lost. Another VR2 was installed to replace it two weeks later.

Tagging

Fourteen manta rays were tagged with acoustic pinger tags between 29 July 2005 and 16 October 2007 (Table 7.2). Eight of these tagged manta rays were female and six were male. Of these individuals, ten of the manta rays tagged were mature based on visual examination (Chapters 5,6). The maturity status of the remaining four female rays was unknown. The disc width of the tagged rays ranged from 3.0 to 4.5 m. Three of the female manta rays were pregnant at the time of tagging. During a pilot study, three dummy tags and one VR-16 tag were recovered with ease off *M. birostris* var. A being cleaned at Manta Reef using a hooked line cutter. These tags had been attached from seven to 24 days. Other than the removal of some of the superficial colouring on the ray's dorsal surface, no other cuts or infections were observed at the time of removal. None of the VR-16 tags from the main study were recovered.

Some of the tags were observed on individuals at the study reefs after deployment including a tag (#1512) that had been attached for 62 days (Fig. 7.20a). Although this tag was fouled with algae and small encrusting barnacles it was found to be transmitting effectively. Some pigmentation on the dorsal surface had been removed by the tag and the denticles in the area felt worn. However, the individual appeared to be in good condition and the insertion point did not appear infected. Some tags were shed after a relatively short period of time after tag insertion (Table 7.2). Manta ray number 500 was re-sighted at one of the observed cleaning stations on 25 February 2008, 141 days after tag insertion. This female ray had shed her tag (#1511), but the attachment site was still visible, with a short remnant of the attachment cable protruding through the skin. The attachment site on this individual also appeared to be free from infection (Fig. 7.20b). Two other re-sighted individuals had no visible infections or scar marks apparent at the original attachment site (Fig. 7.20c). These two rays, (#248 and #306), lost their tags prior to re-sighting events 148 days and 778 days after they were initially tagged.

Table 7.2 Occurrence of tagged individuals at cleaning station reefs as recorded by underwater acoustic receivers. Maturity status: M = mature; M^P = maturity assessed through pregnancy.

ID #	Sex	DW	Maturity	Date Tagged	Tag #	Min time of tag-Attachment (days)	Number of days recorded at sites	Total time of site occupancy (min)
305	F	4.2 m	M^P	29/07/2005	1501	35	2	61
306	F	4.2 m	M^P	29/07/2005	1502	1	1	20
248	F	4.1 m	----	23/10/2005	1503	34	7	889
323	F	4.4 m	M^P	23/10/2005	1504	2	2	353
358	M	3.3 m	M	01/12/2005	1505	N/A	0	0
510	F	3.7 m	----	26/03/2007	1506	50	2	521
415	F	4.5 m	----	05/12/2005	1507	132	26	2902
549	M	3.5 m	M	15/10/2007	1508	N/A	0	0
555	M	3.2 m	M	16/10/2007	1509	N/A	0	0
554	M	3.0 m	M	16/10/2007	1510	1	1	112
500	F	3.3 m	----	07/10/2007	1511	1	1	130
435	F	4.2 m	M	26/10/2007	1512	6	3	655
545	M	3.0 m	M	07/10/2007	1513	4	2	69
541	M	3.5 m	M	12/10/2007	1514	1	1	18
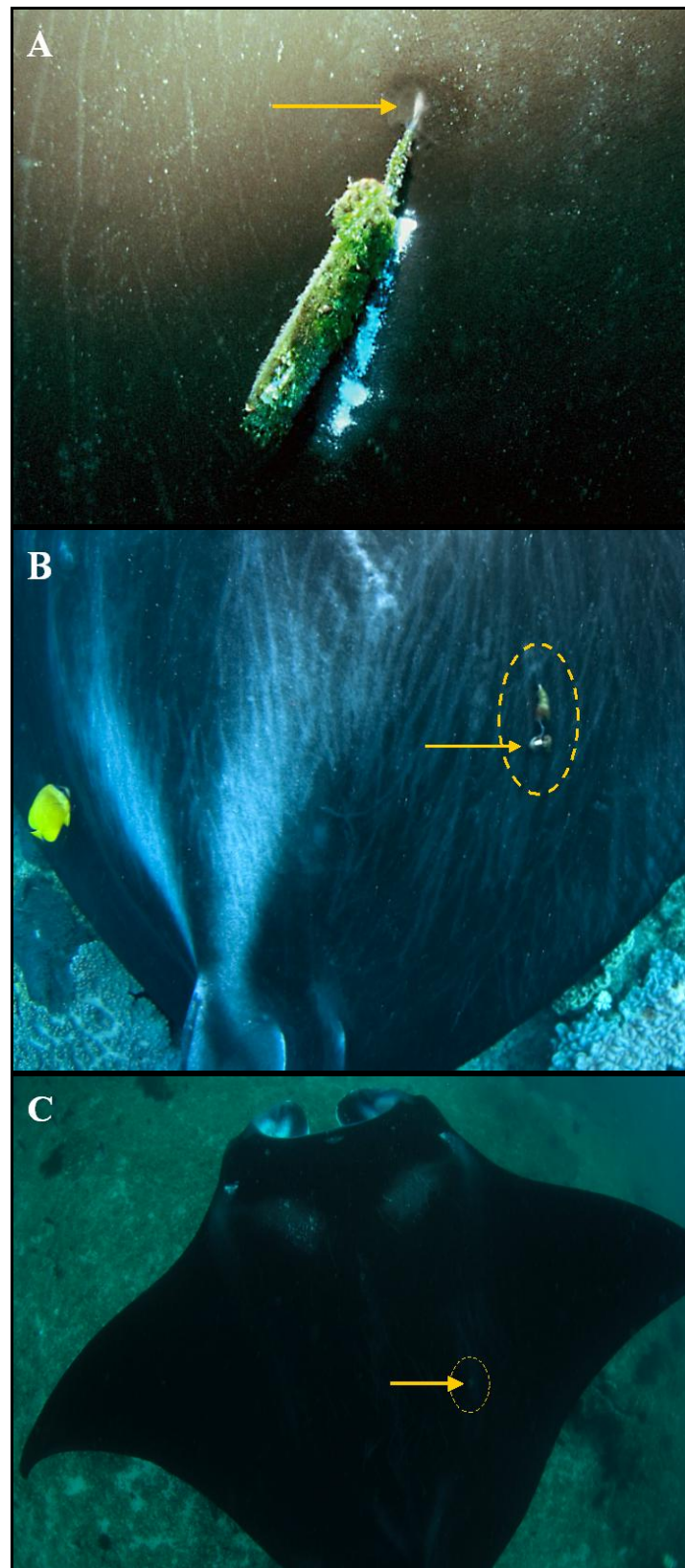
Figure 7.20 Acoustic tags (a) a tag with fouling after two months of attachment, (b) the tag connection left over after a tag had been lost, (c) almost no visible marks on an individual tagged earlier in the year.

Cleaning activity on reefs

Manta birostris variant A used both sites year-round and were almost always observed to be actively cleaning when present on either reef. Both examined reefs dropped off on all sides to sandy bottoms which became increasingly deeper with distance from the reef. The range of receivers in this environment was tested at about 300 meters. Other than the identified cleaning stations which were within the 300 m radius of the receivers, mantas were not seen during the study period to use the immediate area as a feeding station. Additionally, other than passing slowly over the reef, individuals were not commonly seen lingering above or around reefs.

VR2 receivers deployed at the study site(s) recorded data on cleaning events for 11 of the 14 tagged rays. No data were collected for the three remaining individuals. In total, cleaning events on 48 days were recorded, with the tagged rays occupying the two reefs for a total of 5,730 minutes during the deployment periods (Table 7.3). The receivers recorded occupancy events (repeated logging of a unique tag code in excess of 3 minutes) from tagged rays between 00:00h and 19:00h. However, 90% of these events occurred between 7:00h and 16:00h (Fig. 7.21). Factoring in the aggregate time spent on the reefs during each these hourly blocks, tagged individuals showed a similar patter with a small, but distinct increase in occupancy from 10:00h until 12:59h, peaking during the eleven-o-clock hour (Fig. 7.22).

The maximum time a tagged individual was recorded at the study reefs was 518 minutes with a mean occupancy time of only 119.4 minutes (SD ± 119.4). Individuals were recorded re-visiting the same reef on consecutive days as well as switching between the two monitored reefs on consecutive days. The maximum number of consecutive days an individual used the same reef, either Manta Reef or Giant's Castle, was three times and the maximum number of consecutive days an individual used either of the two monitored reefs was four days. The longest period between re-visitation events to the same reef was 46 days. The mean number of days between re-visitation events to either of the monitored cleaning stations was 6.67 days (SD ± 10.01).

Table 7.3 Cleaning event profiles of the four tagged individuals with verified tag retention of over 30 days: Location, date and time of occupancy and time (days) between last detection at either site (MR = Manta Reef, GC = Giant's Castle).

ID #	Reef Site	Date	Time elapsed from last detection (days)	Total daily occupancy (min)
305	MR	29/07/05	N/A	N/A
	MR	14/08/05	16	12
	MR	02/09/05	19	49
248	MR	24/10/05	N/A	165
	MR	29/10/05	5	67
	MR	30/10/05	1	35
	MR	31/10/05	1	321
	MR	19/11/05	19	17
	MR	26/11/05	7	222
	MR	27/11/05	1	62
510	MR	05/12/05	N/A	N/A
	MR	12/01/06	38	512
	MR	24/01/06	12	9
415	MR	26/10/06	N/A	152
	MR	08/11/06	13	7
	MR	24/12/06	46	122
	MR	26/12/06	2	227
	MR	27/12/06	1	116
	MR	28/12/06	1	78
	MR	31/12/06	3	5
	GC	01/01/07	1	12
	GC	03/01/07	2	20
	MR	22/01/07	19	75
	MR	23/01/07	1	27
	MR	24/01/07	1	51
	GC	25/01/07	1	109
	MR	26/01/07	1	16
	MR	31/01/07	5	6
	GC	01/02/07	1	518
	GC	02/02/07	1	416
	MR	08/02/07	6	20
	MR	14/02/07	6	190
	MR	16/02/07	2	98
	GC	22/02/07	6	277
	MR	23/02/07	1	39
	MR	24/02/07	1	162
	MR	01/03/07	5	107
	MR	02/03/07	1	29
	MR	07/03/07	5	23
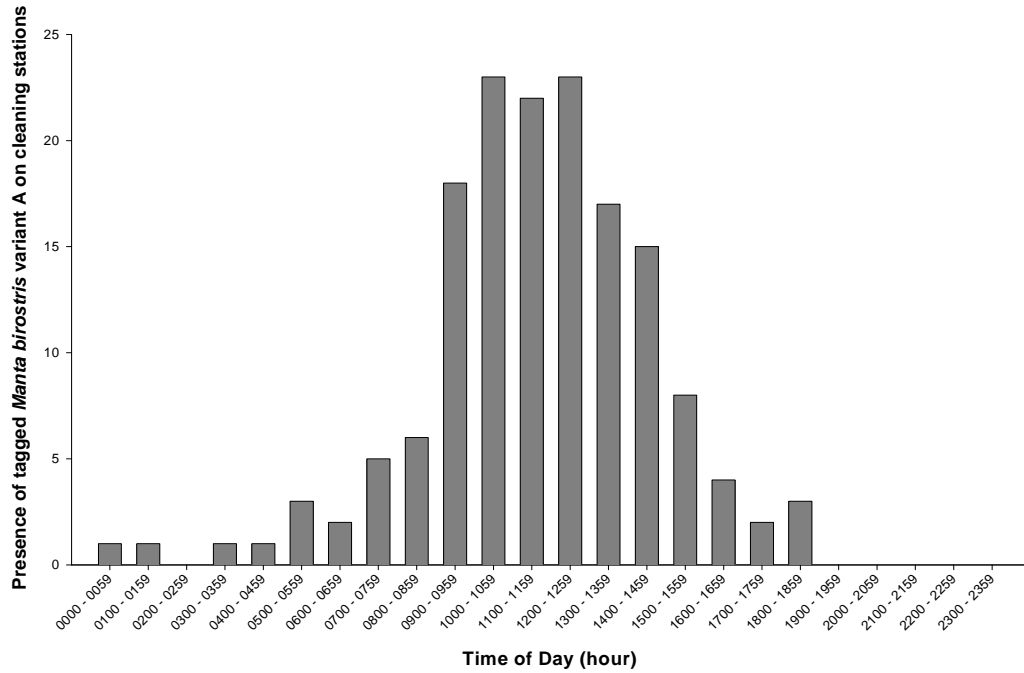
Figure 7.21 Graph showing how occupancy events (n = 155) (events where tags were present in excess of three minutes) were distributed as a function of time of day.
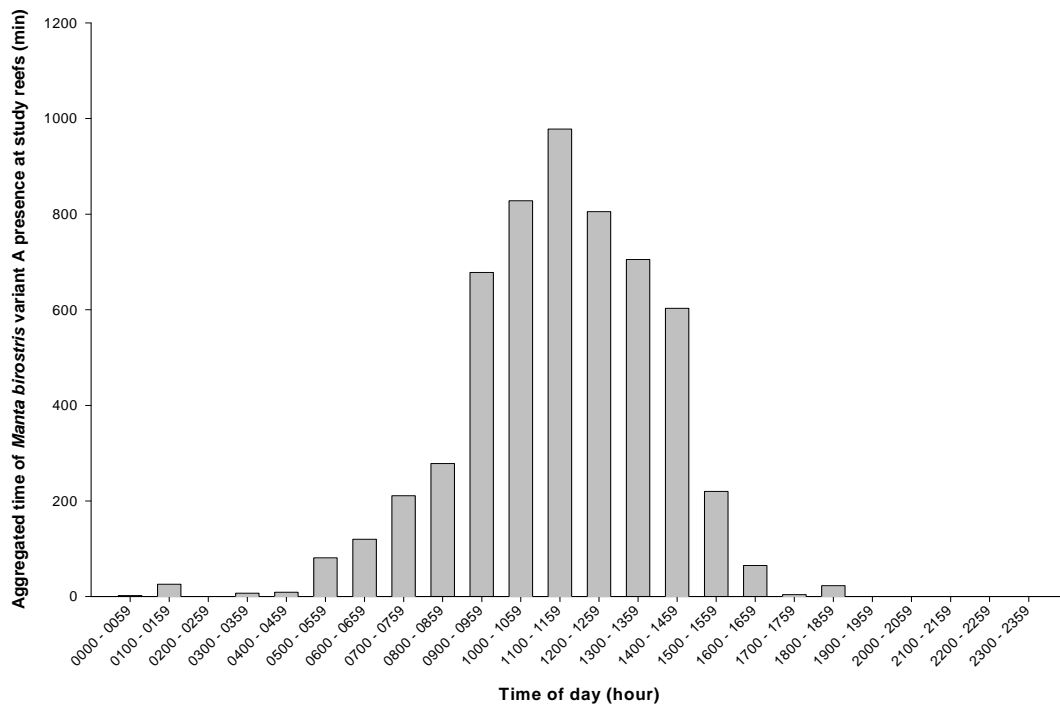
Figure 7.22 Graph showing the time spent by manta rays at the cleaning station reefs as a function of the time of day.

Individual ray #415 had the highest number of re-visitation events and over a 73-day period from late December 2006 to early March 2007 this particular individual visited the two study reefs on 24 different days occupying the cleaning stations for a total of 2,743 minutes (Table 7.3). During this time, the mean number of days between re-visitation events to these two reefs was 1.61 days (SD ± 3.99).

DISCUSSION

Manta birostris variant A frequently visited specific inshore reefs in southern Mozambique to be cleaned by small reef fishes. At these designated 'cleaning stations' individuals were observed to actively solicit cleaning by host cleaner fishes. When approaching cleaning stations, individuals faced into the current, reduced swimming speed and exhibited stereotypical behaviour including posturing and gill flaring. Though not all client fish stereotypically pose, posing may alert host fish to the client's willingness to participate in cleaning activity (Losey 1972; Côté et al. 1998). Kuwamura (1976) proposed that preferred clients may not have to pose in order to get cleaned and Grutter and Poulin (1998a,b) and Bansemer et al. (2002) postulated that because fish size is a good indicator of parasite load, larger fish should pose less. Observations of *M. birostris* var. A at cleaning stations in Mozambique suggest an alternate motive, as individual rays almost always posed before and during cleaning activities, despite being both the largest and most common species being cleaned at the monitored reefs. It is proposed that posturing is not a prerequisite of a cleaning interaction, but rather the position itself may make heavily parasitised or wounded areas easier to access, increasing the efficiency of the host cleaner fishes. For a dorso-ventrally flattened species like *M. birostris* var. A, which passes slowly and close to the reef while cleaning, angled posturing may expose areas with infections, parasites, or bite wounds to cleaner fishes. Findings by Côté et al. (1998) lend support to this argument, suggesting that posing facilitates the act of cleaning itself encouraging cleaner fish to clean for longer periods of time. Posturing may also serve to generate lift while individual rays hover at stations being cleaned. As manta rays are negatively buoyant, individuals wanting to maintain their position to allow cleaner fish time to clean may use posturing and the prevailing current to stabilize themselves so they do not sink.

While seven different species of reef fish were observed cleaning *M. birostris* var. A in southern Mozambique, not all of these species were necessarily engaging in mutualistic cleaning behaviour. Cheating by host fish species has been documented to occur during cleaning interactions (Poulin and Vickery 1995). *Odonus niger* for instance typically incited a negative, uncooperative reaction from *M. birostris* var. A when it attempted to remove build-up from the tip of the tail region. It is therefore possible that *O. niger* may be an unsolicited 'cheater' using the manta ray's slowed swimming and posturing as an opportunity to sneak an unwanted bite. Additionally this hypothesis may explain why *O. niger* only targets the tip of a manta ray's tail, the furthest point from the manta ray's body.

Aside from the seven species of cleaner fish observed in the study there may be additional species actively cleaning *M. birostris* var. A in southern Mozambique. For instance, *M. birostris* var. A was occasionally seen to be associated with a single to several remora and suckerfish (*Remora brachyptera, Echeneis naucrates*). Dietary studies have revealed that remora and suckerfish often ingest copepods living on their elasmobranch hosts as a major part of their diet (Feder 1966; Cressey and Lachner 1970). While this may indeed occur, the suckerfish on *M. birostris* var. A in Mozambique were never observed cleaning manta rays in any respect and were thus not included on the cleaner species list for this region.

None of the reef fishes at the study sites appeared reliant on *M. birostris* var. A as a food source. In Mozambique, as well as at other aggregation sites around the globe, manta rays come and go sporadically or seasonally (Homma 1999; Duffy and Abbott 2003; Dewar et al. 2008; Luiz et al. 2008). When manta rays were absent, the cleaner fishes on the reefs were observed foraging for other food items. However, when *M. birostris* var. A returned, cleaner fishes attended to the rays as soon as they approached the stations, an observation that may suggest cleaning large clients, like manta rays, is more efficient than foraging for other food sources. Cleaning observations additionally revealed that host fish species were predominantly foraging on different regions of the manta ray's body. Resource partitioning is one of the central processes promoting diversity in ecological communities by facilitating the coexistence of either closely related or ecologically equivalent species (Hutchinson 1959; Schoener 1974; Pratchett 2005). Host fishes that often target similar food items

when cleaning (Grutter 2002) may be classified as ecologically equivalent species. Intra/inter-specific competition probably plays a minor role when few host fishes are present during cleaning events. However, when many host cleaner fishes are present, as observed in this study, resource partitioning may result from competitive interactions between these fish species. Similar observations made by Konow et al. (2006) in Indonesia indicated that when cleaning large adult *Mola mola*, five different species of reef fishes (two butterflyfish species *Chaetodon kleinii* and *Heniochus diphreutes*, an angelfish *Pomacanthus imperator*, and two wrasse species, *Labroides dimididatus* and *Thalastoma lunare*) focused their cleaning efforts on different regions of the fish's body. One possible explanation for this behaviour is that host fish species are simply co-existing while sharing a large, common resource (Connell 1983) and it is their various morphological modifications (e.g. mouth size or shape) that result in their specialist diets. However, this is not likely to be the case in the current study, as observations over several years have revealed that in the absence of one or many cleaner fish species, species that typically focused their foraging efforts on a particular body region would sometimes expand their efforts to include multiple regions or various food items (Fig. 7.18). Resource partitioning may therefore be the major driving force behind the diversity of host fishes and their behavioral cleaning interactions with *M. birostris* var. A at the monitored reefs in southern Mozambique.

As planktivores, manta rays are subject to demanding metabolic requirements. In captive aquaria, *M. birostris* var. A reportedly consume up to 12% of their body mass a week (Homma 1999). Time spent at cleaning stations is essentially time lost for feeding, which can be considered to be a negative cost. Such a cost would presumably need to be balanced out by an equal or greater benefit (Côté 1998, 2000). Thus, frequent or extended time periods dedicated to being cleaned suggests that this activity is important to the overall health or fitness of individual rays. Unlike some land animals that may be cleaned by an opportunistic bird while eating grass (e.g. zebras, *Equus quagga*, and oxpeckers, *Buphagus* spp.), these pelagic rays in southern Mozambique must seek out cleaning stations and host cleaner fishes in order to receive this service. During the study period, *M. birostris* var. A were never seen to be cleaned at any other time except when at cleaning stations. Equally, when present on cleaning station reefs, *M. birostris* var. A were always actively being cleaned and were never seen feeding. The interpretation of these observations is that manta rays,

as clients, are actively seeking out cleaning interactions with reef fishes and the benefits derived from these cleaning events are likely to be worthwhile for them, an example of true mutalistic symbiosis (Poulin and Grutter 1996).

While the specific advantages derived by a client fish are often a subject of debate (Losey 1972; Grutter 1996; Poulin and Grutter 1996), it has been noted that some species may rely on cleaning interactions to regulate their ectoparasite levels or minimise the risk of infections (Gorlick et al. 1978). In some parts of its global distribution, *M. birostris* var. A appears not to be heavily parasitised (Cressey and Lachner 1970, Marshall unpublished data), but in the present study all individuals examined exhibited heavy ectoparasite loads, particularly of caligid copepods. Gnathid isopods were also identified in the buccal cavity and on the gill arches of manta rays killed in fisheries in the area. Gnathid isopods, in addition to caligid copepods, are among the most common parasite type found in the diets of cleaner fish species (Grutter 2002; Caira and Healy 2004). While a study by Arnal et al. (2001) stated it was unclear if copepods were ingested by cleaner fish, direct observations in southern Mozambique of *T. lunare* ingesting caligid copepods on the supra-branchial region of *M. birostris* var. A have provided evidence that some host cleaner fish species do target ectoparasites, like copepods.

Some studies have successfully demonstrated that cleaner fish hosts not only remove but control parasite loads on fish (Grutter 1999; Cheney and Côté 2001; Grutter and Lester 2002). It has also been proposed that some cleaner fish species assist in wound healing by removing infected or necrotic tissue (Limbaugh 1961; Hobson 1971). A study by Foster (1985) was the first to successfully demonstrate that the attentions of cleaner fish promoted wound healing in teleosts by helping to eliminate fungal or bacterial infections. In reef fishes wound healing may play a small or less significant role than parasite removal, as injured fish are not commonly observed. However, the observed population of *M. birostris* var. A in southern Mozambique commonly exhibited predatory shark bite injuries, with over 75 percent of the population bearing signs of new or healed shark bite marks (Chapter 8). Foster's 1985 study found that wounded fish spent more time at cleaning stations having their cuts and abrasions tended to than fish without injuries. With the majority of the identified population in Mozambique bearing wounds from shark bite attacks and with the most abundant

species of cleaner fish (*C. kleinii*) targeting these injuries, it is plausible that the promotion of wound healing may be of particular importance in cleaning interactions at this location. To determine if the high incidence of bite marks in the observed population in southern Mozambique influences the amount of time these rays spend being cleaned by host fishes, comparative studies are needed at other *M. birostris* var. A aggregation sites where wounds from shark attacks are less frequent or less severe.

