

Conservation and Population Ecology of Manta Rays in the Maldives

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Abstract

This multi-decade study on an isolated and un-fished population of manta rays (*Manta alfredi* and *M. birostris*) in the Maldives used individual-based photo-ID records and behavioural observations to investigate the world's largest known population of *M. alfredi* and a previously unstudied population of *M. birostris*. This research advances knowledge of key life history traits, reproductive strategies, population demographics and habitat use of *M. alfredi*, and elucidates the feeding and mating behaviour of both manta species.

M. alfredi reproductive activity was found to vary considerably among years and appeared related to variability in abundance of the manta's planktonic food, which in turn may be linked to large-scale weather patterns such as the Indian Ocean Dipole and El Niño-Southern Oscillation. Key to helping improve conservation efforts of *M. alfredi* was my finding that age at maturity for both females and males, estimated at 15 and 11 years respectively, appears up to 7–8 years higher respectively than previously reported. As the fecundity of this species, estimated at one pup every 7.3 years, also appeared two to more than three times lower than estimates from studies with more limited data, my work now marks *M. alfredi* as one of the world's least fecund vertebrates. With such low fecundity and long maturation, *M. alfredi* are extremely vulnerable to overfishing and therefore needs complete protection from exploitation across its entire global range. With similar life history traits assumed for the lesser known *M. birostris*, adopting the precautionary principle, the same highly protective approach is justified.

Through characterising habitat use of *M. alfredi*, it appears that their use of shallow coral reefs may be a function of behavioural thermoregulation and predator avoidance, with cleaning stations acting as focal gathering points where social behaviour is undertaken, such as courtship and mating. The frequent use of shallow protected lagoons by juvenile *M. alfredi* suggests these sites may act as nursery areas.

The study underscores the importance of long-term research on long-lived species with conservative life history strategies if important ecological and management questions are to be answered.

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I hereby declare that this thesis is my original work and contains no material previously written or published by another person, or submitted in any form for another degree or diploma at any university or institution. I have clearly stated the contribution of others to my thesis above.

I consent to this thesis being made available through the University of York Library under the appropriate UK copyright laws and the University of York regulations.

Chapter 1

General introduction

1.1 Population and conservation ecology

The study of the relationships between living organisms and their environment is essential to understanding the vital connections between plants, animals and the physical and chemical world around them (Molles 2012). Understanding these relationships requires ecological knowledge of how plants and animals reproduce, grow and exploit their habitat, and of which biotic and abiotic factors define a species existence and shape its evolutionary past and future (Dodds & Whiles 2010). Ecological knowledge helps us predict how species are likely to be affected by changing climatic and environmental conditions, and determine the ecological ramifications of increasing anthropogenic pressures such as exploitation (Courchamp et al. 2006; Estes et al. 2011; Fenberg & Roy 2008; Lande 1998; Walther et al. 2002).

The oceans are vast, covering 71% of the Earth's surface they generate about 50% of global primary production and support a large proportion of biodiversity on the planet (Field et al. 1998; Pimm et al. 2014). However, because of their size and inaccessibility, scientific exploration in the Ocean has lagged behind the study of terrestrial systems (Segar & Segar 2007). In the last few decades however, advances in technology have led to a proliferation of new scientific studies and insight into the marine world, often revealing how human impacts are increasingly threatening life in the ocean (e.g., reviewed by Roberts 2013). Largely free for all to use and benefit from, an open access resource for anyone with the means, thousands of years of exploitation of our seas has resulted in a tragedy of the commons (Costanza 1999; Hardin 1998; Mansfield 2004; Roberts 2008). Threatened by overfishing, ocean acidification, climate change, habitat destruction, pollution, and invasive species (Dulvy et al. 2003; Hoegh-Guldberg et al. 2007; Hoegh-Guldberg & Bruno 2010; Jambeck et al. 2015; Pauly et al. 2002; Roberts 2013; Sorte et al. 2010; Worm et al. 2006), biodiversity in the World's oceans is in peril and ecosystems are being disrupted (Dulvy et al. 2004; Jackson et al. 2001; Myers & Worm 2005; Myers et al. 2007; Sala & Knowlton 2006; Worm et al. 2006).

In the light of increasing anthropogenic exploitation of marine megafauna populations (e.g., Ferretti et al. 2010; Heithaus et al. 2008; Lewison et al. 2014; Lewison et al. 2004; Worm et al. 2013), conservation and population ecologists are increasingly attempting to define the life history characteristics and traits of their subjects in order to aid conservation (Cortés 1998; Young et al. 2006). Characterising the reproductive behaviours, fecundity, age at maturity and survivorship of a species, and understanding how these traits have been shaped

by natural selection, is fundamental to conservation ecology (Heithaus et al. 2008). Knowledge of a species' life history characteristics and demographics enables the development of population viability analysis, from which it is possible to predict a species' vulnerability and the likely impacts of anthropogenic threats to its survival.

Unlike many teleosts which can be quick to recover from instances of overfishing, elasmobranchs (sharks and rays) are often unable to recover quickly from sustained fishing pressure due to their low productivity (Dulvy et al. 2014a; Dulvy et al. 2008), and are consequently at high intrinsic vulnerability to over-exploitation (Cortés 2000; Dulvy et al. 2014a; Dulvy et al. 2008). However, compared to the other major megafauna groups in our oceans, only in the last few decades have ecological studies on these animals proliferated. Aided by a growing interest in sharks and rays by the general public, and spurred on in the light of growing commercial exploitation, new technologies, such as satellite and acoustic telemetry, advances in genetic techniques, and in-water video and photo-ID techniques, to name a few, have driven research on this group of fishes (Couturier et al. 2012). This thesis uses some of these research tools to investigate one of the most vulnerable and poorly studied groups of large vertebrates, the manta rays (Couturier et al. 2012; Dulvy et al. 2014b).

1.2 Taxonomy and distribution

Currently the ~1,160 recognised species of extant elasmobranchs are divided roughly in half, with ~510 species of sharks and ~650 species of rays (Aschliman et al. 2012; Naylor et al. 2012). This predominantly marine group is one of the oldest vertebrate lineages, going back over 400 million years (Compagno 1990). The first rays appeared in the oceans ~150 mya, radiating from a common ancestor with the sharks. Their flattened body shape was originally an adaptation to a bottom dwelling existence, although many of the Myliobatiformes, including the manta rays, have now reverted back to a more pelagic mode of life (Aschliman et al. 2012; Carrier et al. 2004).

Manta rays are amongst the most conspicuous and charismatic of marine creatures. These zooplanktivorous pelagic elasmobranchs belong to the Mobulidae family which currently comprises nine species in the genus *Mobula* (Notarbartolo-di-Sciara 1987; Rafinesque 1810) and two in the genus *Manta* (Bancroft 1829). Mobulids first appear in the fossil record around 28 mya (Aschliman et al. 2012; Cicimurri & Knight 2009; Poortvliet et al. 2015). Mobulid rays are differentiated from other rays by their highly specialized filter feeding behaviour, whereby via enlarged mouths and modified branchial appendages, commonly referred to as

gill plates, they strain zooplankton, fish spawn and small fishes from the sea (Compagno & Last 1999; Couturier et al. 2012; Hartup et al. 2013; Notarbartolo-di-Sciara 1987).

The genus *Manta* was re-classified in 2009 (Marshall et al. 2009) when the monospecific genus was split into the two species of manta currently recognized: the reef manta (*Manta alfredi*) (Krefft 1868) and the oceanic manta (*Manta birostris*) (Walbaum 1792). A third putative species, the Caribbean manta (*Manta c.f. birostris*), has also been proposed (Marshall et al. 2009). *M. alfredi* are widely distributed throughout tropical and sub-tropical waters of the Indo-West Pacific within 32° of latitude north and south (Couturier et al. 2012; Kashiwagi et al. 2011) (Appendix I), while *M. birostris* is also distributed throughout the tropics, it is also found in sub-tropical and temperate waters within 41° of latitude north and south (Kashiwagi et al. 2011) (Appendix I).