Data from the fourteen tagged manta rays provided valuable clues about the fine-scale habitat use of individuals in this population. During the study period, nine other cleaning stations along a 60 km stretch of coastline were identified through exploratory SCUBA dives. Many of the manta rays observed at these other sites had been previously identified at the two study reefs (Marshall, unpublished data). One identified individual was seen on several of these reefs within a 43 km stretch of coastline, suggesting manta rays in this area may rotate their use of cleaning stations. Anecdotal observations and photographic studies on manta rays suggest that individual rays may use multiple cleaning stations in a region (Dewar et al. 2008; Stevens pers. comm.), although Dewar et al. (2008) recently demonstrated that individual mantas may show extreme site fidelity or preference to particular reefs or cleaning stations. Dewar et al. (2008) also show that manta rays have clear diurnal patterns of habitat use and often use stations on a daily basis between sunrise and sunset. With only two of the potentially dozens of cleaning stations along this stretch of coastline being monitored during this study, it is likely that a high proportion of the identified population is cleaning at some site along the coast each day. Factors that may influence site selection have not been determined, but considering the importance of feeding time to a planktivore's daily schedule, it seems likely that individuals may select cleaning stations closest to their daily foraging grounds. The observations made during this study suggest high site fidelity by *M. birostris* var. A to this stretch of coastline and regular if not daily use of local cleaning stations.

The acoustic tagging study revealed a distinctly diurnal pattern in the cleaning activity of *M. birostris* var. A with individuals most commonly using the inshore reefs between 7:00 am and 4:00 pm. Almost no significant visitation events were recorded between sunset and sunrise. These findings are similar to those reported by Dewar et al. (2008) which also note a distinct diurnal pattern in the visitation of *M. birostris*

var. A to cleaning stations in the Komodo National Marine Park, Indonesia. While the average occupancy time of acoustically-tagged individuals was 119 minutes per day, individuals were recorded to spend up to 518 minutes (over 8.5 hours) a day being cleaned at the study reefs. The reason for such extreme variation was not determined, but may be a good focus for future research efforts as various factors (e.g. parasite loads, infections and bodily injuries) undoubtedly play a part in the time that an individual would spend at a cleaning station. The number or efficiency of host cleaner fishes on cleaning stations may also be a contributing factor. Lastly, unintentional effects of marine-tourism, such as boating traffic and scuba diving may also modify manta ray behaviour, such that they may avoid heavily trafficked cleaning stations or reduce the time they spend at these critical habitats. Changes in the behaviour of *Manta birostris* var. A, including adverse reactions to numerous divers on cleaning reefs were observed during the study (Marshall, unpublished data). The most severe reaction observed was individual *M. birostris* var. A abandoning cleaning stations when large groups of divers approached too close. The fact that the same individual rays reappeared and resumed cleaning activity after the diver groups had surfaced, lends further support to the claim that human activity on the reef can influence the natural behaviour of *M. birostris* var. A at these critical habitats. With diving tourism increasing in Mozambique, further investigation is clearly needed. Given the economic importance of manta ray focused tourism in several countries, and the potential impacts of high diver visitation to key habitats, this information is relevant to the management of manta rays populations at this and other key aggregation sites.

Chapter 8

The frequency and effect of shark-inflicted bite injuries to *Manta birostris* variant A off the coast of southern Mozambique

INTRODUCTION

Sharks are top or apex predators in marine ecosystems (Optiz 1996; Cortés 1999), but while the majority of shark feeding events may result in the complete ingestion or mortal injury of prey, not all predatory activity results in direct fatalities. Non-fatal attacks can cause injuries ranging from those of negligible effect, such as superficial scarring (Naessig and Lanyon 2004) to those that are subsequently fatal (e.g. from blood loss or sepsis resulting from wound infection) (Ames et al. 1996; Stewardson 1999). Individuals that escape predatory attacks may exhibit physical signs of the encounter long after the attack in the form of cuts, scratches or abrasions or even distinguishable bite marks, which may allow for interpretation of attack frequency. Injuries can cause major shifts in the behaviours, habitat use, activity budgets, locomotor performance, reproductive success, life history strategies and mortality rates of prey organisms and may play an important role in the regulation of population size (Le Boeuf et al. 1982; Harris 1989; Lima 1998; Stewardson 1999; Heithaus 2001a).

Excepting human analysis, lasting evidence of non-lethal predatory attacks by sharks has most commonly been reported for cetaceans (Long and Jones 1996; Heithaus 2001a,b; Naessig and Lanyon 2004) and seals (Le Boeuf and Crocker 1996; Long et al. 1996; Stewardson 1999; Bertilsson-Friedman 2006), although similar studies have also been conducted on other marine mammals (Ames et al. 1996), birds (Randall et al. 1988; Johnson et al. 2006), reptiles (Long 1996; Fergusson et al. 2000; Heithaus et al. 2002) and elasmobranch fishes (Fitzpatrick et al. 2006). Previous studies on predation injuries were largely opportunistic, using recent or historical information from animals washed up on beaches (Ames et al. 1996; Long and Jones 1996; Long 1996; Long et al. 1996) or observations of single attack events (Gibson 2006). In only a few cases were seasonal or annual occurrences of shark bite injuries explored at the population level (Heithaus 2001b; Naessig and Lanyon 2004; Bertilsson-Friedman 2006) or were the resulting wounds incurred by recognized individuals studied over time (Fitzpatrick et al. 2006). Detailing what happens at the level of individuals through time, through repeat observations of recognisable individuals may help to

better understand the threat and effects of predation to populations and may additionally contribute to the understanding of how wounds heal.

While chondrichthian fishes form an important part of the diet of numerous sharks species (Cortés 1999), few have been observed in the field with significant tissue loss, such as parts of fins (Fitzpatrick et al. 2006), suggesting that survival after attacks is unusual (Strong et al. 1990; Chapman and Gruber 2002). Manta rays, appear to be an exception, with individuals worldwide exhibiting tissue loss due to predatory shark attacks (Homma 1999; Ito 2000; Ebert 2003; Marshall unpublished data). Non-lethal attacks by sharks, such as *Carcharhinus amblyrhynchos* (grey reef shark) and *Carcharhinus galapagensis* (Galapagos shark), have been observed in the wild on free-swimming manta rays (pers. obs. A. Marshall) as well as a lethal attack by a bull shark on a 2.5 m disc width (DW) manta ray (Ebert 2003), but such sightings are opportunistic and rare. Attacks on living manta rays entangled in nets or mooring lines by *Galeocerdo cuvier* (tiger shark) have also been photographed by divers (Appendix 2). In the absence of sufficient direct observations, data on fresh wounds and bite marks provide the best insight into the frequency of predatory shark attacks on manta rays. Beyond this, an understanding of the effect of shark attacks may be important in the interpretation of differences in natural mortality and behaviour of different populations.

The incidence of non-lethal shark-inflicted wounds on *Manta birostris* variant A in southern Mozambique was documented over a three-year period while monitoring an identified population. The aims of this study were: to examine the frequency of shark-inflicted injuries in relation to time of year, sex and body region affected, to evaluate the rate at which new injuries occur over time and to assess how wounds heal after attacks. This is the first examination of the frequency or effect of predatory shark attacks on manta rays.

METHODS

Manta rays and their associated scars were observed at cleaning stations on inshore reefs where small reef fish would remove parasites, clean infections, and tend to bite wounds (Chapter 7). Wounds and bite mark scars were examined and photographed at

these cleaning stations as individuals were easily approached while being cleaned. Measurements of bite marks could sometimes be made while mantas hovered almost motionless above the reef. When possible, the width and depth of both fresh bite wounds and healed bite marks were measured (± 1 cm) using a measuring tape while individual rays were cleaning on the reef (Fig. 8.1). Partial bite marks or those that were not distinct in shape proved difficult to measure in the field, thus only uniformly crescent-shaped bite marks were selected for measurement and comparison.

Figure 8.1 Measuring a bite mark along the trailing edge of a manta ray at a cleaning station.

Identification and measurement of individual rays

Individual *M. birostris* var. A were examined for predation injuries at two major study sites over a three-year period, from March 2003 to March 2006. Individual rays were photographically identified using techniques covered in Chapter 5. Observations were conducted while SCUBA diving during daylight hours between 7:30 am to 3:00 pm. Information about predator-mediated injuries, such as photographs and measurements, were collected at this time. Individuals were sexed to enable sex-specific analysis of bite wound frequency (Chapter 5).

Bite injuries

Using modified criteria from Long and Jones 1996, crescent-shaped scarring as well as other distinctive wounds in which flesh had been removed (Fig. 8.2a,c-h) were considered for this study. Other minor marks, such as scrapes or pigment discolouration, although common, were not included in the analysis as they were generally superficial, not always quantifiable (i.e. number of bite attempts) or of indeterminable origin (Fig. 8.2b). Recorded bite marks were placed into one of three descriptive categories based on their appearance: 'Fresh', indicated that the wound was either bleeding and/or the exposed flesh was still vascularised and bright red in colour (Fig. 8.2c,g); 'Healing', referred to any wound where pink tissue was still visible, but scar tissue was prominent (Fig. 8.2d,e,h) and; 'Healed', described a bite mark covered entirely by scar-tissue and where red or pink flesh was no longer present (Fig. 8.2a,f). The seasonal occurrence of fresh bite marks was examined and normalized by dividing the total number of fresh bite injuries seen in each of the calendar months by the total number of rays recorded in these same months. These monthly data were then pooled and compared against all other calendar months.

To facilitate the examination of bite mark location, the body of the ray was initially divided into six zones, the left and right sides of the animal (Fig. 8.3) each which contained three regions, the head (region 1), the anterior half of the pectoral fins (region 2), and the posterior half of the pectoral fins including pelvic fins (region 3) (Fig. 8.3). The occurrence of bites marks in these six zones was recorded for each identified individual based on visual examination and photographic images of the entire body.

Statistical analysis

The number and position of bite marks were examined with respect to the sex of manta rays, using Chi-square (χ^2) analysis to compare the bite mark frequencies between two or more groups. Yates' correction factor for continuity was applied to all chi-square tests where there was one degree of freedom to prevent Type I error (Zar, 1996). Significance was accepted at $P < 0.05$.
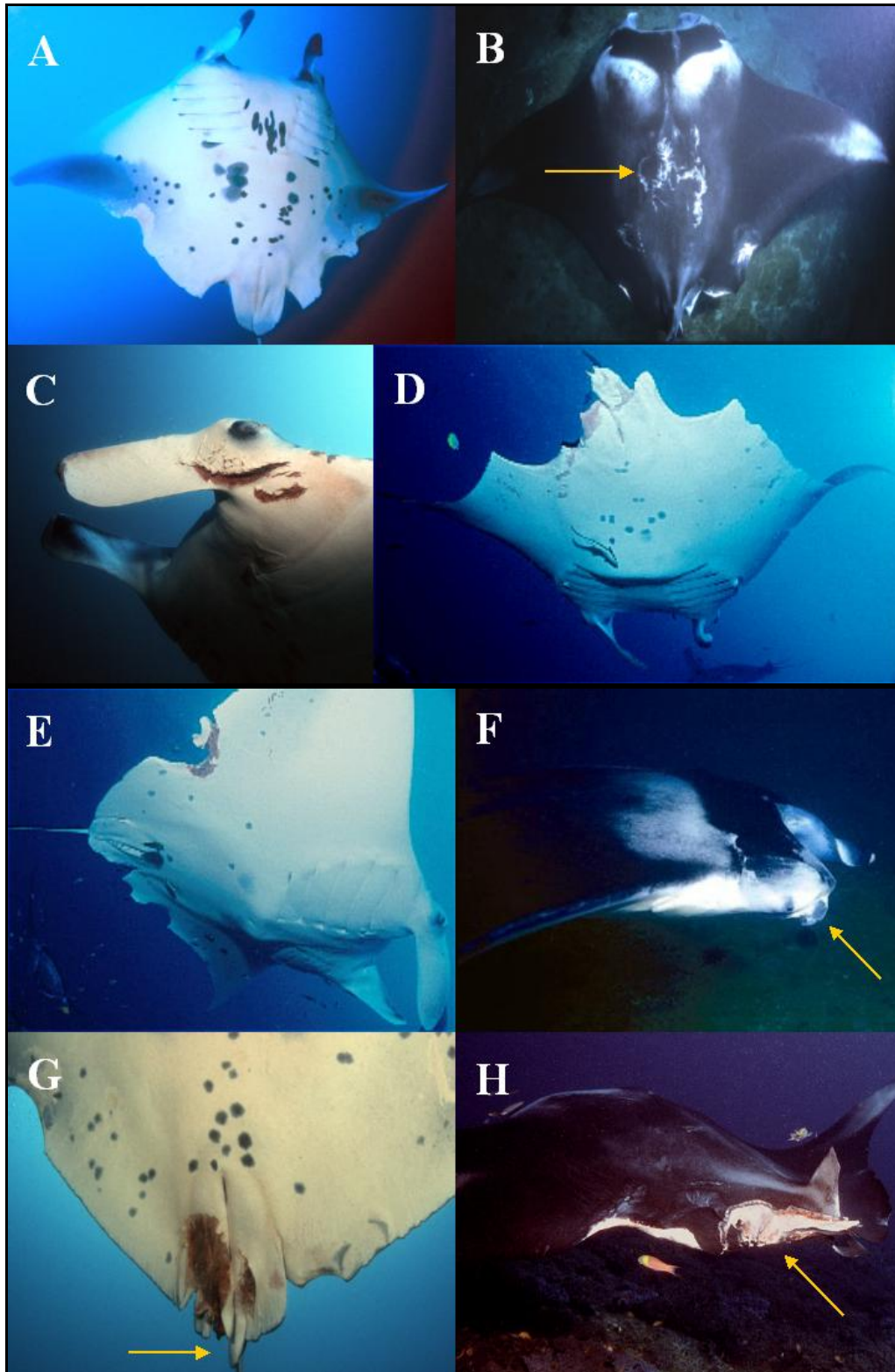
Figure 8.2 Bite wounds and scars on *Manta birostris* variant A: (a) multiple shark bite marks along the trailing edges of pectoral fins; (b) scars on the dorsal surface from an unsuccessful shark attack; (c) fresh bite wound; (d) large healing wounds; (e) fresh incomplete bite mark with loose flesh hanging from the wound; (f) severed cephalic fin; (g) fresh bite wound to the pelvic fins, resulting in the loss of a clasper; (h) extensive injuries to the trailing edge of pectoral fins.
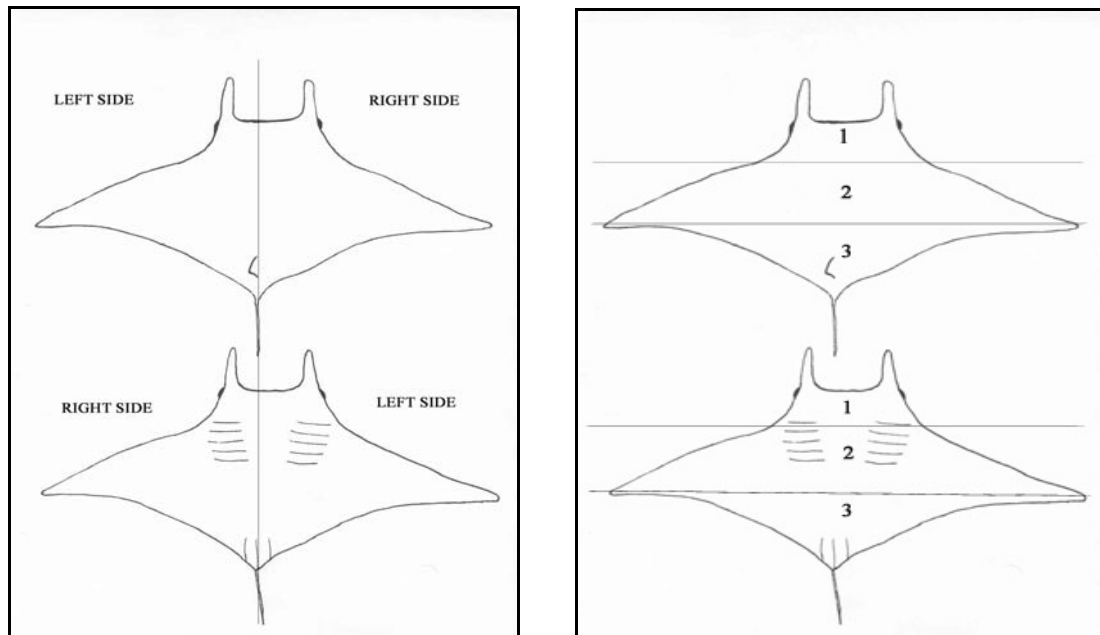
Figure 8.3 Zones of manta ray body used for bite mark distribution analysis.

RESULTS

Bite mark appearance

During the three-year study period, a total of 571 different shark bite wounds were photographed and examined. With the exception of when additional bites or tissue loss occurred to areas already containing bite marks or scars, evidence of the original mark remained in all re-sighted individuals over the duration of the study period, in some cases almost three years (see Fig. 8.4a-d). Even in cases where fresh injuries healed between re-sighting events, the bite marks always remained visible and similar in shape and size to the original wound.
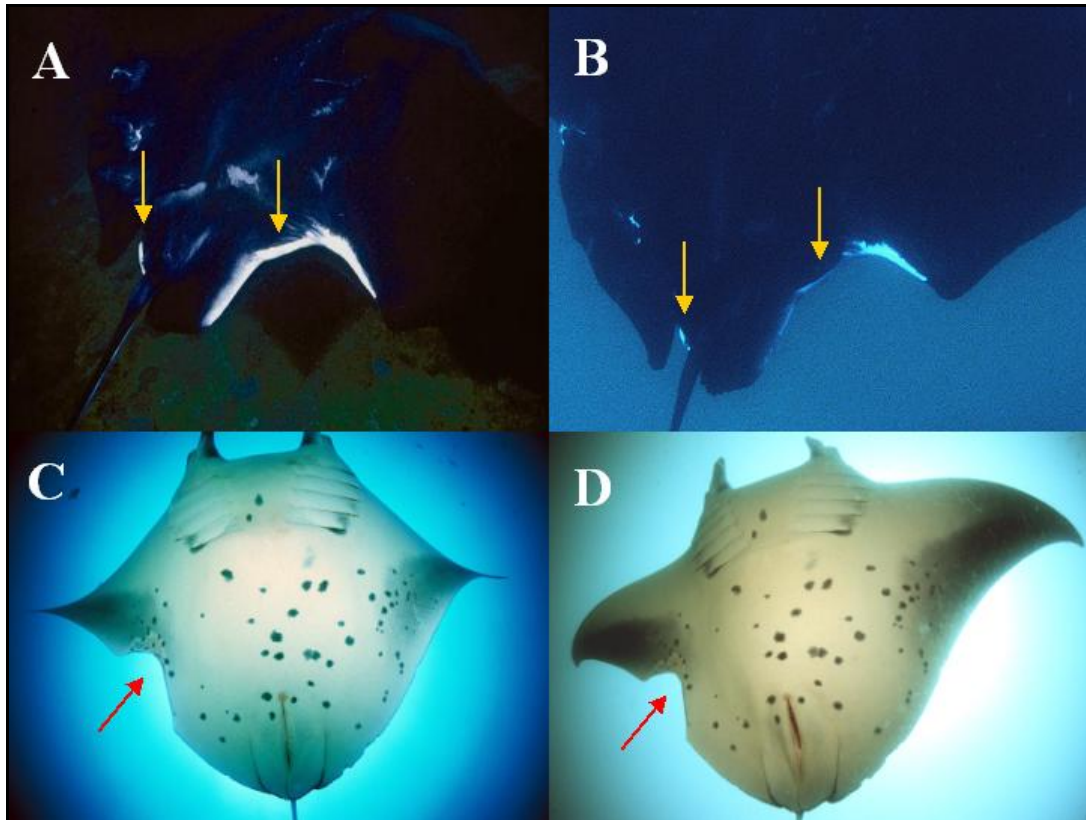
Figure 8.4 Negligible changes in bite marks over time: (a) manta #018 with bite mark in 2003; (b) same bite mark on manta # 018 in 2006; (c) bite mark on manta #340 in October 2003; (d) same bite mark on manta #340 in November 2006.

Occurrence in population

A total of 371 identified *M. birostris* var. A were examined for predatory scaring in the period between May 2003 to March 2006. Overall, 76.3% (n = 283) of manta rays identified exhibited shark-inflicted bite marks. Of these rays, 62 (21.9%) were male and 221 (78.1%) female. The proportions of male (70.5%) and female (78.1%) manta rays in the population with predatory bites were not significantly different (χ^2 = 0.204, df = 1, P = 0.651) (Fig. 8.5).

Of the 283 rays with discernable bite mark injuries, the number of bites per individual ranged from one to four for males and from one to seven for females (Fig. 8.6). The mean number of bite marks per individual was 1.54 ± 1.37 for the entire sampled population and 2.02 ± 1.22 for scar bearing rays. The bite frequency median and mode for the whole population was one for both sexes (Fig. 8.6). The percentage of
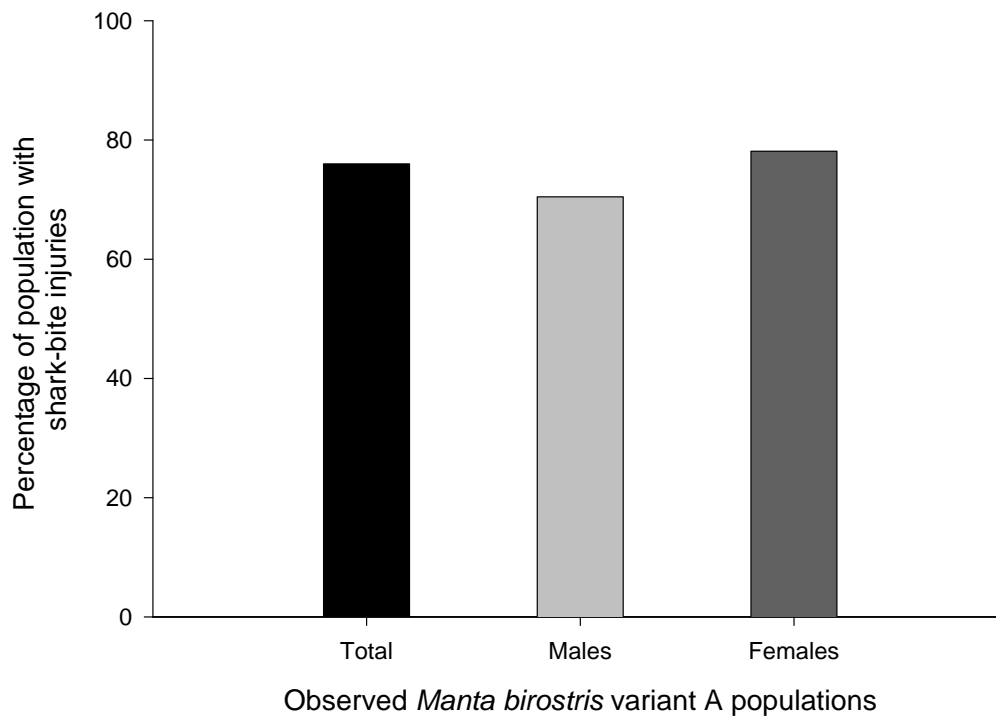
Figure 8.5 Percentage of the total, male, and female observed populations of *Manta birostris* variant A with bite marks.

the entire population with two or more bites was 42.6% while the percentage of predatory scar bearing rays that had two or more bites was 55.8%.

Of the 371 individuals identified in during this study, 138 (37.2%) were re-sighted on at least one occasion, with a total of 302 re-sighting events made. The longest time period between the re-sighting of an individual was 945 days. Observations of the 19 re-sighted male rays resulted in the addition of only a single new bite wound, while the re-sighted female rays (n = 119) accrued ten new bite wounds during the study period.