1.3 Conservation challenge

Manta rays are slow-growing, large-bodied animals which have among the lowest fecundity of all elasmobranchs (Dulvy et al. 2014b). Their small, highly fragmented populations are sparsely distributed across the tropics and their global population size is uncertain. In the subpopulations, identified numbers are typically estimated in the 100s, although some regions support several thousands (Couturier et al. 2014; Deakos et al. 2011; Kashiwagi 2014; Kitchen-Wheeler et al. 2011; Marshall et al. 2011a). Collectively, low fecundity, small size of subpopulations, migratory and aggregating behaviour, make manta rays particularly vulnerable to over-exploitation in fisheries and extremely slow to recover from any form of depletion (Dulvy et al. 2014b).

The greatest threat to *Manta* spp. is excessive targeted and incidental take in fisheries (Croll et al. 2015; Dewar 2002; Lewis et al. 2015; White et al. 2006), increasingly driven by the international trade in gill plates for use in an Asian health tonic purported to treat a wide variety of conditions (O'Malley et al. 2016). Of particular concern is the exploitation of this species from within critical habitats, where numerous individuals can be targeted with relatively high catch-per-unit-effort (Couturier et al. 2012). Other anthropogenic threats include incidental bycatch, boat strikes, and entanglement in marine debris, mooring and fishing lines (Couturier et al. 2012; Deakos et al. 2011; Marshall & Bennett 2010). Less directly, climate change, pollution, habitat degradation and irresponsible tourism practices are also likely to threaten manta rays throughout their range (Deakos et al. 2011; Marshall & Bennett 2010).

Although tourism is likely to be negatively impacting upon manta rays, in terms of economic appeal, their value in this industry is high, driving protective legislation for these species nationally and internationally (Lawson et al. 2016). The global revenue generated from the manta ray tourism industry is estimated to exceed USD 140 million annually (O'Malley et al. 2013). In the Republic of Maldives alone, direct revenue from manta dive and snorkel excursions was estimated to generate over USD 8.1 million per year during 2006 – 2008 (Anderson et al. 2011a).

For such intrinsically vulnerable species, even small negative pressures exerted upon a population are likely to have severe consequences for the population's survival. As a result of this, and in response to the growing threat of the gill plate trade, both species of manta rays were listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) in March 2013. Both *Manta* spp. are also listed on the Convention on Migratory Species of Wild Animals (CMS) and national protective legislation exists for both species in 16 range states (Croll et al. 2015; Lawson et al. 2016).

Despite growing protective measures, manta rays remain extremely vulnerable to exploitation. It is therefore crucial that areas of critical habitat are identified and protected; areas such as mating, feeding, birthing and nursery grounds, and those key migratory corridors that mantas regularly travel along. Continued access to these sites is essential, as is minimisation of disturbances affecting fitness and survival (Block et al. 2011; Heithaus 2007; Heupel et al. 2007; Hyrenbach et al. 2000; Knip et al. 2010; Norton et al. 2012). However, without accurate ecological knowledge of manta ray life history characteristics, the true impact of direct and indirect anthropogenic pressures on these species will be difficult to quantify and effectively mitigate (Powles et al. 2000).

Due the long-lived nature of these species, it is imperative for their conservation that long-term datasets are established and maintained to develop reliable life history information (Clutton-Brock & Sheldon 2010; Willis et al. 2007). However, due to the challenges of undertaking fieldwork in the marine world, both financially and logistically, multi-decadal studies are hard to realise (Strayer et al. 1986). However, long-term observational studies that follow individual animals over the course of a generation or lifespan, recording their life history traits and behavioural activity, are not only able to more accurately answer pressing conservation questions, but also add valuable insight into the selective pressures which shape reproductive strategies, and possible social structures and dominance hierarchies within the population (Jacoby et al. 2012; Jacoby et al. 2014; Mourier et al. 2012). More

widely, this knowledge can be applied to the other elasmobranch species, helping to shape the direction of future studies.

1.4 Thesis synopsis

This thesis reports the findings of an eleven year research project, using individual-based longitudinal observation records from a large population of *M. alfredi* in the Maldives. In addition, I also report findings from observations of the rarer of the two species, *M. birostris*, enlarging knowledge of this more elusive creature. Populations of these species have never supported a fishery in the Maldives and they are currently protected from fishing, offering the opportunity to examine the species under near natural conditions (Anderson et al. 2011b; EPA 2014). A lack of long-term dedicated studies on manta rays has meant little empirical data exists on their life history traits and demography. The population of *M. alfredi* in the Maldives is unique in providing year-round access to thousands of individual mantas across a large geographical range which encompasses hundreds of feeding and cleaning aggregation sites of both adults and juveniles.

The main goal of this thesis research is to describe habitat use, population demographics, life history traits, and reproductive and feeding strategies of *M. alfredi* using photo-ID techniques and behavioural observations from a long-term empirical study in the Republic of the Maldives. A secondary goal of the study is to describe the feeding and reproductive behaviour of *M. birostris*.

In Chapter 2 I characterise habitat use, population demographics, age and size at maturity, longevity, and young of the year abundance. In this chapter I report on efforts to build a photo-ID database of the *M. alfredi* population in the Maldives to analyse and document these traits through repeat sightings of individuals throughout the eleven years; recording newborn, size, maturity status and sex for each individual.

Manta ray courtship and mating behaviour is rarely observed and although the major stages of mating have already been described from a handful of observations (Marshall & Bennett 2010a; Yano et al. 1999), further investigation of the detail is still required, especially in the courtship stages. The aim of Chapter 3 is to elucidate the entire courtship and mating behaviour of both manta species using behavioural observations, video and photographic records, supplemented with data from other sites globally, to enhance the knowledge of these events. Data from Chapter 3 provided structure for investigation of the reproductive strategies of *M. alfredi*, which is one of the primary objective of Chapter 4.

Current estimates of manta ray fecundity suggest a biennial reproductive strategy is the norm (Deakos 2011; Kashiwagi 2014; Marshall & Bennett 2010a). However, this conclusion is based on a few studies of a relatively small number of individuals across a relatively short time in the majority of instances. Given the life history and species characteristics of manta rays, even small increased mortality rates or reduced fecundity are likely to have significant consequences for a population's survival, particularly in light of exploitation. Therefore, in Chapter 4 I determine the fecundity of *M. alfredi* in the Maldives by using repeat photo-ID sightings of a core group of 150 mature females across a decade to accurately document pregnancies and reproductive activity. Throughout the entire adult population recorded in the study, annual and inter-annual variations in fecundity and reproductive activity recorded are also examined in relation to variability in ocean productivity.

With nearly all field research publications on manta rays to date focused solely or predominantly on manta ray cleaning station aggregation sites, the feeding strategies employed by manta rays have yet to be defined. The main objective of Chapter 5 is therefore to elucidate all of the different feeding strategies exhibited by both manta species by creating an ethogram based upon behavioural observations and photographic records. To seek correlations which might further explain the different feeding strategies employed, variations in the feeding behaviours between sexes, maturity status, and with changing prey density, are also investigated at key *M. alfredi* feeding aggregation sites. Manta breaches (i.e. leaps) during feeding events are also analysed to seek explanations for this behaviour.

Chapter 2

Population demographics, habitat use
and life history characteristics of the
reef manta ray (*Manta alfredi*) in the
Maldives