Bite mark distribution

As there was no significant difference between the proportion of bite injuries to the right and left sides of the body (χ^2 = 0.632, df = 1, P = 0.427) data from each side of the body were combined in further analyses. The distribution of bites between the three remaining regions of the body was not random (χ^2 = 1019.52, df = 2, P < 0.001),
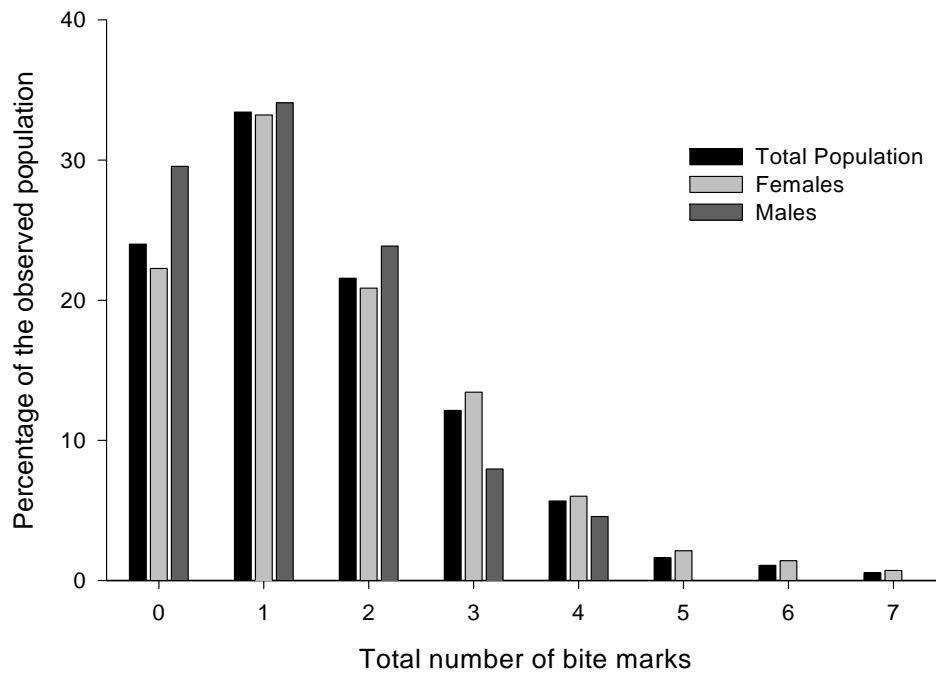
Figure 8.6 Percentage of the observed populations of *Manta birostris* variant A in southern Mozambique with or without multiple bite marks.
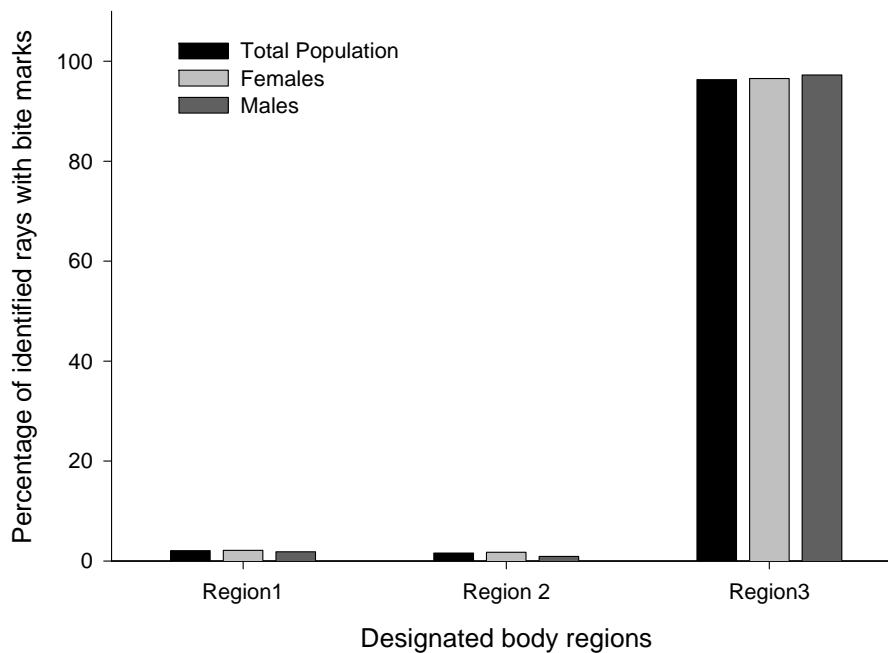
Figure 8.7 Percentage of individuals in the total, female, and male populations of *Manta birostris* variant A with bite injuries in each of the designated body regions.

with the majority of wounds (96.3%, *n* = 550) occurring on the posterior section of the body (region 3) along the trailing edge of the pectoral fins (Fig. 8.7).

Tissue loss from shark bites were relatively uncommon in both region 1 (2.1%, *n* = 12) and region 2 (1.6%, *n* = 9). There was no significant difference between males and females in regard to the proportion of bite wounds in each region (χ^2= 0.421, df = 2, P = 0.810). A closer examination of bite injuries in the third region revealed that the majority of bites (98.1%) occurred along the edges of the pectoral fins, affecting both dorsal and ventral surfaces. Of the remaining bite marks, four wounds were located mid-body on the ventral body surface and seven were mid-body on the dorsal surface.

With the majority of the predatory bite marks distributed along the posterior edge of the pectoral fins, the trailing section of the body at times appeared severely mutilated (Fig. 8.8a-d). Despite the severity of the trauma, many of these wounds were healing or completely healed at the time they were photographed. Some of the injuries to the trailing edge of the pectoral fins affected the pelvic fin region, resulting in the removal of one or both of the claspers of male rays (Fig. 8.8e-h).

Bite mark shape

The dimensions of the observed bite marks varied markedly in size and shape (Fig. 8.9). The maximum width of healed bite marks examined was 51 cm and the minimum width was 9 cm (Table 8.1). The only fresh wounds that were examined measured 24, 27 and 33 cm with depth radiuses of 11, 12, and 12 cm respectively. When an identified individual exhibited more than one bite mark, it was not uncommon for bite marks to be different in overall size or shape (Fig. 8.9).

Wound healing and seasonality of attacks

Of the 283 mantas rays with bite injuries, ten individuals were observed to have fresh wounds and 24 individuals were observed to have wounds in various states of healing. In contrast, a total of 266 individuals were observed to have healed bite marks. Of the total 571 bite marks recorded during the study, 1.9% (n = 11) were classified as fresh,

6.3% (n = 36) were healing, and 91.8% (n = 524) were healed. Only one of the ten manta rays with fresh wounds had more than one fresh bite. Of the 24 manta rays with healing wounds, 18 (75%) had a single healing wound at the time of sighting, three (12.5%) had two healing wounds, two (8.3%) had three healing wounds, and a single individual (4.2%) had four healing wounds (Table 8.2).

Several manta rays bearing fresh bite wounds were noted to have healed on subsequent sightings. In these instances, fresh wounds took no more than 225 days to completely heal and in one instance a bite completely healed in under 126 days. Additionally, during the study period, four identified rays were re-sighted 148-246 days after the initial sighting with new, yet healed bite marks.

When data from the three-year study period were combined, fresh shark-inflicted injuries were seen in six of the 12 calendar months (Fig. 8.10). With only 11 fresh bite marks recorded during the study, no significant seasonal effect could be determined, however the attacks did not exclusively occur in any one month or season.
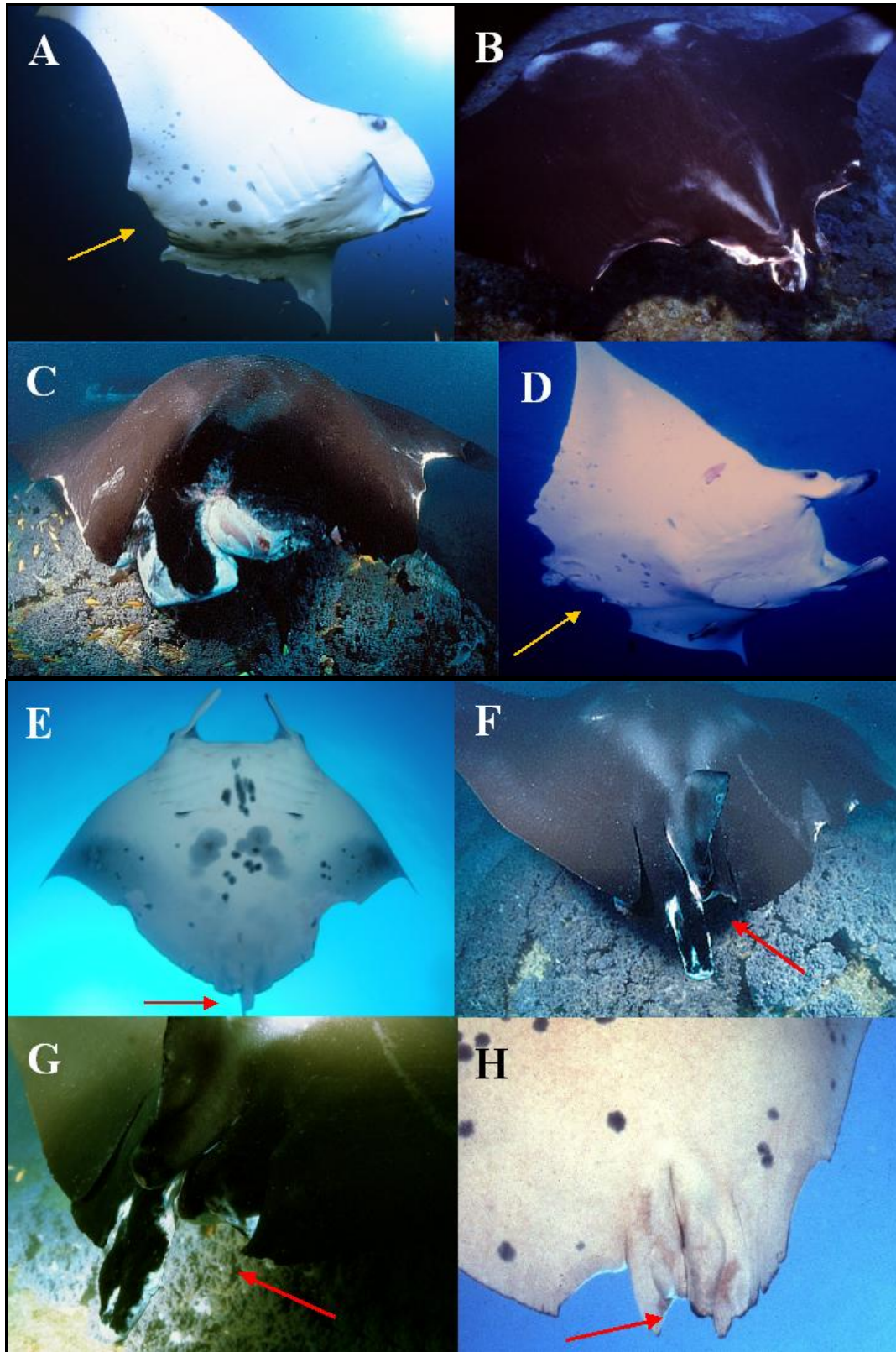
Figure 8.8 Injuries to the trailing edge of the pectoral and pelvic fins; (a-d) Extensive injuries to female mantas including the almost complete removal of pelvic fin area; (e-h) injuries to the trailing edges of male rays including the disfigurement or complete amputation of one or both claspers.
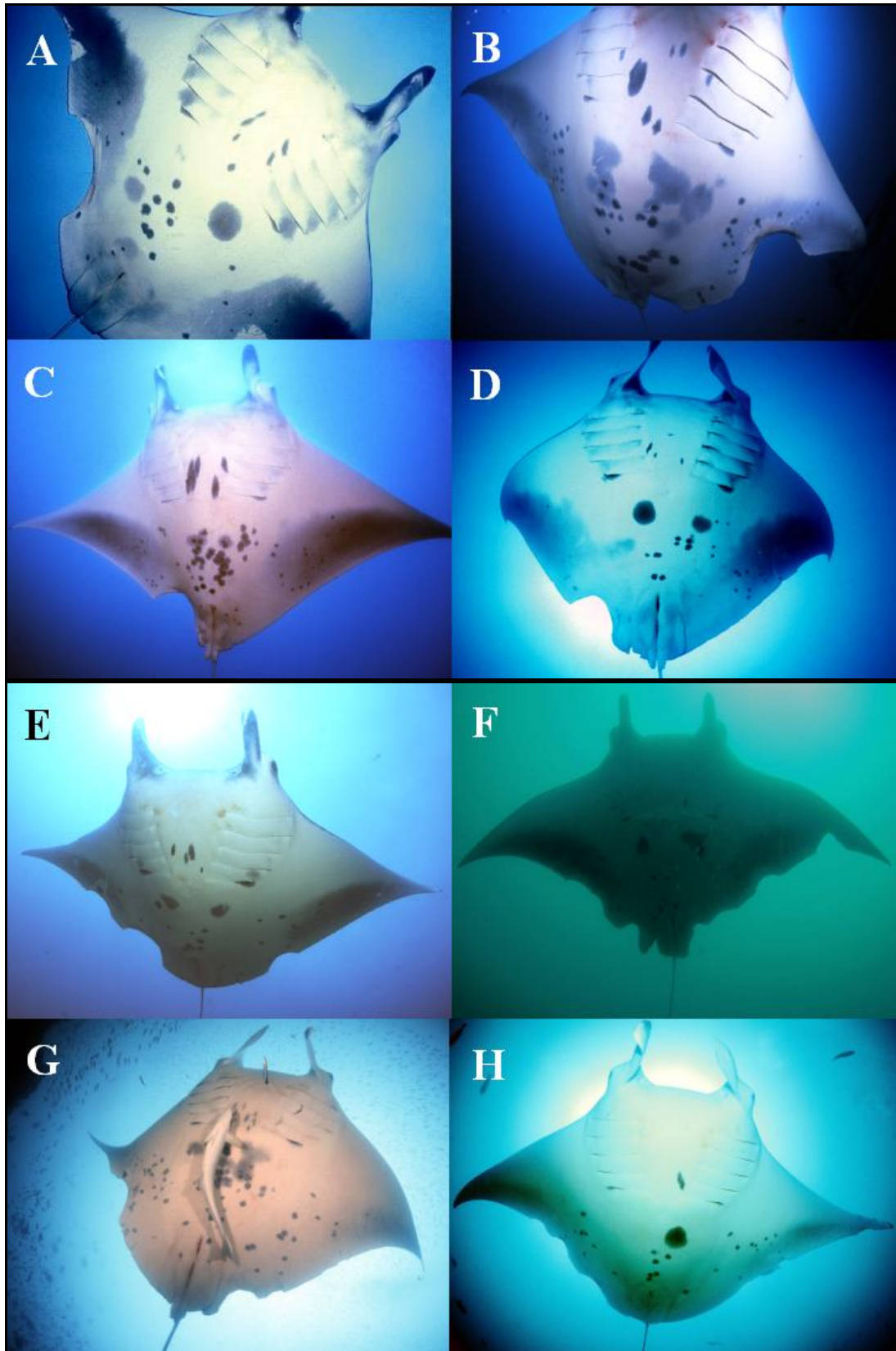
Figure 8.9 Size and shapes of bites on identified individuals: (a-h) Range in size and shape of bites marks on both male and female rays.

Table 8.1 Measurements of predatory bite marks on *Manta birostris* variant A in southern Mozambique.

Manta ID #	Sex	Size	Maturity	Date Measured	Status of Bite	Bite Mark Width	Bite Mark Depth
066	F	~4 m	M	14/01/2005	Healed	33 cm	~17 cm
099	M	~4 m	M	18/05/2004	Healed	29 cm	~14 cm
100	F	4.3 m	M	18/05/2004	Healed	09 cm	~04 cm
108	M	~4 m	M	20/05/2004	Healed	43 cm	~18 cm
412	F	~4 m	---	13/03/2006	Healed	51 cm	~19 cm
307	F	4.2 m	M	30/07/2005	Healed	16 cm	~06 cm
105	F	4.5 m	M	17/02/2006	Fresh	24 cm	~11 cm
113	F	~4 m	---	20/05/2004	Fresh	27 cm	~12 cm
307	F	4.2 m	M	30/07/2005	Fresh	33 cm	~12 cm

Table 8.2 New or unhealed bite marks on identified *Manta birostris* variant A in southern Mozambique between March 2003 and March 2006.

ID #	Sex	Last date recorded	Date of recorded bite	Status of Bite(s)	Number of Bites	Notes
001	M	26/01/2005	01/11/2005	F	1	N/A
019	F	N/A	19/08/2003	HL	2	Similar stage of healing
023	F	29/11/2003	05/11/2004	HD	2	N/A
059	M	N/A	03/01/2004	HL	1	N/A
074	F	05/12/2004	16/12/2006	HL	1	N/A
078	F	08/05/2004	17/01/2005	HL	1	N/A
083	F	12/05/2004	20/02/2006	HL & HD	6	3 healing & 3 healed
086	F	N/A	12/05/2004	F & HL	3	2 fresh & 1 healing
105	F	19/05/2004	17/02/2006	F	1	N/A
106	F	N/A	19/05/2004	F & HL	2	1 fresh & 1 healing
113	F	N/A	20/05/2004	F	1	N/A
147	F	N/A	27/11/2004	HL	3	Similar stage of healing
168	F	N/A	29/11/2004	HL	1	N/A
201	F	N/A	22/12/2003	HL	2	Similar stage of healing
212	M	N/A	16/12/2004	HL	1	N/A
214	F	N/A	16/12/2004	F, HL	2	1 fresh & 1 healing
216	F	N/A	16/12/2004	HL	1	N/A

Table 8.2 Continued.

ID #	Sex	Last date recorded	Date of recorded bite	Status of Bite(s)	Number of Bites	Notes
237	F	N/A	21/04/2005	HL	1	N/A
257	F	N/A	13/01/2005	HL	1	N/A
267	F	N/A	24/12/2004	HL	1	N/A
271	F	N/A	15/01/2005	HL	1	N/A
279	F	N/A	18/01/2005	HL	4	Similar stages of healing
283	F	N/A	18/01/2005	HL	2	Similar stages of healing
292	F	N/A	28/12/2004	HL	1	N/A
293	F	N/A	19/01/2005	HL	1	N/A
296	M	N/A	21/01/2005	HL	1	N/A
306	F	N/A	29/07/2005	HL	1	N/A
307	F	03/12/2004	30/07/2005	F	1	2 healing on first sighting
326	F	28/01/2004	28/10/2005	HL	1	N/A
351	M	N/A	28/11/2005	F	1	N/A
369	M	N/A	29/12/2005	HL	1	N/A
373	M	N/A	02/01/2006	F	1	N/A
380	F	N/A	27/01/2006	HL	1	N/A
394	M	N/A	16/02/2006	F	1	N/A
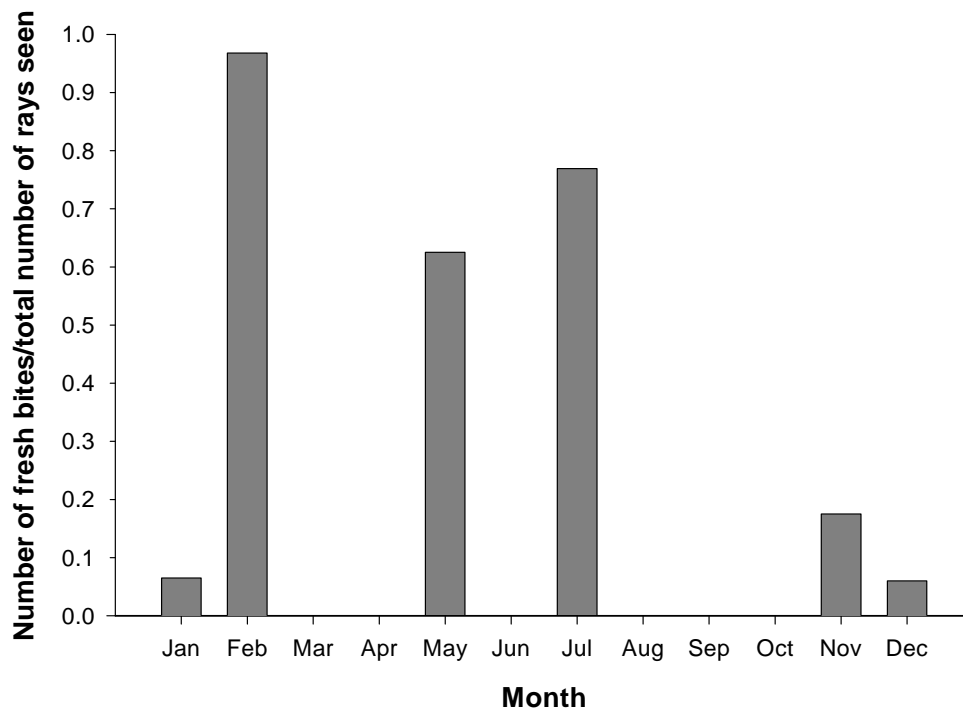
Figure 8.10 Normalised occurrence of fresh shark bites by calendar month. Number of individuals observed: January (n = 153), February (n = 31), March (n = 16), April (n = 6), May (n = 64), June (n = 1), July (n = 13), August (n = 12), September (n = 3), October (n = 28), November (n = 114), December (n = 168).

DISCUSSION

Occurrence in the observed Mozambican population

The examined population of *Manta birostris* variant A off the southern Mozambican coast exhibits a high incidence of non-lethal shark attack bite injuries with about three-quarters of the sampled population affected. The incidence of visible, shark-mediated bite marks in this population appears higher than in other well-documented populations of manta rays around the world, where predatory scars, like bite marks, appear less common (Ito 2000; Acker pars. comm.; Deakos pers. comm.; Stevens pers. comm.; Marshall unpublished data).

None of the bite injuries appeared to change markedly in shape or size over the three-year study period. It was noted, however, that scrapes and tooth rake marks that were not included in the study, often disappeared or changed colour or shape over the study period (Fig. 8.2b, 8.4a,b).

Bite mark distribution and method of attack

Bite mark evidence from this study suggests that sharks most commonly attack *Manta birostris* variant A from the rear of an individual, with over 96% of bite injuries occurring on the posterior section of the body. While direct comparisons are difficult, reports indicate that many cetaceans and other marine mammals sustain shark-bite injuries to the posterior body, with sharks most frequently approaching from the rear to avoid both visual and, in the case of odontocetes, sonar detection (Long and Jones 1996; Long et al. 1996; Naessig and Lanyon 2004). Manta rays, with their laterally placed eyes and elongated pectoral fins, may have a blind spot posteriorly, making approaches from the rear potentially easier for predators. During the three-year study period only a single attack on a manta ray by a grey reef shark was observed in Mozambique in 2003. The shark approached from the rear of the animal but was not able to secure a hold on the posterior margin of the pectoral fin, resulting in an unsuccessful attack.

Only one of the observed manta ray individuals showed evidence of multiple fresh bites, indicating that a single bite wound may be the most common result of a shark attack. However, 25% (n = 6) of all individuals with healing wounds had two or more bites in a similar stage of healing, with a single individual having four distinct bites healing at the same time. Despite these observations, it was impossible to determine if these bites came from the same shark or were inflicted during the same attack. Direct observations of sharks attacks on smaller rays, like stingrays and eagle rays, have revealed that some species, like hammerhead or bull sharks will debilitate their prey with an initial bite before returning to make repeated attacks which result in the death and ultimate consumption of the ray (Appendix 3, Strong et al. 1990; Chapman 2002). As these observations have been on rays smaller in size than *M. birostris* var. A, it is not known if a shark could have a similar effect on a manta ray. Observations of multiple healing wounds on *M. birostris* var. A may also reflect the susceptibility of injured rays to further predatory attacks from sharks other than the initial attacker, particularly if the initial injuries were severe or debilitating (e.g. blood loss or damage to an eye) or if the individual was more conspicuous or attractive to predators (e.g. trailing blood).

Bite wound sizes

Bite injury dimensions based on direct measurements and qualitative assessments of photographic images clearly indicated a large range in bite size and shape (Table 8.1, Fig. 8.9a-h). This strongly suggests that different sized sharks and most likely different species of sharks were responsible for the observed bite marks.

Fresh wounds provide the best indication of the minimum size of the jaws used to make an observed bite mark. In this study, however, the vast majority of the bite marks observed were healed. Ashhurst (2004) reported that elasmobranch cartilage cannot repair itself and while none of the bite marks during the short study period were noted to have altered dramatically in size or shape, using healed bite marks to infer the size or species of the attacking shark may potentially be misleading. Bite wounds made when a manta ray is small in size may change in size and shape with the subsequent growth of that individual. Bigg (1982) while confirming that notches out of dorsal fins in killer whales, *Orcinus orca*, are permanent also reported that the shape of the notch could elongate, becoming shallower, as the fin grows over time. As such, inferences of shark size from healed bite wounds should be made with care, as they might not be entirely accurate.