2.1. Abstract

Manta rays (*Manta alfredi*) are known to have low reproductive rates, small highly fragmented populations, and aggregating behaviour which make them particularly vulnerable to fishery over-exploitation. Many areas of their life history remain poorly understood, without which effective management and conservation plans cannot be devised. From January 2005 through to December 2015 and sporadically during the two previous decades, using photo-ID techniques and behavioural observations, over 8,000 surveys for manta rays were undertaken in the Republic of Maldives. A total of 38,804 photo-ID sightings of 4,000 individuals were recorded in 21 of the Maldives' 26 atolls at 239 different sites. The overall population sample consisted of 1,945 males, 2,010 females, and 45 for which sex could not be determined. Sex ratio, maturity status and size class varied among site types and location. The smallest individual seen had an estimated disc width of 140 cm, while the average for young of the year was 160 cm. Males reached maturity at a disc width of ~270 – 280 cm and females at ~320 – 330 cm, with maximum observed sizes of approximately 310 cm for males and 360 cm for females. Age at maturity, using photo-ID recapture sightings, growth rates and external indicators of maturation, was estimated to be 11 and 15 years for males and females respectively, increasing the generation span estimate from 11 of 16 years and the vulnerability estimation for this species. Three individuals were sighted over periods exceeding 20 years, the longest at 24. Ratios between age at maturity and longevity, and disc width relationships, may characterise the life history strategy of *M. alfredi* as large species which grow relatively quickly to full size, enabling long reproductive lifespans. These findings validate the importance of undertaking long-term studies on species with conservative life history strategies if important ecological questions are to be answered.

Keywords: age and size at maturity, longevity, young of the year, photo-ID, cleaning stations

2.2. Introduction

Zooplanktivorous mantas are the largest of all rays. These pelagic elasmobranchs belong to the Mobulidae family which currently comprises nine species in the genus *Mobula* (Notarbartolo-di-Sciara 1987; Rafinesque 1810) and two in the genus *Manta* (Bancroft 1829). Reef manta rays (*Manta alfredi*) (Krefft 1868; Marshall et al. 2009) are widely distributed throughout the tropical and sub-tropical Pacific and Indian Oceans, although populations are highly fragmented (Couturier et al. 2012; Kashiwagi et al. 2011) by resource and habitat needs (Anderson et al. 2011b; Braun et al. 2015; McCauley et al. 2014). *M. alfredi* occur in

shallow waters along the coastal reefs of continents and around remote oceanic islands and archipelagos (Kashiwagi et al. 2011; Marshall et al. 2011b), venturing offshore and into the mesopelagic zone (Braun et al. 2014; Braun et al. 2015; Jaime et al. 2014).

Long-term sighting records of *M. alfredi* at established aggregation sites suggest this species is highly philopatric, often undertaking short seasonal migrations between favoured sites (Braun et al. 2015; Couturier et al. 2011; Couturier et al. 2014; Deakos et al. 2011; Dewar et al. 2008; McCauley et al. 2014). Site fidelity and migrations have been linked to areas of high primary productivity and prey density (Anderson et al. 2011b; Armstrong et al. 2016; Jaime et al. 2014), and may also vary by sex and age-class (Couturier et al. 2011). The island nation of the Maldives is strongly influenced by the South Asian monsoon (Gischler et al. 2014), which drives currents that enhance primary productivity on the leeward side of the atolls through deep-water upwellings (Doty & Oguri 1956; Sasamal 2006), bringing nutrient rich water into the euphotic zone (Sasamal 2007). These productive seas support a large year round population of *M. alfredi* which migrate across the archipelago with the changing monsoons to exploit the richest zooplankton feeding grounds (Anderson et al. 2011b; Kitchen-Wheeler et al. 2011). Within the Maldives *M. alfredi* use a variety of shallow reef habitat, favouring productive channels and lagoons which concentrate their prey, they often frequent cleaning stations (Côté 2000; Feder 1966; Losey Jr 1972; O'Shea et al. 2010) situated nearby (Kitchen-Wheeler 2010).

Manta rays are ovoviviparous matrotrophs (Dulvy & Reynolds 1997; Wourms 1977), they give birth to a single large pup (Beebe & Tee-Van 1941b; Bigelow & Schroeder 1953; Coles 1916; Compagno & Last 1999) and occasionally twins (Marshall 2009). In the wild, newborn pups are approximately 130 – 150 cm in disc width (Kashiwagi 2014; Marshall & Bennett 2010a), although larger pups of 180 – 190 cm disc width have been born in captivity (Okinawa Churaumi Aquarium 2010). During copulation the male bites down hard on the end of one of the female's wing-tips, inserting one of his paired claspers into her cloaca (Marshall & Bennett 2010a; Yano et al. 1999; Chapter 3). These bites often result in permanent mating scars to the female's left pectoral fin tip (Marshall & Bennett 2010a).