Predator identity

Comprehensive analyses of shark diets have shown that about a third of the 149 species studied consumed chondrichthyans, with rays featured prominently in the diet of species where over 10% of their intake comprised chondrichthyan species (Cortés 1999; Heithaus 2001a; Simpfendorfer et al. 2001). Thirteen species of large predatory sharks, known to consume chondrichthyan fishes as a part of their normal diet (Cortés 1999; Heithaus 2001a; Compagno et al. 2005) are present off the eastern coast of Mozambique (Compagno 1984; Cliff and Wilson 1994). However, dietary data from dissections of some of some of these species including *C. plumbeus* (sandbar shark), *Isurus oxyrinchus* (shortfin mako) and *C. limbatus* (blacktip shark) inhabiting the waters off southern Africa suggest they may not target large prey such as manta rays (Cliff et al. 1990; Stillwell and Kohler 1992; Dudley and Cliff 1993). Thus the potential attackers of manta rays in this region include: *C. amblyrhynchos* (grey reef shark), *C. albimarginatus* (silvertip shark), *C. amboinensis* (Java shark), *C. leucas* (bull shark), *C. longimanus* (oceanic whitetip), *C. obscurus* (dusky shark), *G. cuvier*

(tiger shark), *Sphyrna mokarran* (great hammerhead), *Carcharodon carcharias* (great white), and *Hexanchus griseus* (bluntnose sixgill). Of these species, *C. leucas*, *G. cuvier, C. carcharias,* and *C. amblyrhynchos* have been seen at or in the vicinity of the study reefs in Mozambique. Of the eleven potential shark candidates, only *C. leucas* has been reported to have manta ray remains in stomach contents (Cliff and Dudley 1991) and *G. cuvier* and *C. amblyrhynchos* have been witnessed attacking or consuming manta rays (Ebert 2003 and Marshall pers. obs.).

From the size of the bite marks on *M. birostris* var. A in southern Mozambique it is likely that no one species of shark is responsible for the injuries sustained by this population. From the characteristic shape and size of many of the bite marks it is likely that *G. cuvier* is responsible for some of the observed wounds, while large *C. leucas*, common in this region, is a likely candidate for other large bite wounds. Both these species are known to attack large bodied prey and show considerable geographical variation in their diet, often based on the abundance of prey items at a given location (Heithaus 2001a).

Frequency of injuries

As only quantifiable injuries with removed tissue were considered in this study, the reported frequency of shark-inflicted wounds on manta rays should be considered a minimum estimate. However, the data presented do reflect the proportion of individuals in the observed population, which were involved in serious attacks by sharks. There are only a few studies which estimate predation frequency or comment on the rates of non-lethal predation injuries in known individuals or populations. For marine mammals, Nasseig and Lanyon (2004) reported single scars appearing on three humpback whales over periods ranging from about one to five years and Heithaus (2001b) estimated that more than one shark attack per dolphin would be expected over a 20-30 year lifespan. While it is not possible to determine from such a short observation period the rate of shark predation on *M. birostris* var. A in southern Mozambique, fresh shark bite injuries were not commonly observed. As shark inflicted bite marks appear to be permanent in manta rays, with bite scarring accumulating over an individual's lifespan, the rate of predation in the observed population may not be as high as initially expected (Heithaus 2001a). Data from this study does suggest however that individual *M. birostris* var. A in this region will

likely be the victim of a shark attack during the course of its lifetime, however, at this time it cannot be determined what proportion of these attacks will result in fatalities.

Effects on local manta ray population

While it is evident from the sampled population that many individuals survive shark attacks, it is not clear how many individuals, particularly smaller juvenile rays, either are killed by sharks or succumb to their injuries at a later time. Also unclear is how shark inflicted injuries may subsequently continue to adversely affect individuals after the initial attack. The vast majority of shark bite injuries in this study caused damage to the posterior pectoral and pelvic fin region (Fig. 8.7). As a result, damage to the claspers of male rays was not uncommon, ranging from superficial cuts and abrasions to partial or complete loss of one or both claspers (Fig. 8.8e-h). The loss of both claspers would render a male ray reproductively non-functional. Serious or debilitating injuries may also have an affect on a male ray's ability to participate in mating activity (Heithaus 2001a). In addition to male rays, many female rays in the population also sustained gross injuries to the pelvic fin region, resulting in disfigurement and the formation of extensive scar tissue around the cloaca (Fig. 8.8a-d). In extreme cases, such extensive disfigurement may hamper or prevent clasper insertion during mating attempts or even inhibit waste excretion (Clarke 2008). During the short study period, two pregnant females which survived major shark attacks (evidenced by severe fresh injuries) were documented days after their attacks. During these re-sighting events the females were no longer pregnant and may have aborted their pups (mid-term) as a result of either their attack or extensive injuries (Chapter 6). Manta rays that have been harpooned or caught in nets have been documented to abort their pups during the traumatic and often fatal events (Coles 1916; Appendix 4), a behaviour which is also common in other batoids (Snelson 1989, 1990). Furthermore, Harris (1989) reported that serious injuries to organisms may delay the mean age at first reproduction or prevent females from mating while recovering. While shark bite injuries may not always end a fatality, the injuries inflicted by sharks may negatively impact an individual's health, their ability to reproduce, or their reproductive behaviour (Ainley et al. 1981; Le Boeuf et al. 1982; Heithaus 2001a).

The high incidence and severity of the shark inflicted injuries to *M. birostris* var. A in southern Mozambique may also play a role in the daily cleaning behaviour of individuals in this population, with wound healing and the prevention of secondary infection potentially playing a factor in the amount of time individuals need to spend on cleaning stations (Heithaus 2001a) (Chapter 7). While additional and certainly longer-term observations are needed to clarify and quantify interactions between manta rays and sharks in Mozambique, this study provides an interesting preliminary look at the occurrence and potential repercussions of these interactions in this region.

Chapter 9

Population structure, biology and ecology of a photographically identified population of *Manta birostris* variant B in southern Mozambique

INTRODUCTION

The ability to comprehensively manage and protect a species or a discrete population is not only predicated upon the ability to distinguish between cryptic or closely related species in the field, but the compilation of enough biological, ecological and behavioural information to both properly assess its conservation status and implement appropriate management strategies (Simberloff 1998; Golding and Timberlake 2003; Mace 2004). The discovery of a new putative species in the genus *Manta* with a sympatric distribution to *Manta birostris* variant A in southern Mozambique raises biological questions as well as conservation and management concerns. One of the quickest ways to investigate the differences in closely related species in the field may be to examine them in areas were they co-occur. In southern Mozambique the two putative species of *Manta* are not only sympatric, they are regular visitors to critical habitats such as feeding areas and cleaning stations along the coastline. This rare overlap in their distribution (see Chapter 4) allowed the collection and comparison of data on both *M. birostris* var. A and *M. birostris* var. B from the same region. With specimens of both putative species difficult to access, data collection in the field may be the easiest and most cost efficient method of determining the most salient biological and behavioural differences between these two variant types. The application of advanced technologies such as biotelemetry and molecular genetic techniques may then be applied to help to clarify and resolve other issues.

The aim of this five-year study was to gather data on *M. birostris* var. B to compare and contrast to those collected on the sympatric population of *M. birostris* var. A (Chapters 5–8). Additionally, the biological and behavioural trends seen in *M. birostris* var. B in Mozambique were further compared to those of a population of *M. birostris* var. B in Mexico to enable diagnostic traits, which differentiate the two putative species, to be confirmed in another region.

METHODS

The same two field sites, Manta Reef and Giant's Castle, used to observe the population of *M. birostris* var. A in southern Mozambique were used to examine the

population structure and behaviour of *M. birostris* var. B. Field seasons which started in the month of May each year and ended the following April were completed in 2003-2004, 2004-2005, 2005-2006, 2006-2007, and 2007-2008 and henceforth are referred to as Year 1, Year 2, Year 3, Year 4 and Year 5.

To help facilitate more robust comparisons between the biology and behaviour of *M. birostris* var. B and *M. birostris* var. A, data collected on *M. birostris* var. B in the Revillagigedo Archipelago, Mexico (Chapter 2) during two fieldtrips in November 2006 and 2007 were compared to those collected in Mozambique. Observational data, measurements, samples and photographs collected at this field site in Mexico were obtained using the same methods outlined below.

Photographs and measurements

The same techniques used for the photographic identification (Photo ID) of *M. birostris* var. A (Chapter 5) in Mozambique were used to identify individual *M. birostris* var. B. During each encounter, a picture of the ventral surface of the manta ray was taken (Fig. 9.1a) and, if possible, one was also taken of the dorsal surface (Fig. 9.1b) and of any other characteristics or marks that aided identification. Although natural spot patterns could occur across the majority of the ventral surface of *M. birostris* var. A (Chapter 5), spot patterning almost never occurred in the area between adjacent gill slits in *M. birostris* var. B (Figure 9.1c inset 1). The most distinct and variable spot patterning on the ventral surface of *M. birostris* var. B was posterior to the fifth pair of gill slits and anterior to the opening of the cloaca (Fig. 9.1c inset 2). However, to allow for comparisons with individual *M. birostris* var. A, it was considered appropriate to use a standardised area for both species. For this study, the region was defined as a rectangular area that extended posteriorly from the anterior margins of the first pair of gill slits to, and including, the pelvic fins (Fig. 9.1d). Black and white composite sketches were created of each identified individual and were used to facilitate the matching of re-sighted individuals. Sketches were updated with additional information about the animal (e.g. new bite marks or scars) whenever they were re-sighted. The collection of re-sighting data and the comparison of images followed techniques used for *M. birostris* var. A (Chapter 5).

The sex of an individual was determined by the presence or absence of claspers (Fig. 9.1e,f). The maturity status of males and pregnancy in female rays were assessed
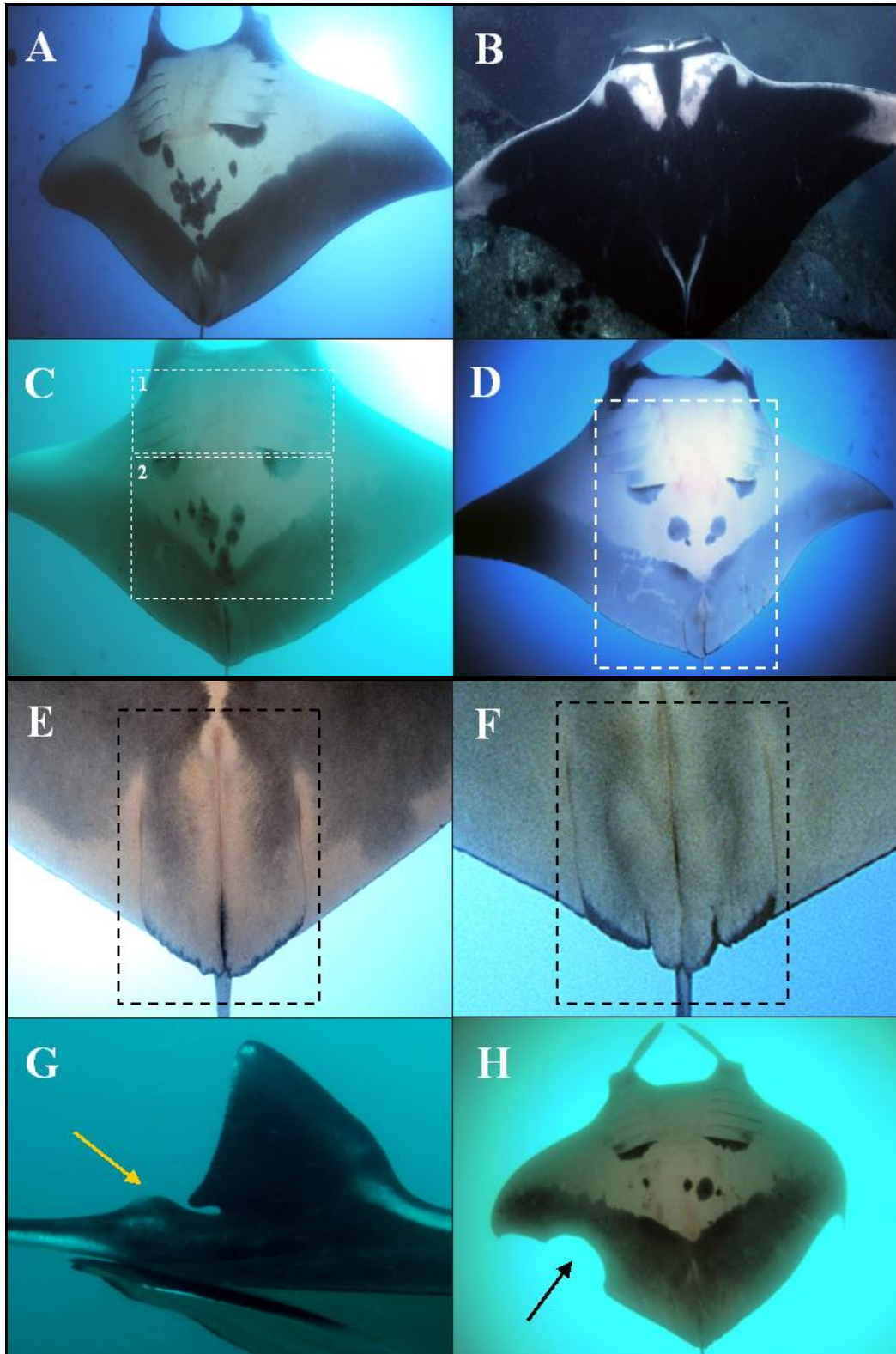
Figure 9.1 Distinctive markings on *Manta birostris* variant B: (a) natural makings on the ventral surface; (b) natural makings on the dorsal surface; (c) variation in ventral markings inset 1: no natural markings between the gill slits, inset 2: greatest variability in the natural markings; (d) standardized area of ventral surface for ID shots; (e) female with boxed area showing no claspers on the pelvic fins, (f) mature male with boxed area showing claspers on the pelvic fins; (g) lateral view of the reduced spine on the tail at the base of the dorsal fin; (h) distinctive bite wounds from sharks on the body of a female ray.

using the same methods described for *M. birostris* var. A (Chapter 5). Characteristic features used to identify *M. birostris* var. B, such as the cartilage encapsulated spine on the tail were noted in each identified individual (Fig. 9.1e). After appropriate identification photos were taken, a size estimate of the animal was obtained using one of two methods. The distance between the tips of the pectoral fins of a manta ray (disc width (DW)), was either estimated using known lengths of divers swimming immediately above or below the ray as it hovered above the reef or were estimated with a parallel laser beam system (Chapter 5). In order to compare to data collected for *M. birostris* var. A, size class estimates were used rather than exact measurements. Based on the smallest and largest individuals encountered, five size classes were used: <3 m DW, 3.0 – 3.9 m DW, 4.0 – 4.9 m DW, 5.0 – 5.9 m DW and >6.0 m DW.

Sightings and identification

On each dive, SCUBA divers executed a defined, uni-directional transect that bounded all monitored cleaning stations. The total number of *M. birostris* var. B seen along the reef transect during the dive was recorded. Photographic images (still or video) taken along the transect were subsequently examined and the total number of identifiable individuals observed per minute of the dive was determined.

To examine seasonal trends, both the total number of rays and the total number of photographically identifiable rays seen per minute of observational dive time were calculated for each dive and each was pooled separately by calendar month. Data were combined for the entire five-year study and mean numbers of rays compared across calendar months. For the month of November, five years of data were combined; for the months of October, December, January and February, four years of data were combined; three years of data were used for the months of March, May and September, for the months of July, August, and April, two years were combined and July was represented by a single year's data. The primary approach, referred to as sighting per unit effort (SPUE), delivered the most realistic indication of manta ray abundance at the study sites for each month of the year, as it reflected the total number of rays counted along the transect including those which were present and could be easily distinguished but could not be definitively identified using photography. The latter approach, identifications per unit effort (IPUE), represents the minimum number of rays seen on the transect reef per dive without the possibility of

recounts and offers an evaluation of the efficiency and accuracy of using photographic techniques to estimate abundance or occupancy.

Biological and behavioural comparisons to *M. birostris* var. A

Information on the predatory scaring of each identified individual (*M. birostris* var. B) was collected from observations and photographs in order to compare to the frequency of occurrence of bite marks in *M. birostris* var. A (Fig. 9.1h). Bite marks were categorised according to Fig. 8.3 and data were collected using the same methodology described in Chapter 8. The presence or absence of a scar or bite mark was also often used to further confirm the identification of an individual whose ventral surface had already been matched to an individual in the Mozambican database. If the bite mark was fresh or uniformly crescent in shape and if the individual could be approached closely while at a cleaning station, measurements of these bites were also taken using a measuring tape (Fig. 9.2a). The presence or absence of skin infections around the gill slits (Chapter 7) was also noted during the identification process and compared against the occurrence in *M. birostris* var. A (Fig. 9.2b).
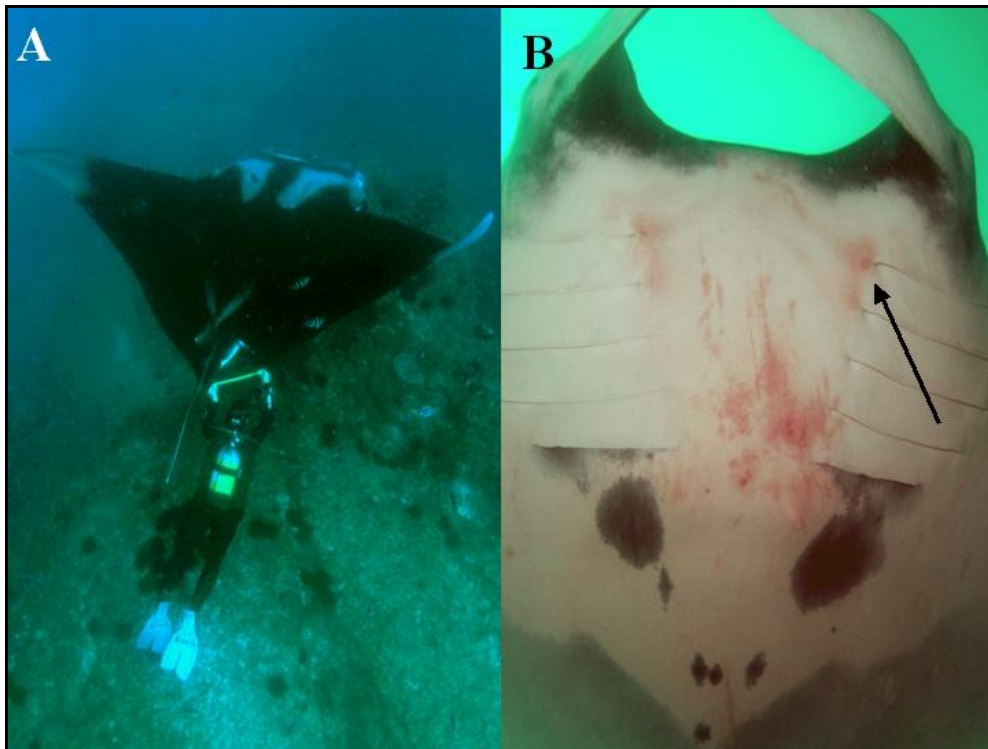
Figure 9.2 Field notations for *Manta birostris* variant B: (a) measurements of shark inflicted bite marks; (b) presence of infections around the gill slits.

The frequency and seasonality of mating behaviours and the timing and duration of pregnancies were recorded. Mid-to-late term pregnancies in rays were identified by swelling of the abdomen, which was apparent from both dorsal and ventral viewpoints. Cleaning behaviour was also monitored at the study reefs in a similar manner to that applied to *M. birostris* var. A (Chapter 7). As only the most northerly cleaning station at Manta Reef (Chapter 7) was used by *M. birostris* var. B, cleaning data could only be compared to *M. birostris* var. A at this single location. In total, 12 separate cleaning bouts were examined at this cleaning station; for each one, the number and species of host fishes involved, the body region of the manta ray targeted, and the swimming behaviour and posturing of *M. birostris* var. B was recorded. The presence and distribution of parasites was also examined in five individuals using the same body surface regions used for *M. birostris* var. A (Chapter 7).

Statistical analysis

Chi-square (χ^2) analysis was used to compare re-sighting ratios, sex ratio data and interspecies data. Yates' correction factor was applied to all Chi-square tests where there was one degree of freedom. Seasonality data, both SPUE and IPUE, were examined using the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks as data were non-normal and could not be normalised. Analyses were conducted using Sigmastat (SigmaStat® S.P.S.S.). Significance was accepted at $P < 0.05$. Box plots were used to graphically represent median values, with the box representing the 25% and 75% confidence intervals (CI) and the whiskers the 5% and 95% CI. The mean is also displayed (for reference) as a dotted line, outliers are indicated by solid dots, and the numbers over the boxes indicate the number of sampling events (dives).

Population size

Mark-resight data from 2003 to 2008 were examined using five separate annual re-sighting periods. These annual re-sighting periods corresponded to the photographic survey's field seasons (e.g. Year 1, Year 2, etc.). As the assumption of population closure is violated when temporary emigration occurs (Otis et al. 1978), the open population Jolly-Seber model (Schwarz and Arnason 1996) was applied, using the POPAN option in the program MARK version 4.3 (White and Burnham 1999), to estimate super population size from the identified individuals of *M. birostris* var. B at the monitored aggregation site. As sample size was small and re-sightings were rare,

sex data were not analysed separately and only the super population ($\hat{N}*$) was estimated. Four models were fitted within the program to estimate $\hat{N}*$ for *M. birostris* var. B for the entire sample period. In all four of the models employed, capture probability (*p*) was kept constant to prevent estimates from being confounded. Some models allowed for variability in time while others held time constant. In some models, β (probability of entry into the population) was kept constant with respect to time. In other models when β was allowed to vary with time, parameters were fixed at the value of 1 to account for extremely high confidence intervals (CI), which had caused them to be essentially inestimable. Models were fitted using the logit link function for $\hat{\phi}$ and $\hat{p}$, the identity link function for $\hat{N}$, and the multinomial logit link function to constrain the set of $\hat{\beta}$ parameters to ≤ 1 (White and Burnham 1999). To correct for small sample sizes, the Akaike's Information Criterion (AIC$_c$) (Burnham and Anderson 2002) was employed to compare between the fitted models.

RESULTS

Effort and photo identification

A total of 436 dives (327.8 hours) on the two study reefs were made over a five-year period from May 2003 to March 2008. In the first year, 1665 minutes of effort were completed at the field sites, in the second, third, fourth and fifth seasons, 4635, 4410, 5220 and 3735 minutes were spent at the sites respectively.

A total of 101 individual *M. birostris* var. B were positively identified during the study period. Partial identifications occurred where, due to obstruction, the ventral coloration pattern in the standardised area of some individuals was not entirely visible and therefore, according to photo identification criteria used in this study, the individual could not be confidently identified. More partial identifications occurred for *M. birostris* var. B than for *M. birostris* var. A (9.9% as opposed to <1%) as *M. birostris* var. B were more often accompanied by schools of suckerfish that obscured the standardised ventral area used for identification (Fig. 9.3a-d).

Unlike in other areas of their distribution where a melanistic form occurs, no black *M. birostris* var. B were seen in the observed population in southern Mozambique. In

contrast the two field trips to the Revillagigedo Archipelago in Mexico revealed a 1:2.4 (n = 16) and a 1:4.3 (n = 31) ratio of melanistic to normally coloured individuals (Fig. 9.4a-d).

Distinctive spot patterning in the standardised area on the ventral surfaces of all re-sighted individuals (*M. birostris* var. B) in Mozambique did not alter during the study period (Fig. 9.5a-d). Bite marks also retained their overall shape and size during the study period (Fig. 9.5a-d). Natural spot patterns in *M. birostris* var. B were less extensive than in *M. birostris* var. A (Fig. 9.6a-d), with the majority of spots occurring centrally on the ventral abdominal region, posterior to the fifth gill slits (Figure 9.6a-b). The spot patterning in *M. birostris* var. B also appeared less elaborate and not as variable as in *M. birostris* var. A. The ventral spot patterns of individuals with standard colouration (not including the melanistic form) in Mexico were similar in colour and design to those observed in Mozambique.

Size distribution

Individuals in the observed population of *M. birostris* var. B in Mozambique were all over 4 m DW, with most individuals ranging in size from 4.0 – 4.9 m DW to 5.0 – 5.9 m DW (20.8% and 77.2% respectively) (Fig. 9.7). Only two (2%) individuals, both females, were over 6 m DW. When analysed by sex, the vast majority of the females (84.3%) were between 5.0 – 5.9 m DW whereas, the majority of males (75%) were between 4.0 m and 4.9 m DW, suggesting sexual dimorphism in size or less probably the segregation of the largest male size class.

Sex ratios and reproductive maturity

Of the 101 *M. birostris* var. B identified in Mozambique, 12% were male and 88% were female (sex ratio 1:7.4), indicating a highly significant sex bias ($\chi^2 = 58.7$, df = 1, P < 0.0001). When examined by year, a significant difference in the sex ratio of sighted individuals was found for all years (Fig. 9.8), with a bias towards females (ratios of 1:6, 1:9.5, 1:6.5, 1:5, 1:18) (chi-square: P = 0.0075, P = 0.0006, P = 0.0045, P = 0.0067, P = 0.0001). Observations of *M. birostris* var. B in Mexico did not reveal a significant difference in the sex ratio (1:2.2) for the 2006 fieldtrip ($\chi^2 = 2.25$, df = 1, P = 0.1336), although sample size (n = 16) was small, but a highly significant
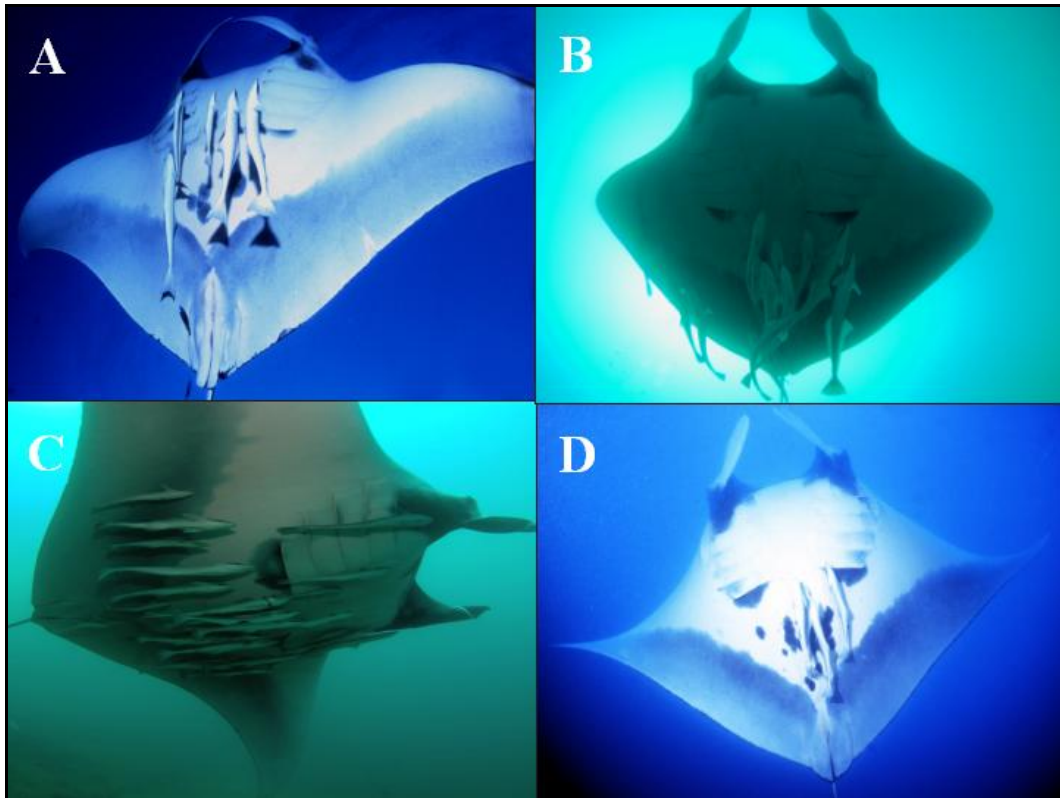
Figure 9.3 Remoras on *Manta birostris* variant B: (a-d) remoras obscuring view of natural markings on observed individuals in southern Mozambique.
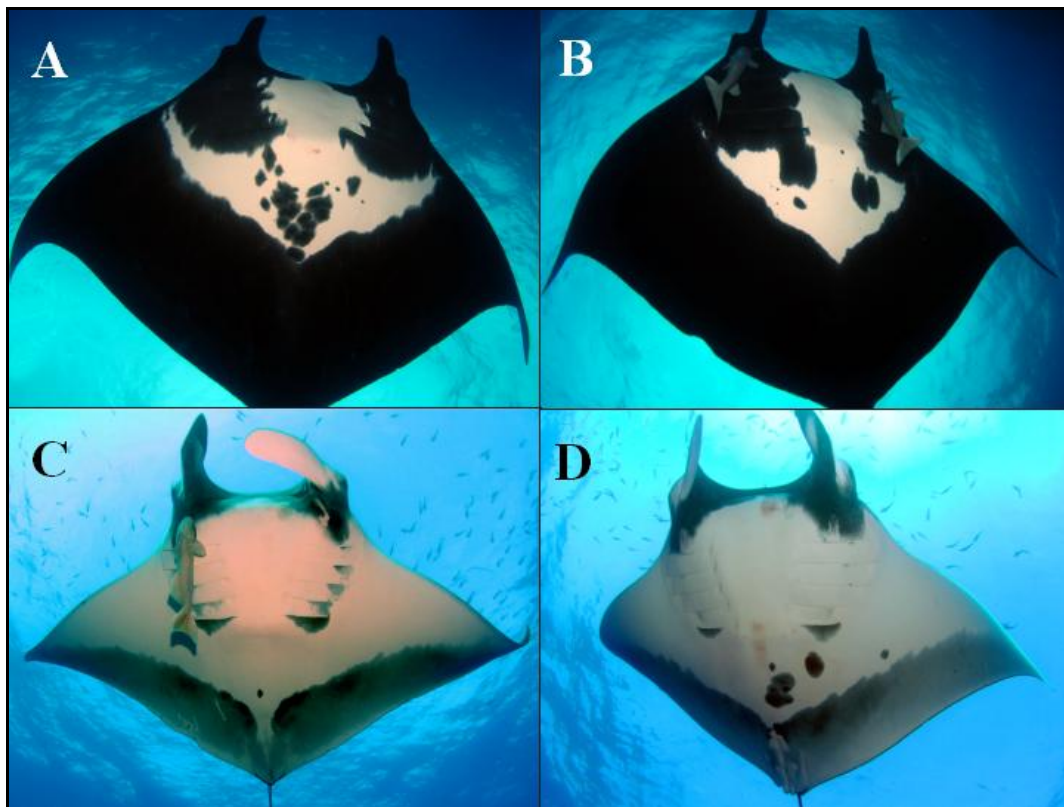
Figure 9.4 Colour forms of *Manta birostris* variant B: (a, b) black colouration; (c, d) standard colouration.

difference in the sex ratio for individuals observed during the 2007 fieldtrip (n = 31) with a very strong bias towards females 1:9.3 (χ^2 = 20.16, df = 1, P < 0.0001).

Of the 12 male *M. birostris* var. B identified in southern Mozambique, nine were mature, and three were classified as 'sub-adult' at the time of sighting as their claspers were more or less flush with the posterior pelvic fin margin, did not look or feel fully calcified and lacked evidence of reproductive use (e.g. no reproductive scarring on clasper tips). Aside from the sub-adults, which appeared to be transitioning into maturity, no juvenile individuals were seen in Mozambique. The three sub-adult rays observed were estimated to be approximately 4.0 m DW suggesting the size at maturity for the observed population in Mozambique may be around this size.
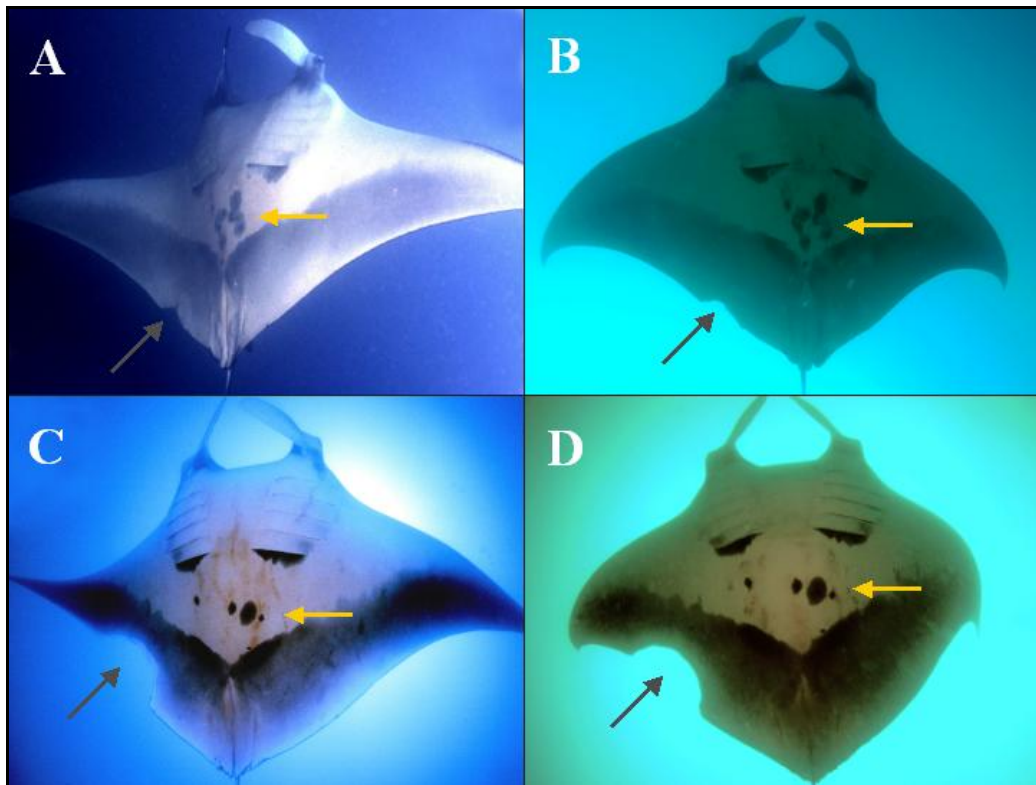
Figure 9.5 Example of unchanged spot patterning and bite marks in re-sighted individuals. Identification image of individual #317 in: (a) 2003 and (b) 2008; Identification image of individual #415 in (c) 2004 and (d) 2007.
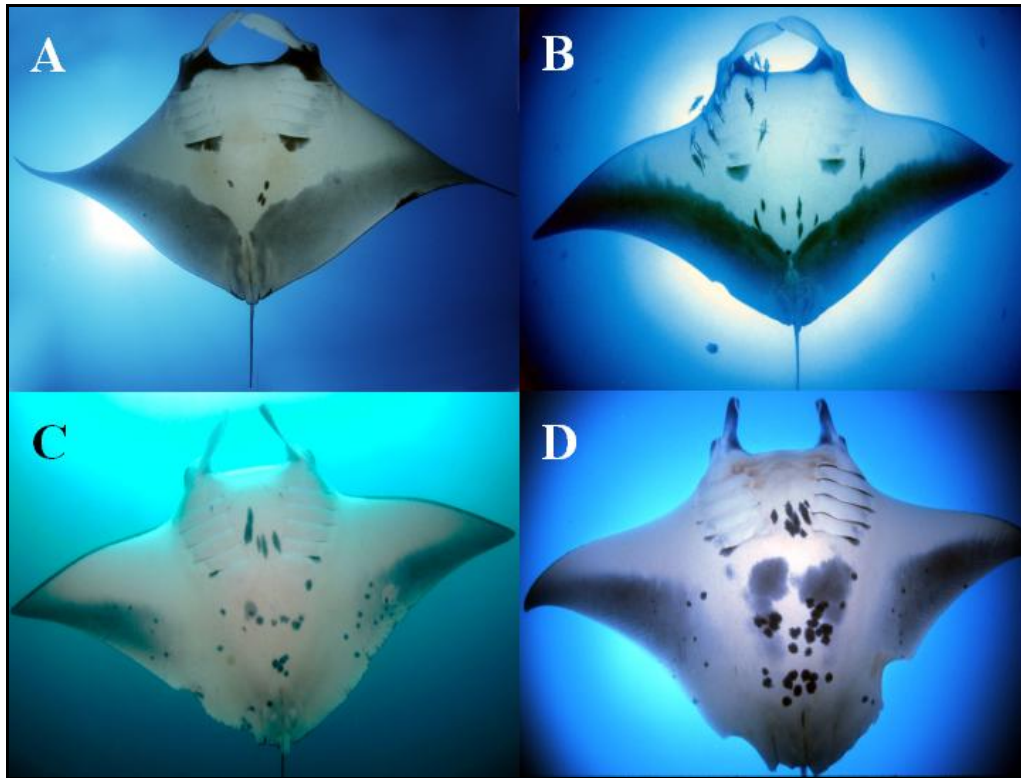
Figure 9.6 Natural colouration and spot patterning in *Manta birostris* variant B: (a) male and (b) female, and in *Manta birostris* variant A (c) male and (d) female.
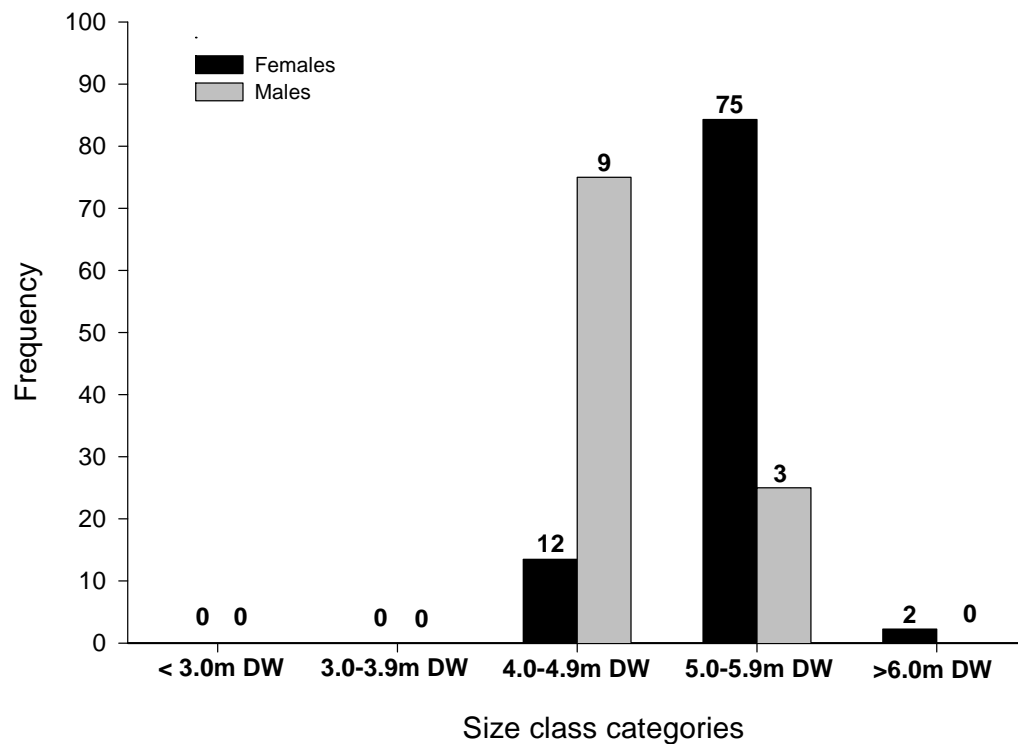
Figure 9.7 Size distribution of identified male and female *Manta birostris* variant B. Numbers above the bars represent sample size.
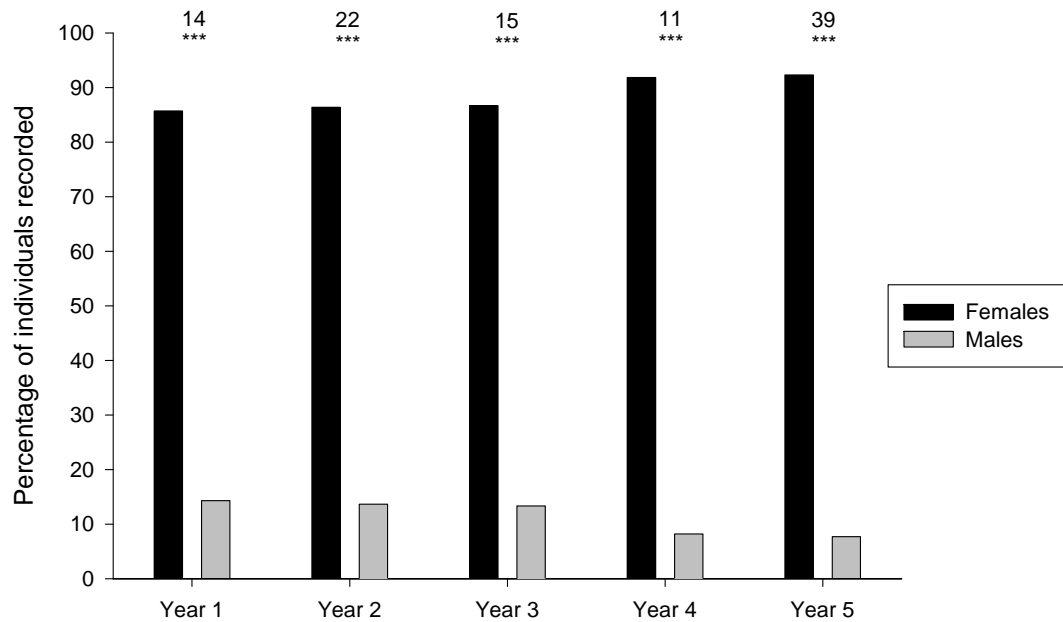
Figure 9.8 Percentage of male and female *Manta birostris* variant B recorded in each year. Numbers across the top indicate total number of individuals recorded that year and *** indicates significance to the $p < 0.001$ level.

Manta birostris variant B was observed to have consistently smaller claspers compared to *M. birostris* var. A (Fig. 9.9a-f, 9.10a,c-d), with the claspers of all mature males in Mozambique reaching only slightly past the pelvic fins. In contrast, while the difference was visibly subtle, the claspers of *M. birostris* var. A extended approximately twice as far as the claspers in *M. birostris* var. B. Although small in overall size, the claspers of *M. birostris* var. B had reproductive scarring (Fig. 9.10a) and were evidently full size, as the claspers of the single re-sighted male did not change in size or appearance over a four-year period.

Observations of claspers of *M. birostris* var. B in Mexico confirmed that mature claspers in this putative species do not extend much further than the posterior margins of the pelvic fins (Fig. 9.10b, 9.11b,d). The majority of the males (71%) observed in this region were immature (n = 7). Immature individuals had small, uncalcified claspers similar in appearance to the immature males of *M. birostris* var. A (Chapter 6, Fig. 9.11a,c). The claspers of mature rays observed in Mexico (n = 2, 29% of identified male rays) were similar in size to those observed in Mozambique and had
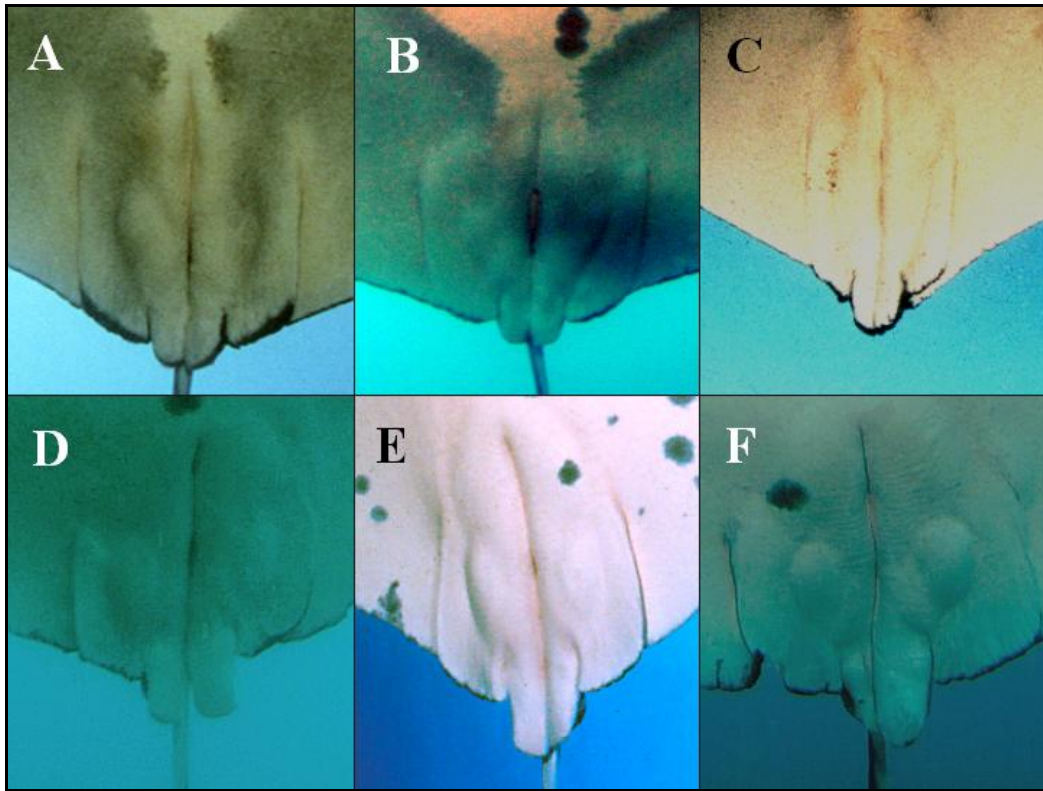
Figure 9.9 Differences in the size and shape of claspers (mature males) of the two putative species (ventral view): (a-c) *Manta birostris* variant B; (d-f) *Manta birostris* variant A.
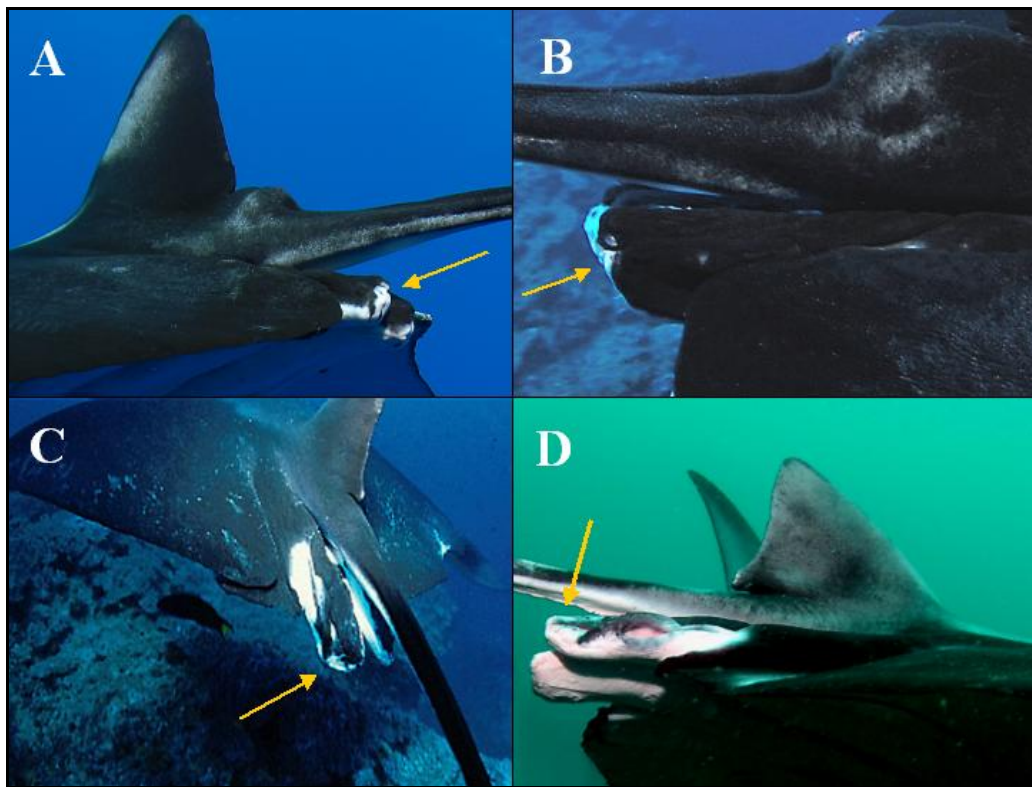
Figure 9.10 Differences in the size and shape of claspers of the two putative species (dorsal and lateral view): (a) *Manta birostris* variant B, Mozambique; (b) *M. birostris* var. B, Mexico; (c, d) *Manta birostris* variant A in Mozambique.
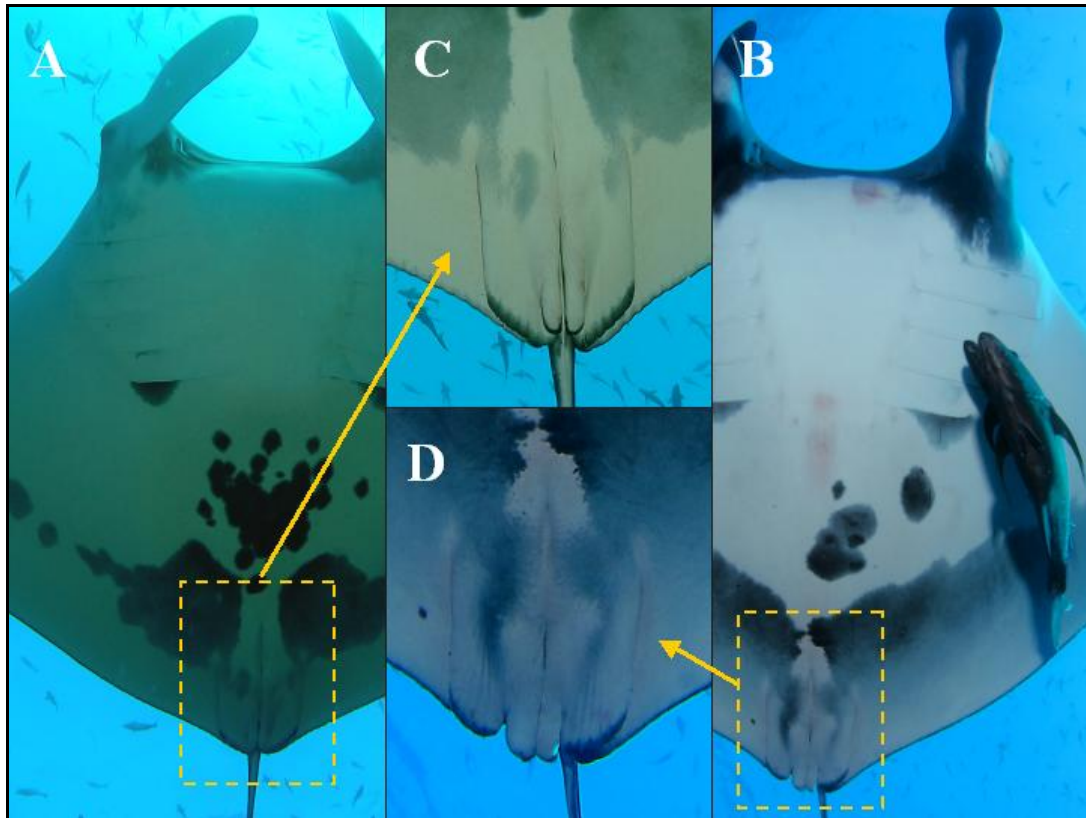
Figure 9.11 Differences in the size and shape of claspers of *Manta birostris* variant B in Mexico: (a, c) immature; (b, d) and mature.

reproductive scaring towards the tips suggesting that they had previously engaged in mating events (Fig. 9.10b).