Both manta ray species are sexually dimorphic, such that the sexes can be distinguished by the greater disc widths' in females and the presence of external sexual organs called claspers in males (Marshall & Bennett 2010a). In a study by Deakos (2010) on an population of *M. alfredi* which visited a cleaning station in Maui, Hawaii, the disc widths of accurately measured adult females were as much as 20% larger than in adult males. In the

aforementioned Hawaii study the disc widths of adult females were 324 – 364 cm and 260 – 303 cm for adult males, although size estimates in Mozambique suggest disc widths of more than 500 cm for females (Marshall et al. 2009). For *M. alfredi* in Hawaii, Deakos (2010) gave a conservative estimate for size at maturity in females of 337 cm disc width, where the latter was based on evidence of pregnancy and mating scars. In the same study, using clasper length as a basis for maturity in males, the size at which 50% of the population matured was between a disc width of 270 – 280 cm. In adult male *M. alfredi*, the externally visible presence of paired clasper glands can also be used to determine maturity (Marshall & Bennett 2010a). Age at maturity for both sexes is poorly documented. However, based on photo-ID resightings and clasper extension in four individuals, estimates for male *M. alfredi* from Hawaii (Clark 2010) (N = 2), and Japan (Kashiwagi 2014) (N = 2), suggest maturity is attained at 3 – 6 and 4 – 9 years respectively. The Japan study's estimate of age at maturity for females were also quite variable, ranging from 8 – 15 years, based on a sample of nine individuals.

Reliable methods of ageing individuals are necessary for effective management of any exploited elasmobranch (Cailliet et al. 2006). With no other ageing method for *M. alfredi* available, photographic records show they can live for at least 30 years (Clark 2010; Couturier et al. 2014; Homma et al. 1999; Kashiwagi et al. 2010) and are estimated to reach about 40 years, although maximum longevity remains unknown (Couturier et al. 2012). Natural mortality in *M. alfredi* is thought to be low, and current estimates of life history parameters put generation span [female age at maturity (estimated as 10 years), plus gestation time] at 11 years (Dulvy et al. 2014b).

M. alfredi appear to have among the lowest fecundity of all elasmobranchs (Dulvy et al. 2014b) and their small, highly fragmented populations make the global population size hard to estimate. In known subpopulations, numbers are typically estimated in the 100s, although they can reach up to several thousand (Couturier et al. 2014; Deakos et al. 2011; Kitchen-Wheeler et al. 2011; Marshall et al. 2011a). The low rate of reproduction, long maturation time, small size of subpopulations, and aggregating behaviour of mantas make them particularly vulnerable to fishery over-exploitation, from which they would be extremely slow to recover (Dulvy et al. 2014b). The greatest threat to *Manta* spp. are targeted fisheries, increasingly driven by the international trade in their gill plates which are used in Asian medicine for a health tonic purported to treat a wide variety of conditions, but for which evidence is unfounded (Lewis et al. 2015; O'Malley et al. 2016). In regions where targeted fisheries occur, the rate of manta ray population reductions appears to be high, with recent studies demonstrating declines of up to 86% (Lewis et al. 2015; Rohner et al. 2013).

Thousands of mantas and mobulas also get caught annually as bycatch in other fisheries, although accurate data is lacking (Croll et al. 2015).

The global revenue generated from the trade in manta ray gill plates is estimated at USD 5 million annually (S. Heinrichs, pers. comm.; O'Malley et al. 2016). However, in terms of economic appeal, the value of these species to the tourism industry is much higher. Indeed, the global revenue generated from the manta ray tourism industry is estimated to exceed USD 140 million annually (O'Malley et al. 2013). In the Republic of Maldives alone, direct revenue from manta dive and snorkel excursions was estimated to generate over USD 8.1 million per year during 2006 – 2008 (Anderson et al. 2011a).