Using pregnancy as a definitive indicator of maturity in females, only 2.2% (n = 2) of the identified rays in Mozambique were classified as mature. Both of these individuals exhibited mating scars on their left pectoral fin tip. In addition to these individuals, 14 other identified females exhibited reproductive scarring on their pectoral fin tips (Fig. 9.12a,b), suggesting that at least 18% of the identified females were mature. However, most of the other observed female rays were in the same size class (5.0 – 5.9 m DW) as the two pregnant females and those bearing reproductive scars, suggesting the majority of the observed female rays in Mozambique were likely mature. Similar to *M. birostris* var. A there was a strong bias in the location of mating-related scars on the pectoral fins of mature female rays, with over 87.5% of observed reproductive scars in female rays occurring on the left pectoral fin tip only ($\chi^2 = 9.0$, df = 1, P = 0.0027).

The appearance of mid-to-late term pregnancies in *M. birostris* var. B was similar to that seen for *M. birostris* var. A, with pregnant rays exhibiting distended dorsal and ventral abdominal surfaces (Fig. 9.12d). Only two pregnant females observed in October 2007 and March 2008 were recorded during the entire study period, thus the seasonal timing of pregnancies and parturition, the gestation period and reproductive periodicity of *M. birostris* var. B could not be determined. No female *M. birostris* var. B in the Mexican population (n = 39) were observed to be pregnant or had reproductive scarring on their pectoral fins.
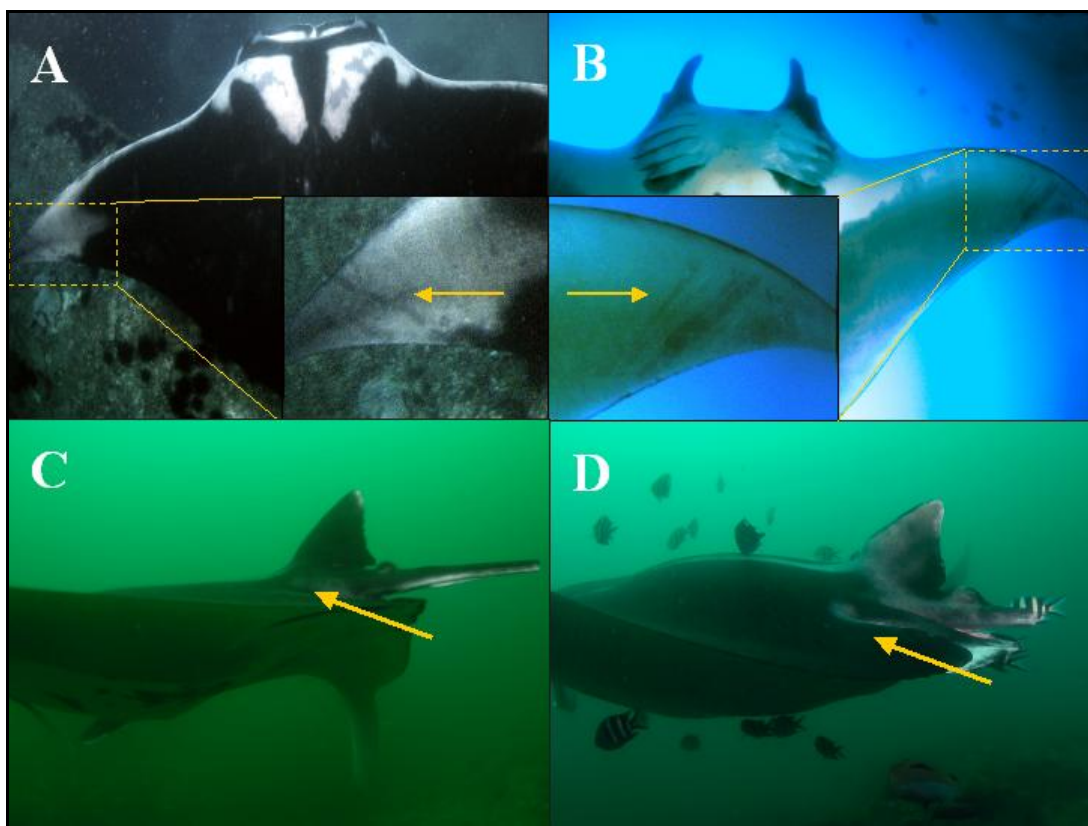
Figure 9.12 Indications of reproductive status in female *Manta birostris* variant B: (a) dorsal reproductive scars; (b) ventral reproductive scars; (c) non-pregnant female; (d) pregnant female.

Occupancy and re-sightings

One hundred and one individual *M. birostris* var. B were identified over the course of the study, with newly identified individuals added to the existing identified population at a rate of about 0.2 rays per transect dive (Fig. 9.13). New individuals were identified in every year, but not in every month sampled. *Manta birostris* variant B

was not seen as consistently on the reefs as *M. birostris* var. A and of the 436 dives to the study reefs, *M. birostris* var. B was not observed on the transects on 343 of those dives. The mean number of individuals seen on the study reefs per dive was 0.51, but occurred at a rate of 2.45 individuals per transect on dives where at least one individual was observed (Fig. 9.14), far fewer on average than *M. birostris* var. A where, comparatively, 6.47 individuals per transect were observed (dives where at least one individual was observed).
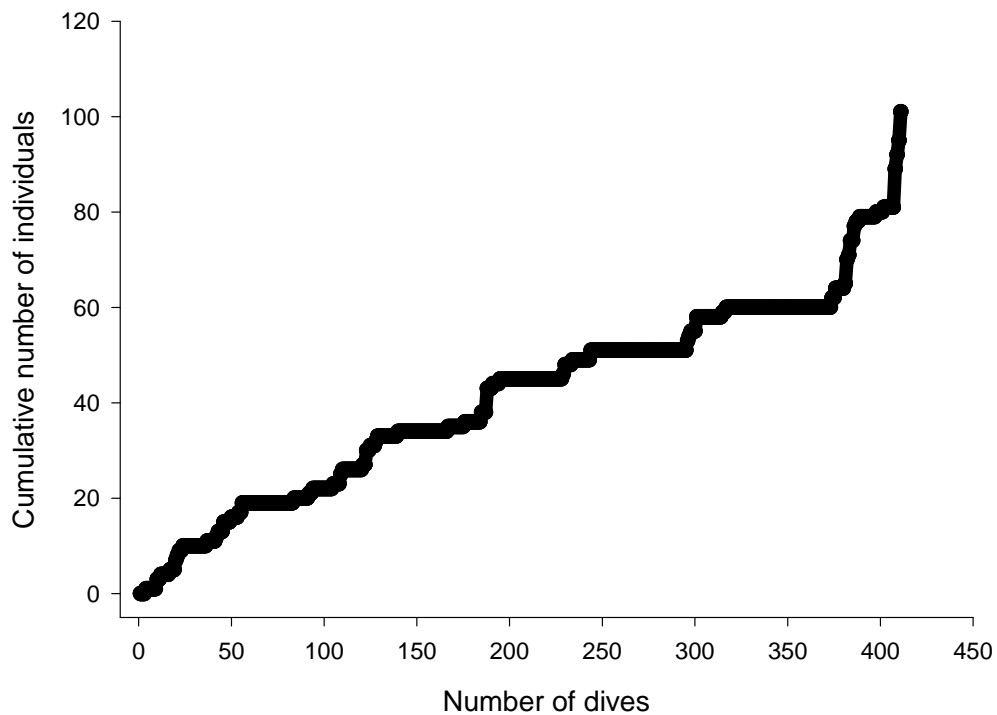
Figure 9.13 Discovery curve indicating the rise in total number of identified individuals over the study period.
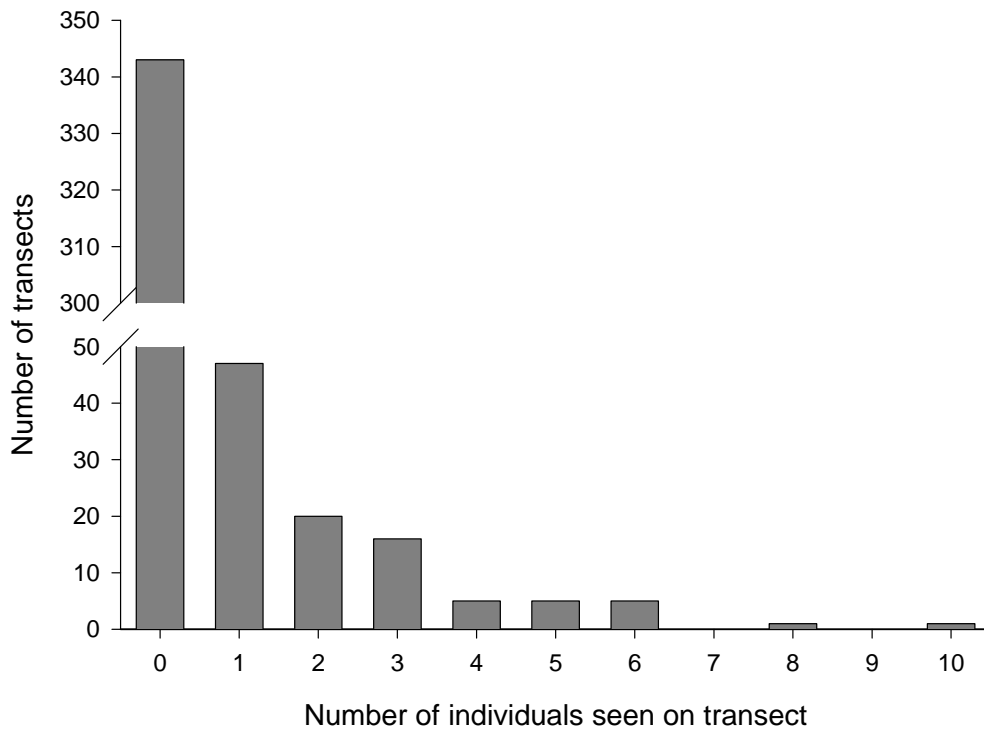
Figure 9.14 Total number of *Manta birostris* variant B seen on each dive transect.
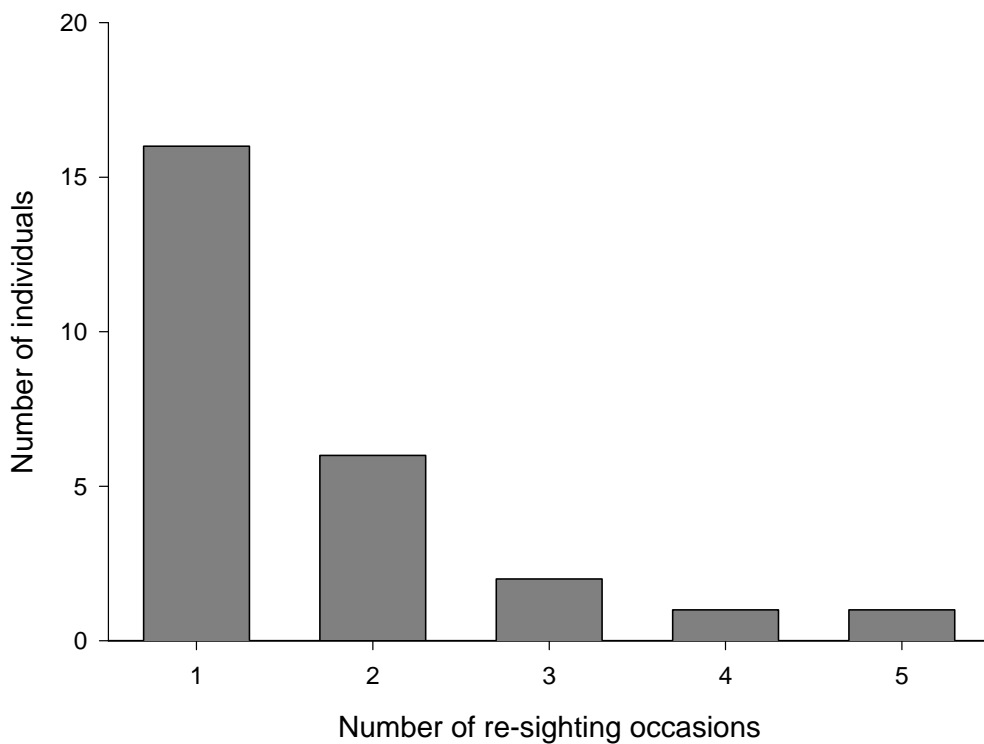
Figure 9.15 Total number of re-sighting occasions for female *Manta birostris* variant B.

Of the 101 individuals identified in this study 17 (16.83%) were re-sighted on at least one occasion, with 27 re-sighting events made in total. Again this was significantly lower than *M. birostris* var. A where 40.5% of the population was re-sighted. Of the total identified *M. birostris* var. B, 91 were sighted only within the initial year of identification, nine individuals were seen in two of the five years of the study and a single individual was seen in three separate years of the study. The mean number of re-sights for an identified ray in the observed populations was 0.27 while the mean number of re-sights for re-sighted individuals only was 1.59.

The longest period between re-sighting events was 1546 days. This individual was a male ray, the only male ray re-sighted during the study period. Periods between re-sighting events for *M. birostris* var. B were either short, under 20 days (n = 12) or long, over 157 days (n = 15) (Table 9.1). Four individuals were re-sighting over 1,000 days after initial identification or a local sighting event.

Twenty-six (96.3%) re-sighting events were of female rays (Fig. 9.15) and of the 89 female *M. birostris* var. B identified, 16 (18%) were re-sighted on at least one occasion as opposed to the single male re-sighting event, which represented 8.3% of the total identified males. The maximum number of re-sights for any individual between 2003 and 2007 was once for males and five times for females, while the mean number of re-sights was 0.08 for male rays and 0.29 for female rays.

In all years, the number of newly identified rays exceeded the re-sighting of previously identified individuals (Fig. 9.16). The ratio of re-sighted individuals to newly identified individuals was not significantly different between any of the five years (χ^2 = 0.877, df = 4, P = 0.928) (Fig. 9.16). While some individuals revisited the observed reefs over periods of time, re-sighting data suggest that in this region the population of *M. birostris* var. B is open and not resident to the immediate area.

Seasonality

Manta birostris variant B was not present throughout the year in any single year of the study, although individuals were seen in each calendar month over the course of the whole study (Fig. 9.17a). *Manta birostris* variant B were observed cleaning at the examined inshore reefs on days when the water temperature was between 20-28˚

Table 9.1 Re-sighting records for individually identified *Manta birostris* variant B during study period.

ID #	Sex	Date of initial sighting	Number of re-sighting events	Number of days between consecutive re-sights
014	Female	16/08/2003	1	915
032	Female	06/12/2003	2	17, 20
080	Female	11/05/2004	5	392, 8, 164, 299, 22
082	Female	12/05/2004	2	1230, 2
091	Female	14/05/2004	3	245, 312, 13
092	Female	14/05/2004	1	180
118	Female	18/06/2004	1	1176
120	Female	25/07/2004	2	158, 15
145	Female	07/11/2004	1	1069
242	Female	30/12/2004	1	15
250	Female	10/01/2005	1	984
317	Male	05/12/2003	1	1546
328	Female	06/11/2005	2	700, 5
448	Female	16/12/2006	1	1
451	Female	17/12/2006	1	444
539	Female	12/10/2007	1	20
546	Female	08/10/2007	1	7
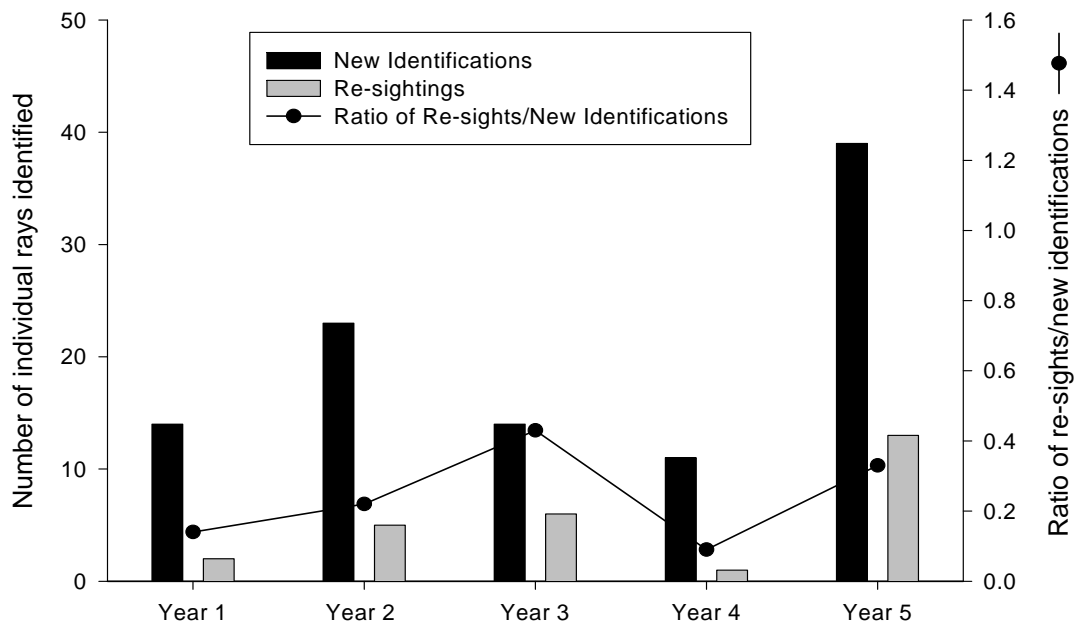
Figure 9.16 Total number of individuals identified and total number of re-sights in each of the years of the study and the ratio of re-sights/new identifications plotted over the five years.

Celsius. The mean water temperature on days when *Manta birostris* variant B was observed cleaning was 24.6° Celsius.

Dive transect effort was consistent over the study period as dive routes were very similar and the conditions and dive profiles did not commonly vary. The maximum number of *M. birostris* var. B seen on a single dive during the study period was ten individuals, a third less than *M. birostris* var. A where a maximum of 30 individuals were sighted. The mean SPUE value (sighting per minute) over the study period was 0.012 and the mean IPUE (identification per minute) was 0.008. A Kruskal-Wallis one-way ANOVA on ranks demonstrated no significant difference in the median values of manta rays seen per minute of dive time (SPUE) ($P < 0.001$), or in the median values of identified individuals seen per minute of dive time (IPUE) between calendar months ($P = 0.339$, $P = 0.719$ respectively) (Fig. 9.17a,b).

A

B
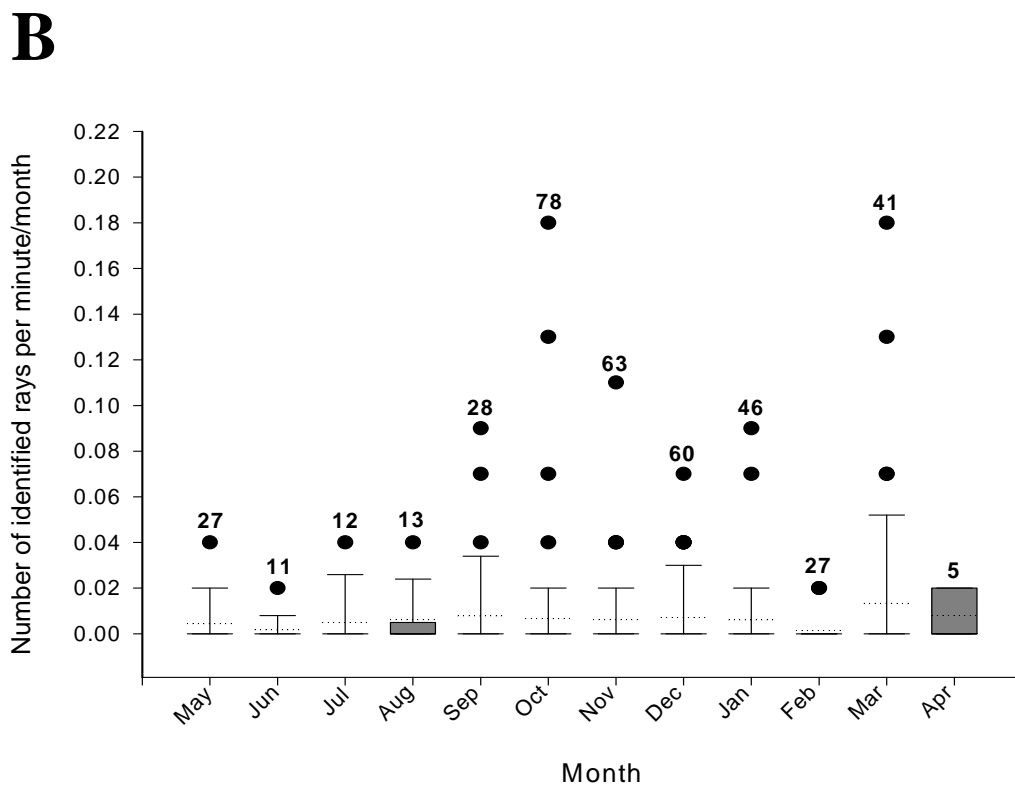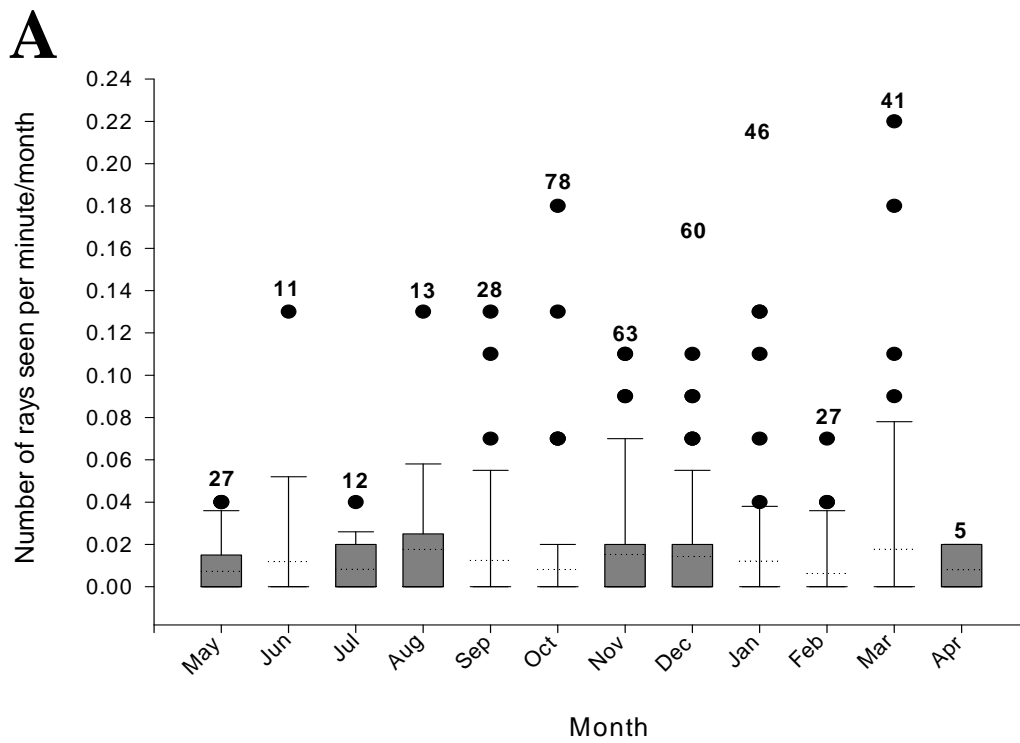
Figure 9.17 Inter-month comparisons: (a) SPUE median number of rays seen per minute of dive effort/month (all years); (b) IPUE median number of rays identified per minute of dive effort/ month (all years) Numbers in graph indicate the total number of dives for each month over the four-year study period. Dotted lines indicate mean.