M. alfredi's vulnerable life history characteristics mean that even small increases in mortality rates or decreases in fecundity could affect population survival. Consequently, and in response to increased demand for gill plates, both *Manta* species (Walbaum 1792) were listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) in March 2013. In November 2014, *M. alfredi* joined *M. birostris*, being listed on Appendix I and II of the Convention on Migratory Species (CMS). However, despite recent protective measures, *M. alfredi* still remain vulnerable to exploitation with many areas of their life history poorly understood (Marshall et al. 2011b). Detailed information on their age, growth, and size and age at maturity are essential to assess fishery impacts and develop sustainable management (Cortés 1998; Cortés 2002). Inaccurate estimates of life history parameters often results in serious errors in the understanding and management of fish populations (Beamish & McFarlane 1983; Fisher et al. 2013).

The aim of this eleven year study was to characterise habitat use, population demographics, age and size at maturity, longevity, and young of the year (YoY) abundance for *M. alfredi* in the Maldives. Knowledge of these life history parameters is critical to effective management of this species globally. *M. alfredi* has never supported a fishery in the Maldives, and in 2014 the species attained complete protection nationally from fishing (Anderson et al. 2011b; EPA 2014). Sightings of both adult and juvenile *M. alfredi* can be virtually guaranteed here throughout the year at dozens of cleaning and feeding sites, which worldwide is extremely unusual.

2.3 Methods

2.3.1 Study area

The 26 coral atolls of the Maldives archipelago extend 870 km from 7° north to half a degree south of the equator in the Indian Ocean (Fig. 2.1). The maximum natural height of the country is 2.4 m above sea level and the atolls have steep outer reef slopes that drop to 2 – 3,000 m. In the central Maldives' the atolls form a double chain, and here the seafloor between the atolls reaches a maximum depth of 500 m. The Maldives exclusive economic zone contains 3.1% of the world's coral reefs and encompasses 916,000 km² of ocean. By contrast the total area encompassed by the 26 atolls is 21,600 km², only 300 km² of which is land (Sea Around Us Project 2014).

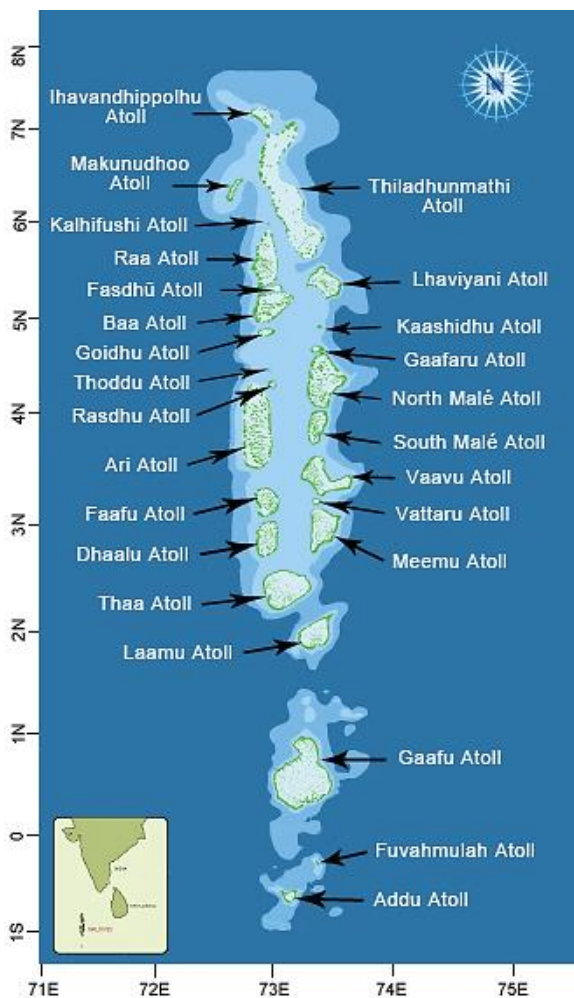


Figure 2.1 Map of the Maldives Archipelago showing the 26 geographical atolls, illustrated in green. Dark blue indicates where the sea floor exceeds 1,000 m; middle toned blue where it is between 500-1,000 m and the lightest blue where 500 m or shallower. The depth of the sea floor inside the lagoon of all the atolls is < 100 m. 1° latitude = 111 km.