Population size estimates

A total of five 'capture' sessions (years) provided super population estimates for *M. birostris* var. B in southern Mozambique. Of the four models fitted, the most parsimonious model was $\varphi(.)p(.)\beta(1)N$ with an AIC_c weight equal to 0.91 (Table 1). This particular model held apparent survival (φ) and capture probability (p) constant and fixed the probability of entry (β) at 1. One of the three other models, $\varphi(t)p(.)\beta(.)N$ did not converge and was abandoned. Additionally the model that held time constant for (φ), (p) and (β) converged but was both weighted inferior to the selected model and did not demonstrate information theoretic support, and was therefore not used for model averaging estimates. The only other model to receive AIC support (0.09) was $\varphi(t)p(.)\beta(1)N$, and was used in addition to the to the most parsimonious model for a model average estimate of derived super population ($\hat{N}^*$) (Table 1). The super population estimate ($\hat{N}^*$) was 599.8 (SE = 174.3, 95% CI = 258.1 – 941.5) with 0.01% variation attributable to model variation. Standard errors were large, suggesting a degree caution should be used in the interpretation of these estimates.

Cleaning behaviour, parasites, and predation

Unlike the identified population of *M. birostris* var. A in Mozambique, of which 76% exhibited bite marks and wounds from sharks, only 34.7% of the observed population of *M. birostris* var. B had bite mark scars ($\chi^2 = 14.81$, df = 1, P = 0.001). There was also a significant difference in the proportion of male (6.9%) and female (27.7%) *M. birostris* var. B with bite marks ($\chi^2 = 12.6$, df = 1, P = 0.0004). As for *M. birostris* var. A there was no significant difference in the distribution of bite wounds to the left and right sides of the body ($\chi^2 = 0.32$, df = 1, P = 0.5716), but there was a highly significant difference in the distribution of bite marks in the three designated body regions ($\chi^2 = 12.6$, df = 2, P = 0.0004), with 94% of injuries sustained to the trailing edges of the pectoral fins. Only a single individual was observed with a fresh bite wound (that was 34 cm across) (Fig. 9.18). The identified population of *M. birostris* var. B in Mexico exhibited an even lower frequency (10.9%) of shark-bite injuries and in all cases only a single wound per individual was observed. All bite mark injuries observed in Mexico were located on the trailing edge of the pectoral fins (region 3).

Table 9.2. Comparison of models from the POPAN analysis used to estimate the abundance of *Manta birostris* variant B. AICc = Akaike Information Criterion for small samples; Delta AICc = difference in the AICc of a model from the minimum AICc model; AICc weight = Akaike weight used in model averaging; φ= apparent survival; p = capture probability; . = time constant; t = time varying by secondary sample; 1 = parameter fixed at the value of 1; g = sex; β = probability of entry; N = size of population.

Analysis	Model	AICc	Delta AICc	AICc Weight	Number of Parameters
POPAN	$\varphi(.)p(.)\beta(1)N$	114.27	0.00	0.91	4
POPAN	$\varphi(t)p(.)\beta(1)N$	118.95	4.68	0.09	7
POPAN	$\varphi(.)p(.)\beta(.)N$	12734.92	12620.65	0.00	3
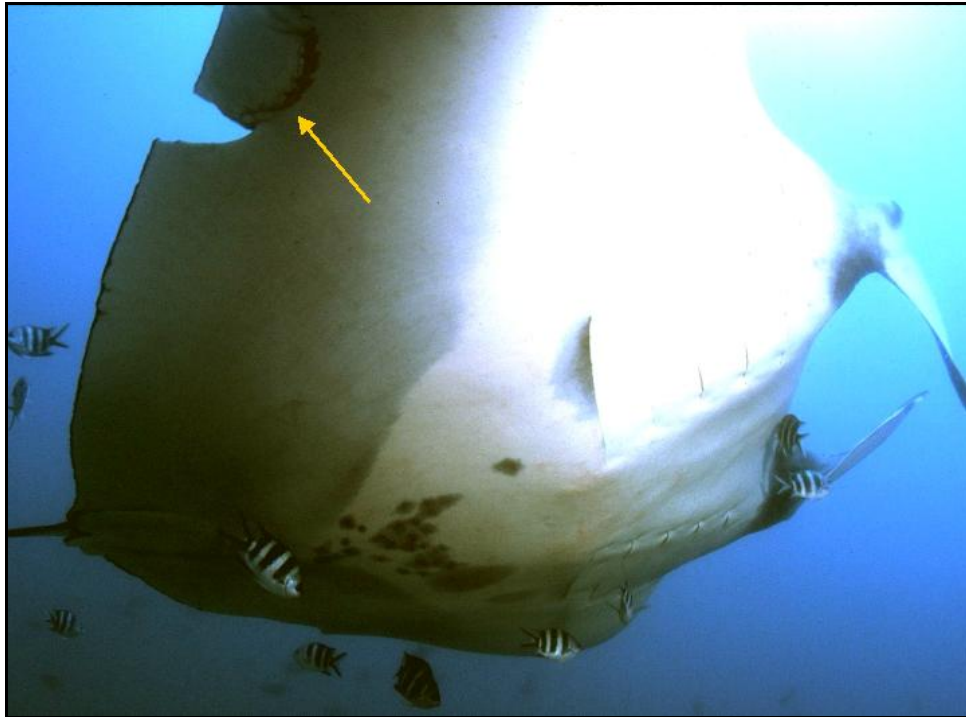
Figure 9.18 Fresh bite wound on *Manta birostris* variant B

Of twelve *M. birostris* var. B examined in Mozambique, only five individuals exhibited visible ectoparasite loads, equivalent to a prevalence of 41.7%, whereas *M. birostris* var. A had 100% ectoparasite prevalence. Of the individuals with ectoparasites, 8% had caligid copepods on their supra-branchial region (region 2), 17% had caligid copepods around their gill slits (region 3) and 25% of individuals had caligid copepods around their face and mouth (region 1). *M. birostris* var. B also exhibited a significantly lower prevalence of infections around the gill slit region (region 3) than *M. birostris* var. A ($\chi^2 = 27.03$, df = 1, P < 0.001). In Mexico, no observed individuals exhibited heavy ectoparasite loads or gill slit infections, however some individuals (n = 3) had small patches of caligid copepods around their mouths or areas with minor irritations such as attachment sites for remora fish (Fig. 9.19 a-c).

Manta birostris variant B was never observed on transects of Giant's Castle reef. Of the three available cleaning stations on Manta Reef, individual *M. birostris* var. B were almost always observed being cleaned at the most northerly station (97.8% of 228 observations). On only five occasions was *M. birostris* var. B observed being cleaned at the southern cleaning station on Manta Reef. Consequentially, *M. birostris* var. B was cleaned predominately by *Abudefduf natalensis* (sergeant major damselfish), the most common cleaner at the northerly station (Chapter 7).
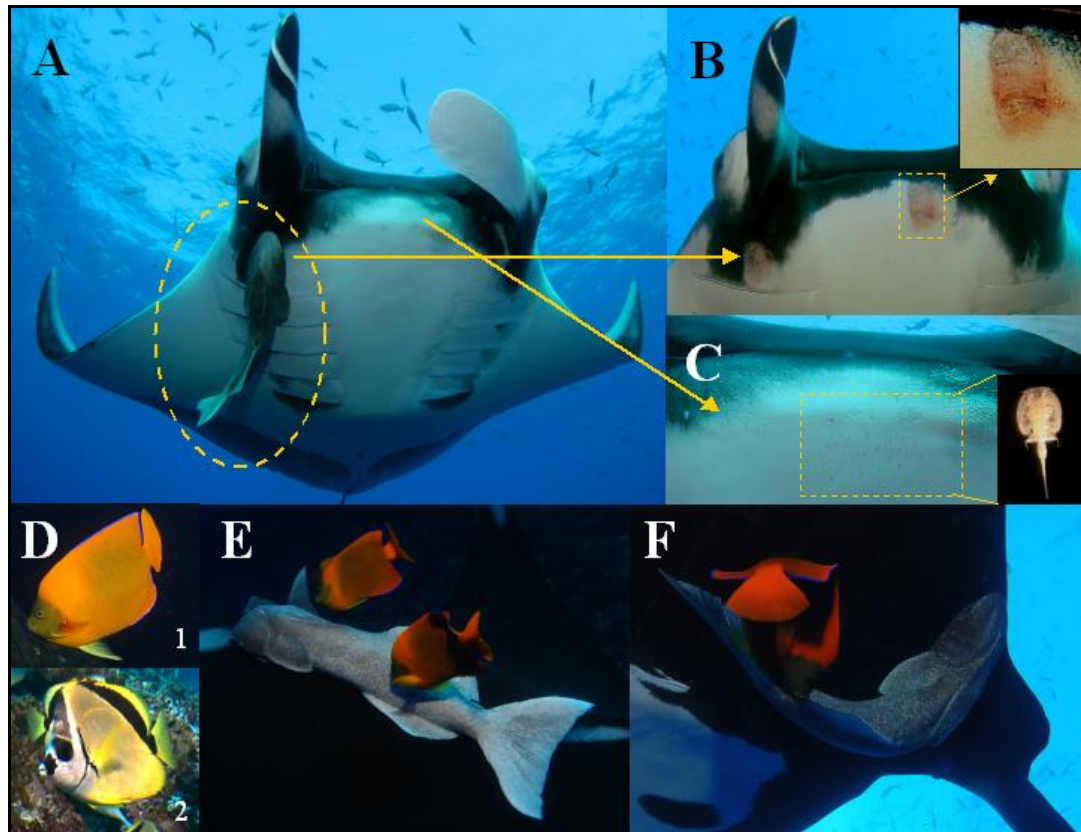
Figure 9.19 Cleaning behaviour of *Manta birostris* variant B at San Benedicto Island: (a) *M. birostris* var. B with remora attached in region 2; (b) irritated remora attachment site in region 2; (c) patch of caligid copepods in region 2 (inset) close-up of copepod (*Lepeophtheirus* sp.); (d) major manta ray cleaners at San Benedicto Island (1) *Holacanthus clarionensis* (2) *Chaetodon* sp.; (e, f) *H. clarionensis* cleaning copepods off remora fish attached to *M. birostris* var. B.

On all twelve focal studies of the cleaning behaviour at this northerly station, *A. natalensis* was the most abundant host cleaner, with as many as 27 and as few as ten individual fish (mean = 16.6) seen at a time cleaning the dorsal and ventral surfaces of *M. birostris* var. B (Fig. 9.20a-d). A single *Pomacanthus rhomboids* (Rhomboid angelfish), was observed cleaning on three occasions (25% of total observed cleaning events) (Fig. 9.20b). No other host cleaner fish species were seen to interact with *M. birostris* var. B at the study sites during the study period.

At San Benedicto Island, Mexico *M. birostris* var. B was also predominately attended by a single host fish species, *Holacanthus clarionensis* (Fig. 9.19d1), but was also cleaned occasionally by an unidentified species of butterflyfish, (*Chaetodon* sp.) (7% of observed interactions) (Fig. 9.19d2). *Holacanthus clarionensis* was occasionally

observed picking off caligid copepods from the ventral surface of *M. birostris* var. B, but it appeared to concentrate it's efforts on the remora fish (*Remora remora*), which attached themselves to the dorsal and ventral surfaces of the manta rays' bodies, as these fish exhibited very high ectoparasite intensities (Fig. 9.19e,f).
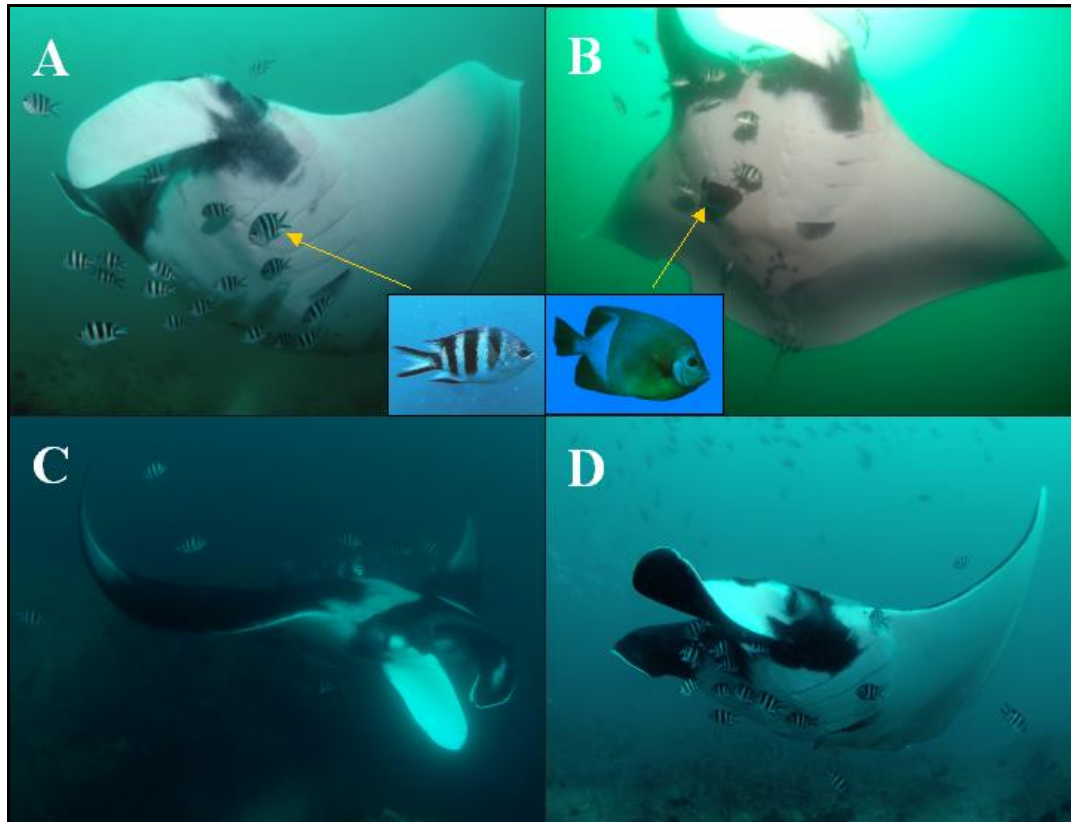
Figure 9.20 Cleaning beahviour of *Manta birostris* variant B at Manta Reef in Mozambique: (a) *M. birostris var. B* being cleaned by *Abudefduf natalensis*; (b) *M. birostris var. B* being cleaned by a single *Pomacanthus rhomboids*; (c) *A. natalensis* cleaning dorsal surface of body; (d) *A. natalensis* cleaning ventral surface of body.

The behaviour exhibited by *M. birostris* var. B during cleaning events in Mozambique was similar to that exhibited by *M. birostris* var. A. In 100% of events, individuals would reduce speed upon approaching the cleaning station, would face into the current and would hover above the reef while being cleaned. However, while being cleaned *M. birostris* var. B and would 'shudder' in apparent response to the tactile stimulation of the host cleaner fish. *Manta birostris* variant A was never seen to exhibit such a response when being cleaned. Similar to *M. birostris* var. A, in 100% of interactions *M. birostris* var. B would make circling passes entering back into the

stations repeatedly. However, in only 41.6% of events did *M. birostris* var. B exhibit angled posturing or flared gill-slit behaviours, which were commonly observed in *M. birostris* var. A (Chapter 7). *Manta birostris* variant B at San Benedicto Island, Mexico also rarely exhibited posturing or flared gill-slit behaviour (13% of cleaning events), but did reduce swimming speed, hovered while being cleaned by host fishes, exhibited shuddering behaviour while being cleaned, and made repeated circling passes to the same area where cleaner fish were present in all observations.

DISCUSSION

Photographic identification

As in the photographic study on *M. birostris* var. A, this study demonstrated that natural spot patterns can be confidently used to identify individual *M. birostris* var. B during discrete study periods at aggregation sites. The only other rigorous photo identification studies on *M. birostris* var. B in the world, in the Revillagigedo Archipelago, Mexico, in Laje de Santos Marine State Park, Brasil, in Ogasawra Island, Japan and off the north island of New Zealand have confirmed the permanence of natural markings in this putative species over periods of up to 20 years (Yano 1999; Duffy and Abbott 2003, Luiz et al. 2008; Rubin pers. comm.). While each individual in this study was able to be uniquely identified using natural markings in the standardised area on the ventral body surface (Fig. 9.1d), the patterning was often less variable than *M. birostris* var. A, with some individuals having similar spot patterns to one another or very few distinctive markings (Fig. 9.6). Another factor that complicated positive identification of *M. birostris* var. B was remoras, which commonly obscured spots on the abdominal region and often cast shadows onto the rays (Fig. 9.3) Consequentially, some manta ray individuals could not be confidently identified or potentially re-sighted. Obscured or inadequate images are an inherent problem of photographic identification studies in the wild (Meekan et al. 2006; Bansemer and Bennett 2008). In this study, only individuals that were definitively identified were added to the database, counted in re-sightings events, used in SPUE and IPUE analysis, or used for population estimates. Therefore, the total number of individuals identified at this location, seasonal data and the estimate of super population size should all be viewed as conservative estimates.

Population composition and re-sightings

In southern Mozambique there was a strong sex bias in observed *M. birostris* var. B, with females out numbering males over seven-to-one. Such an extreme sex bias has not been reported previously for any manta ray population. Photographic cataloguing of *M. birostris* var. B at the Revillagigedo Archipelago, Mexico over the last twenty years revealed a fairly even sex ratio of 1:1.3 (males to females) (Rubin pers. comm.). Field trips to the islands in 2006 and 2007 showed a much higher sex ratio of 1:4.9 with a bias towards females. However, although 46 different individuals were identified during these trips, these data were only collected over two, week-long periods in the month of November each year and could therefore be an artifact of seasonality rather than an accurate reflection of the sex ratio in this region. Reports of *M. birostris* var. B landings in fisheries in Indonesia have shown a very slight bias towards females, with a sex ratios of 1:1.6 (White et al. 2006), however this ratio is not as pronounced as that (1:7.4) observed in southern Mozambique.

Re-sighting data show that females were not only more prevalent in the area but were re-sighted more commonly than males. The longest re-sighting during the study, however, was of a male ray, 1546 days after initial identification in December 2003. However, even the most re-sighted female individual during the study was only seen on five occasions in a five-year period. Most re-sighting events appeared to occur after short periods of time (less than 20 days) or quite long periods of time (over 157 days). The four longest re-sighting events were 1095, 1176, 1230 and 1546 days apart respectively. These patterns strongly suggest that the observed population of *M. birostris* var. B is not resident to the immediate area. While some individuals clearly return periodically to critical habitats such as cleaning stations or feeding areas along this coastline, they may do so only when travelling through the region or when food abundance attracts them to the vicinity. Data on regional occupancy suggest the latter may be true, with multiple *M. birostris* var. B showing up simultaneously, particularly when the water temperature drops, underwater visibility decreases, and plankton blooms are dense and conspicuous (Marshall unpublished data). However, as fewer than 17% of the identified individuals at the aggregation site were re-sighted during the five-year observation period, either the super population size is large or individuals do not utilise this stretch of coastline with particularly high frequency.

Population size estimates

The estimated super population for the monitored aggregation sites was 599.8 individuals, almost six times the number of the individuals identified during the five-year study period (101 individuals, 16.83% of total projected super population). This estimation was supported by both the cumulative growth curve, which did not reach a plateau during the study period, and the annual ratio of new to re-sighted individuals which consistently showed in each year of the study that the majority of observed individuals were newly identified rays. Because the sample size for this population was relatively small and re-sightings events were rare, it is recommended that monitoring at this aggregation site continue to see if longer-term patterns in visitation can be teased out over longer time periods. While the generated population estimate confirms that southern Mozambique is an aggregation site for *M. birostris* variant B, with hundreds of individuals using coastal habitats throughout the year, it also confirms that the population of *M. birostris* variant B is not resident to the immediate area nor do individuals visit this part of the coastline with particular regularity. Re-sightings events of certain individuals do suggest that *M. birostris* var. B may move through the region and utilize habitats along the coast on a cyclical basis (long time intervals) with some individuals being seen several years after their initial identification. Additional photographic effort in the region and beyond may uncover patterns to their visitation over longer time periods or spatial scales (i.e. Mozambican wide). The application of satellite telemetry may also prove useful in determining where individuals travel to when they leave this coastline.

Given the extreme sex ratio of the observed population, the generated population estimate is highly biased and may reflect the super population of female rays in the region rather than the total population. However, this is the only study that has used population modelling techniques to estimate the super population of *M. birostris* var. B from visual mark-resight data collected at an aggregation site. Trends in abundance and distribution of this species remain completely unknown. The longest photographic study of *M. birostris* var. B, from the west coast of Mexico has identified 225 individuals over the past 20 years (Rubin pers. comm.). Clearly additional data and modelling are needed throughout this species' distribution to generate more robust and accurate estimates, particularly those that can additionally estimate survivorship, recruitment, and natural mortality.

Seasonality

Manta birostris variant B does not appear to be resident to the area immediately surrounding the study sites, however individuals were observed throughout the calendar year. Many individuals often appeared at one time when conditions in the area appeared optimal for feeding (i.e. when large plankton blooms were observed) or when cold water upwelling or thermoclines were present along the coast. It hypothesised that *M. birostris* var. B has a broader general temperature tolerance, being found in both tropical and temperate waters. Furthermore, the northerly and southerly distributions of *M. birostris* var. B exceeds the range of *M. birostris* var. A. In Mozambique, *M. birostris* var. B was observed using inshore habitats when the water temperature was between 20-28˚ Celsius. However, there was no significant difference in the seasonal use of the monitored cleaning stations. Independent observations found that during times *M. birostris* var. B were absent from the cleaning stations they were also absent from other critical habitats such as feeding areas (Marshall unpublished data). These data are unlike those reported for other aggregation sites of *M. birostris* var. B, with researchers in some regions reporting clear seasonal visitation patterns for *Manta birostris* variant B (Duffy and Abbott 2003, Luiz et al. 2008).

Biological and behavioural comparisons

The ultimate aim for a study which takes in to consideration the behavioural ecology of a population is to understand how different behaviours can contribute to and influence the reproductive success and survivorship of individuals under different ecological conditions (Krebs and Davies 1993). To that end, the collection of information on habitat use, movement patterns, reproductive biology and behaviour can be used to make interspecific comparison of populations. The biological and behavioural observations made during this study at two geographically separated regions lend support to the claim that these two putative species of manta rays are distinct. While there are clear differences in their over all morphologies (Chapter 4), this study also suggests that there are significant differences in their biology, behaviour and habitat use which may affect their respective populations along this coastline.

Compared to *M. birostris* var. A, *M. birostris* var. B exhibits far fewer bite marks from sharks, have fewer skin infections in the area of their gill slits and have smaller parasite loads. All three observations may be due to differences in their skin and dermal denticle structure (Chapter 4), but may also reflect different habitat use, lifestyles, or behaviours. The fact that *M. birostris* var. B have fewer parasites, infections, or wounds may in turn cause them to visit cleaning stations less frequently or for shorter periods of time than *M. birostris* var. A, resulting in lower rates of inshore sightings. These differences, however, do not explain the apparent partitioning of the cleaning habitat on coastal reefs in southern Mozambique. While small scale resource or habitat partitioning is a common strategy between closely related or competitive species (Platell and Potter 2001; Papastamatiou et al. 2006), observations of the feeding habits of these two putative species suggest that the two feed in similar areas along the coast in southern Mozambique (Marshall unpublished data). There are no obvious major differences between the two study reefs. Giant's Castle is slightly deeper and slightly closer to deeper water than Manta Reef but is not located much farther offshore. Observations outside of this study revealed that *M. birostris* var. B occasionally used other inshore reefs in the area including two other shared cleaning stations with *M. birostris* var. A. The reason for the observed habitat partitioning on Manta Reef is therefore unexplained. While the most northerly cleaning station does differ from the two more southerly cleaning stations in that there is no soft coral cover, the stations are very close together and there are no other obvious differences in habitat. The single most conspicuous difference observed during the study was the presence of sergeant major damselfish, *A. natalensis*, which appeared to clean exclusively at the northerly station. Despite initial suspicions that this cleaner species may be the reason that *M. birostris* var. B preferred this station/reef to other sites or stations, individual *M. birostris* var. B have subsequently been seen cleaning at other inshore reefs by other species of cleaners including *Chaetodon kleinii* and *Labroides dimidiatus*. Perhaps the majority of the time *M. birostris* var. B seek out cleaning interactions with *A. natalensis* which is why individuals seem to prefer sites like the northern station on Manta Reef, however the real reason for this habitat partitioning could not be determined during the course of this study. It is suggested that this phenomenon be explored in future studies, which may expand out to monitor more cleaning stations and shared habitat along the coast.

Another striking difference between the two putative species in southern Mozambique was the absence of observed mating behaviour and fresh mating scars *M. birostris* var. B as well as the observation of only two pregnant females during five years of observation. These data suggest that, unlike *M. birostris* var. A, *M. birostris* var. B does not commonly use this coastline as mating or birthing grounds (Chapter 6).

Lastly, one of the most definitive differences between the two observed populations in Mozambique was the lack of residency of *M. birostris* var. B to the coastline, with individuals exhibiting a far lower re-sighting rate than *M. birostris* var. A. Probably more importantly, the two putative species do not appear to interact behaviourally. Manta rays are commonly observed interacting with conspecifics while feeding (e.g. in feeding chains), while mating (e.g. courtship displays) and while travelling (e.g. in schools) (Yano 1999; Homma 1999; Marshall 2006). However, *M. birostris* var. A and *M. birostris* var. B were never seen to interact with one another and furthermore, the two species appear quite often to segregate themselves, whether purposely or not, through habitat selection as demonstrated in their apparent partitioning of cleaning stations.

Co-existence in Mozambique

The speciation of closely related species may occur from populations that are geographically isolated (allopatric speciation) or from co-existing populations, which are able to maintain reproductive isolation (sympatric speciation) (Harper et al. 1961; Johannesson 2001; Via 2001). For sympatric speciation to occur and furthermore for closely related species to co-exist, it is fundamental that mechanisms such as habitat selection, staggered reproductive seasonality, breeding behaviour or mate selection prevent individuals in populations from mixing during breeding seasons (Cain 1953; Harper et al. 1961; Johnson 1996; Johannesson 2001; Via 2001). Two additional prerequisites for the successful occurrence of closely related, sympatric species are: that both species are tolerant of the environmental regimes that occur in the shared habitat or region and that species do not differ in such a way that the success of one species comes at the detriment or peril of the other (Harper et al. 1961).

An important distinction needs to be made when examining species with sympatric distributions, as individuals with common geographic distributions may not be utilising the same microhabitats or behaviourally intermixing (Bagenal 1951; Cain

234

1953; Harper et al. 1961; Rivas 1964). The proposition that closely related sympatric species may not be occupying the same microhabitats led to more detailed definitions of sympatric distributions, with 'syntopic' distributions designated to describe populations or species that occur together in the same locality, are observed in close proximity, and could potentially interbreed, and 'allotopic' distributions describing populations or species which have similar geographic distributions, but do not occupy the same microhabitats, intermingle, or even come within close contact with one another and therefore could not interbreed (Rivas 1964).

Mozambique is one of only a few sites worldwide where both putative species of *Manta* are known to have sympatric distributions. These two species were seen to use common habitats such as cleaning station reefs and inshore feeding areas. However, the findings of this study indicated that in southern Mozambique, the observed populations show very few similarities with respect to their behaviour, habitat selection or residency patterns; an indication that they have different ecological niches or microhabitats. *Manta birostris* variant A showed more residency to coastal areas with regular sightings of individuals at inshore cleaning stations and feeding grounds, while *M. birostris* var. B was both sighted with less frequently at these inshore habitats and was less philopatric to the area, with individuals not commonly re-sighted. Their respective reproductive behaviours also seemed to differ along this coastline. *Manta birostris* variant A was observed to use the immediate area as both a mating and birthing ground, while *M. birostris* var. B was never seen engaging in reproductive behaviours and only two pregnant females were seen during a five-year study. These apparent differences in their reproductive ecology may help to explain how these two putative species of *Manta* remain reproductively isolated from one another despite their shared habitat and close proximity to one another along this coastline. Initial observations strongly suggest that while these two putative species should be defined as having 'syntopic' distributions, further investigation into their ability to co-exist, through the partitioning microhabitats and potentially mating grounds is needed.

Differences in the movement patterns and behavioural ecology of these two putative species and their presumed use of different microhabitats highlights the potential need for different conservation strategies. Isolating and ultimately understanding these

interspecific differences as well as species-specific vulnerabilities is crucial to their respective management and protection in Mozambique and other regions where they have sympatric distributions. Currently very limited data have been collected on *M. birostris* var. B in any part of its distribution, with most past and current research efforts instead concentrating on the more accessible *M. birostris* var. A. It is strongly suggested that future research efforts focus on this more elusive species, as the current paucity of data hinders the correct designation its conservation status, the ability to manage or restrict fisheries which occur mainly in Central America and South East Asia, and the identification and protection of critical habitats, migratory routes or viable aggregations for ecotourism.

Chapter 10
General discussion

OVERVIEW OF GENERAL FINDINGS

This study has provided fundamental data on the biology and ecology of manta rays. Potentially the most important contribution is the recognition and description of two putative species of *Manta*, a genus that was previously considered to be monospecific (Chapter 4). As both putative species occur in sympatry in southern Mozambique, populations of both *Manta birostris* variant A and variant B were monitored intensively to investigate previously unknown aspects of their reproductive ecology, habitat use and natural predation (Chapters 6, 7 and 8). The resulting information has dramatically enhanced the understanding of the reproductive ecology of *Manta birostris* variant A, providing estimates for important reproductive parameters such as size at maturity, gestation period, fecundity, and reproductive periodicity (Chapter 6). The study also for the first time generated estimates of the effective super population size at a manta ray aggregation site, providing valuable regional data for both putative species (Chapters 5 and 9). As a way forward, research output in the form of standardised morphological and meristic measurements (Chapters 3 and 4), standardised photographic mark-resight techniques (Chapter 5), and a field key to the differentiation of the two putative species (Chapter 4), will aid in future research efforts and facilitate future comparisons of regional data.

TAXONOMIC UNCERTAINTY

The early recognition of potential systematic complications of the genus *Manta* meant that while the central research objectives of this thesis focused on populations of manta rays in southern Mozambique, further comparative investigations were necessary in other areas to explore regional variation. A fishery on the island of Lombok, Indonesia, a bather protection netting program in South Africa and remote islands off the coast of Mexico provided opportunities to inspect and make detailed comparisons of the morphological and beahvioural differences between the two putative species. While target fisheries for both species still exist, limited access to specimens will likely continue to restrict the collection of robust fisheries data. To that end, it is important to be able to distinguish between these closely related species in the field so that data collection and comparative studies can be undertaken on wild populations. Lack of specimens and field observations were certainly responsible for

previously overlooked traits that may have distinguished these two species at an earlier date. Moreover, this research demonstrates that a full revision of the genus is needed with systematic examinations of specimens throughout *Manta*'s range to ensure that other cryptic species do not exist. A global genetic investigation of *Manta* is currently underway (Kashawagi, Marshall, Bennett and Ovendon, in progress) which incorporates not only genetic material from key aggregation sites around the world but photographic records of all individuals sampled, a key component missing from an investigation by Clarke (2002). Preliminary findings of this research have confirmed the divergence between the two putative species described in this thesis.

REPRODUCTIVE PARAMETERS

While manta rays were previously known to be among the least fecund elasmobranch species (Dodd 1983; Musick and Ellis 2005; Walker 2005; White et al. 2006), the limited availability of fisheries data and lack of field studies have prevented a more complete understanding of their reproductive parameters. Extracting this information from elusive and highly mobile individuals in wild populations presents various challenges, and at best may provide only estimations of these parameters. Despite these obstacles, this study contributed key insights into the reproductive biology and behaviour of *Manta birostris* variant A. While mobulid rays are most commonly uniparous (Notarbartolo-di-Sciara 1987b), opportunistic dissections and observations in southern Mozambique have established that *Manta birostris* variant A can carry up to two pups on occasion. This study also confirmed through photographic sight-resight techniques that the gestation period for this putative species is approximately one year, a duration that had only been reported once before in a captive environment. Variation in the reproductive periodicity of this species was also demonstrated. This study confirmed the ability of female rays to produce offspring annually but it also highlighted that pregnancy in consecutive years is uncommon, with many females taking a one to two year resting period between pregnancies. This discovery confirms that fecundity is extremely low in *Manta birostris* variant A and strongly suggests that this species may have a low rate of population growth and hence may be highly susceptible to fishing pressures. While many elasmobranches show bi or triennial reproductive cycles, most species compensate with large litter sizes (Dodd 1983;

Musick and Ellis 2005; Walker 2005). *Manta birostris* variant A is an exception producing most commonly a single offspring per litter. Slow parturition cycles are also often offset by either rapid growth during early stages of life or the early onset of maturity. Unfortunately attempts to age either putative species were thwarted at an early stage by the abnormally low levels of calcification in the vertebral column and greatly reduced centra in individuals of all sizes (Marshall, unpublished data). Age and growth parameters provide crucial information on a species and have important implications in fisheries management (Cailliet and Goldman 2004). For manta rays, which are assumed to be long-lived but for which longevity is unknown, the formulation of age and growth techniques should be rigorously explored.

HABITAT USE AND POPULATION SIZE

The discovery that the two putative species of *Manta* were not only sympatrically distributed along the coastline in southern Mozambique but were utilising similar habitats raised many questions concerning their ability to co-exist. While the limited time frame of the study prevented the examination of long term trends in occupancy and habitat use, the five-year study did demonstrate that the two putative species exhibit marked differences in their respective occupancy of and philopatry for inshore cleaning habitats. This information suggests that *Manta birostris* variant A may be more resident to coastal areas while *Manta birostris* variant B may be more pelagic and migratory. *Manta birostris* variant A and B did show similarities in their observed population compositions, with both species exhibiting extreme biases in their sex ratios, with this study reporting the strongest female biases for any observed population of *Manta*. There were also very few juvenile rays observed of either putative species. These data suggest not only that the observed populations were sexually segregated in favor of females, but additionally suggested a potential ontogenetic shift in habitat use. Future research efforts in southern Mozambique should focus on locating the habitat used by these smaller size classes of manta rays, as nursery ground habitat may be a key area to protect in larger management schemes.

This study provided estimates of the effective super population sizes of the two putative species at the aggregation site in southern Mozambique. While *Manta birostris* variant A was seen with greater regularity and more consistency than *Manta*

birostris variant B, the projected population sizes were 890 and 600 total individuals respectively. The photographic sight-resight program used during this study identified slightly over 50% of the estimated super population of *Manta birostris* variant A but only a mere 16.8% of the estimated super population of *Manta birostris* variant B. These population estimates are the first of their kind using data from a monitored manta ray aggregation site and future studies in other regions would benefit from similar treatments. Even basic models that provide population estimates as well as other standard estimates of apparent survival, capture probability, and birth and immigration offer valuable information for management efforts and may highlight the need for more robust conservation objectives.

DISTRIBUTION AND BIOGEOGRAPHY

This thesis contains a preliminary account of the worldwide distribution of the two putative species of *Manta*. Manta rays have been considered for sometime to be a single, panmictic population. As such, descriptions have been superficial, often without details of their colouration patterns or external features, and records of their distributions lumped into a single cosmopolitan range. A continuing investigation of photographic databases worldwide, including images of manta rays from other researchers, dive industries, tourists visiting aggregation sites, in addition to those available from historical accounts has already revealed that these two putative species may often have allopatric distributions, with only a handful of sites thus far identified as areas with sympatric distributions. These preliminary indications make the data collected in southern Mozambique valuable in that they offer insight into the habitat use and behaviour of these two putative species in a region where their distributions overlap. Additional research is still is still required to expand on and strengthen the findings of this study.

CURRENT THREATS AND FUTURE RESEARCH

The vulnerability of chondrichthyan species, particularly in the context of extinction risks, is strongly related to their respective life history traits (Dulvy et al. 2003; Garcia et al. 2008). The factors that most heavily influence these elevated risks are mainly

slower growth, later age at maturity, higher longevity, a matrotrophically viviparous reproductive strategy and to a lesser extent, larger body size (Garcia et al. 2008). The current study contributes information that suggests manta rays have a suite of conservative life history traits that may make them highly susceptible to rapid population declines as a result of anthropogenic influences such as elevated natural mortality or the degradation of critical habitats.

Fisheries that target manta rays are still in existence, with the meat, skin and cartilage harvested for consumption, trade, and sale (Alava et al. 2002; Dewar 2002; White et al. 2006). Of the fisheries examined to date, it appears that the more pelagic manta ray, *Manta birostris* variant B, may be both the target of most directed fishing efforts and the victim of unintended bycatch (Garcia and Hall 1997; Marshall, unpublished data). For example, in the fishery in Lombok, Indonesia where approximately 250 manta rays are estimated to be killed per annum (White et al. 2006), 100% of the manta rays landings are *M. birostris* var. B. While continued assessment is needed to determine the level of threats facing both putative species, it is unlikely that populations of either species can sustain long-term or intense fishing pressure.

In the face of these anthropogenic threats, the severely limited body of information on these putative species continues to hamper the accurate assessment of their respective conservation status. Insufficient data may also jeopardise future efforts to upgrade *Manta* to a listing on CITES (Convention on International Trade in Endangered Species or Wild Fauna and Flora). To date very limited data has been collected on *Manta birostris* variant B in any part of its distribution, with most past and current research efforts instead concentrating on the more accessible *Manta birostris* variant A. It is strongly suggested that future research efforts focus on this more elusive and potentially migratory species, as comprehensive management strategies may require collaborations between various countries.

CONCLUSIONS

The results of this thesis have already been applied to tourism and coastal management strategies in Mozambique such as the formation of a code of conduct for manta rays and a draft proposal for the protection of *Manta* in Mozambican waters.

Additionally, the results, particularly those related to the reproductive ecology and distribution of these two putative species, will strengthen the IUCN red list assessment for this genus and allow research data to be spit in order to treat the two species individually. It is hoped that the results of this thesis, along with the presentation of necessary long-term objectives and the development of standardised methodologies will encourage new studies and enhance collaborative research efforts on the members of this genus in the future.

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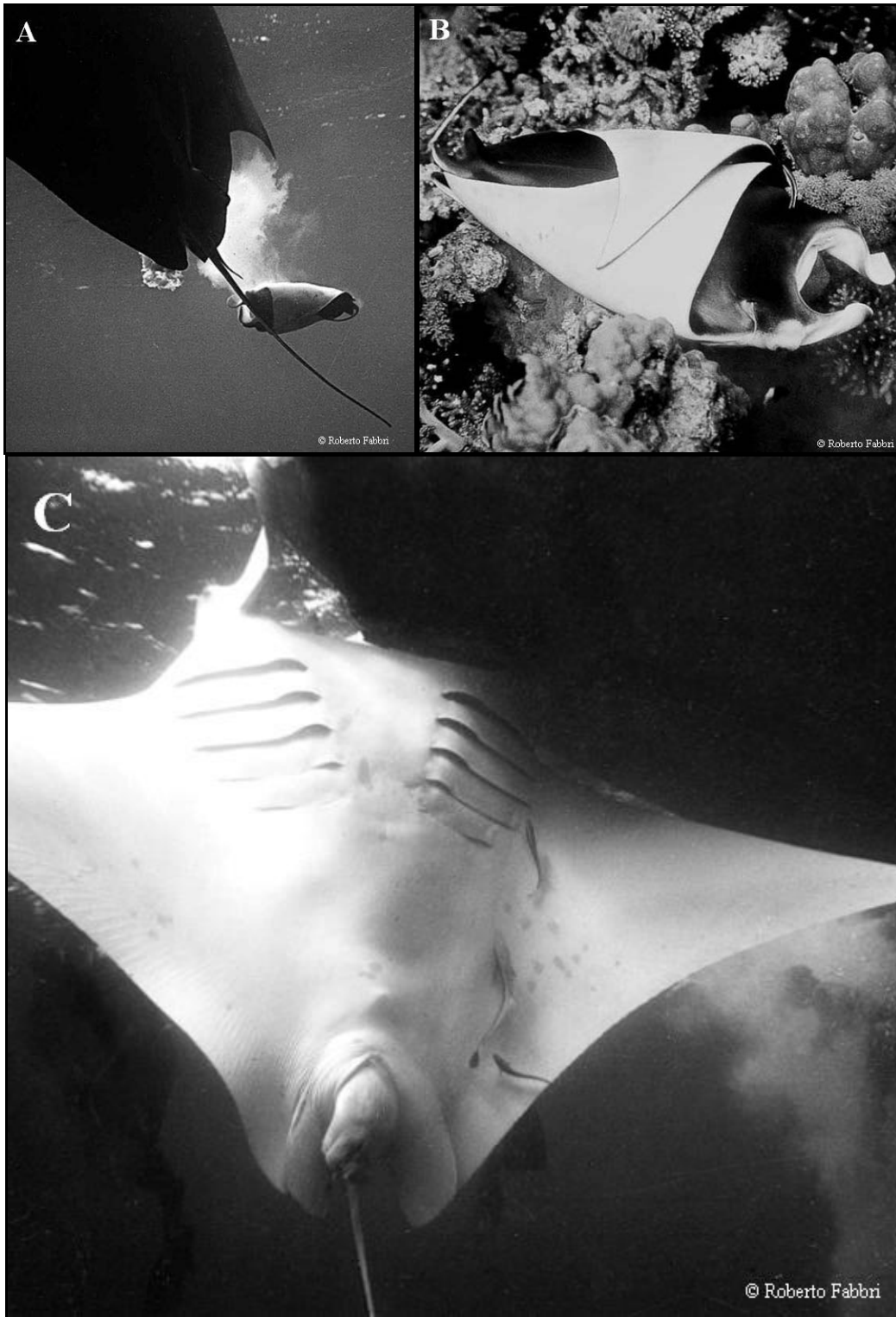
Appendix 1 Female (top) and male (bottom) *Manta birostris* var. B mating mid-water off Ogasawra Island, Japan.

Appendix 2 *Manta birostris* var. A caught in a mooring line in Hawaii being attacked by a tiger shark, *Galeocerdo cuvier*.
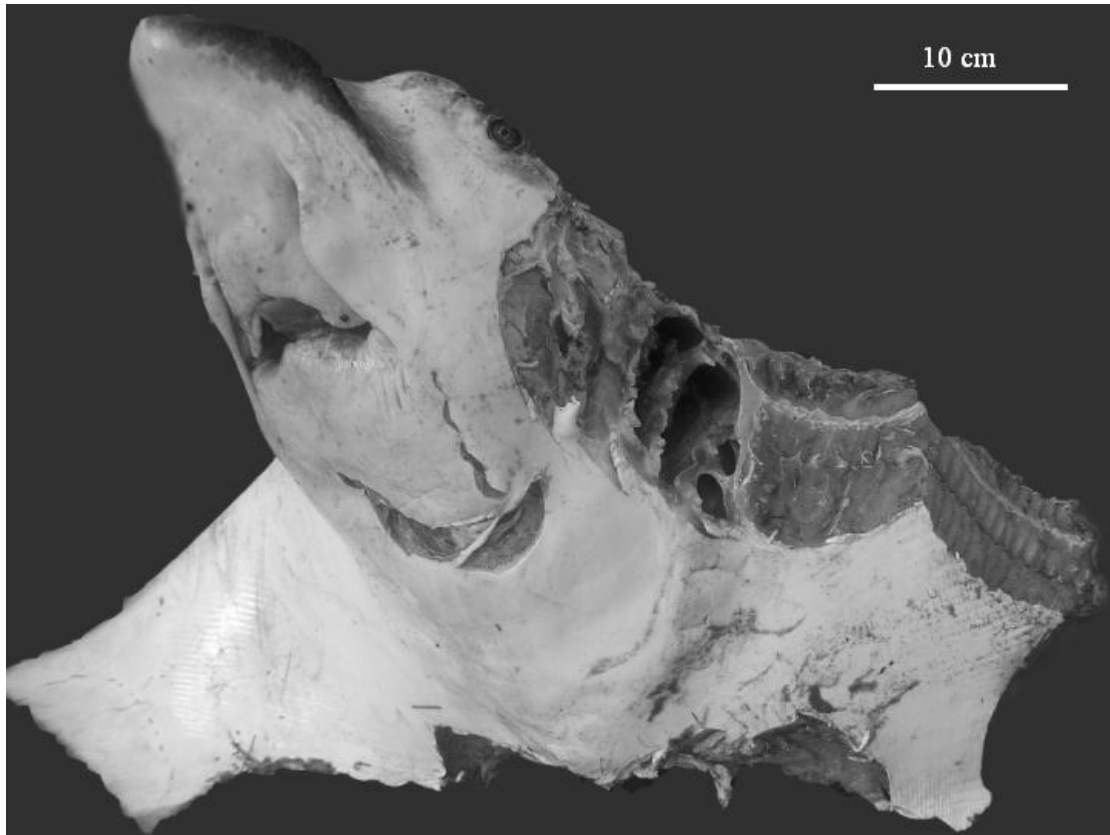
Appendix 1 An eagle ray, *Aetobatus narinri*, off southern Mozambique an obvious victim of a shark attack.

Appendix 1 Female *Manta birostris* var. A aborting premature foetus after being speared by fishermen off the coast of Sudan.

Other Relevant Publications:

Pierce, S.J., White, W.T. and Marshall, A.D (Accepted) New record of the smalleye stingray, *Dasyatis microps* (Myliobatiformes: Dasyatidae), from the western Indian Ocean.

Pierce, S.J. and Marshall, A.D (Submitted) Identifying cryptic threats to whale sharks through the analysis of body scars.

Speed, C.W., Meekan M.G., Rowat D., Pierce, S.J., Marshall, A.D. and Bradshaw, C, J. A. (Accepted Journal of Fish Biology) Scarring patterns and relative mortality rates of Indian Ocean whale sharks.