2.3.2 Study duration, species and photo-ID

During an eleven year study from January 2005 through to the end of December 2015, over 8,000 surveys (see Section 2.3.4 for sampling protocol) for manta rays were undertaken in

the Maldives at 155 sites where *M. alfredi* are known to form feeding and cleaning aggregations. Opportunistic data were also collected at a further 84 sites and likewise at the known aggregation sites for two decades before the systematic study began (Appendix II). The data collected prior to the start of 2005 was submitted to the Manta Trust’s global database by the general public (www.mantatrust.org/make-a-difference/id-the-manta/).

Both *Manta* species occur in the Maldives, but as *M. birostris* was rarely sighted they are not included. Figure 2.2 illustrates how differences in ventral spot patterns and dorsal shading can be used to help distinguish the two manta ray species, although there is often more pattern variation within species than between. *M. birostris* (Fig. 2.2: A dorsal, B ventral) have few, if any spots, usually clustered on the lower abdominal region ventrally, while *M. alfredi* (Fig. 2.2: C dorsal, D ventral) often have spots spread across the posterior edge of the pectoral fins and between the gill slits. Dorsally, the shoulder markings of *M. birostris* often form a black ‘T’ shape, while in *M. alfredi* they are more ‘Y’ shaped. In both species there are sub-populations where a rarer melanistic morph occurs (Fig. 2.2: 1 and 2 ventral, 1 dorsal), although not in the Maldives for *M. alfredi* population, and rarely so for *M. birostris*. Globally most individuals in both species are “chevron morphs” (Fig. 2.2: 3, 4 and 5 ventral, 2 and 3 dorsal), although paler leucistic morphs (Fig. 2.2: 4 dorsal) are also common in the Maldives *M. alfredi* population (Fig. 2.2: C4).

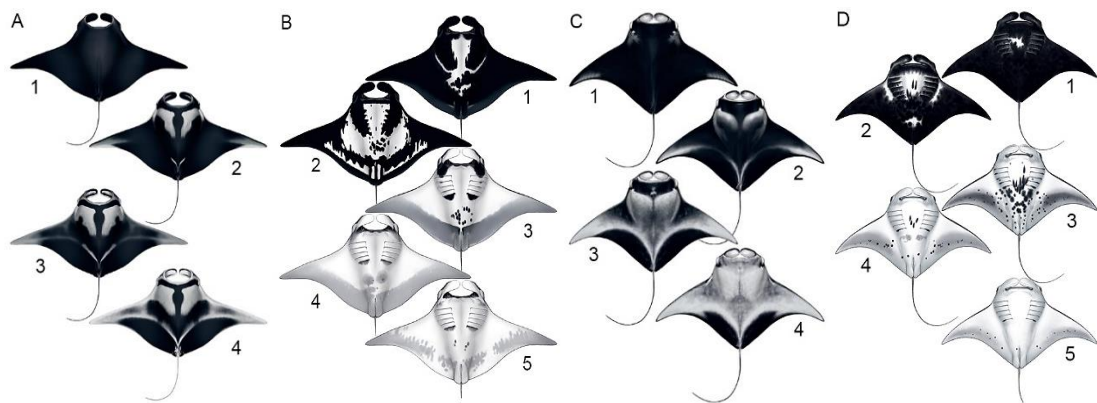


Figure 2.2 Variations in the pattern of ventral black spots (B, D) and dorsal shading (A, C) help distinguish between the two manta ray species. *Manta birostris* (A, B), *Manta alfredi* (C, D). Illustrations © Marc Dando.

Individual manta rays are recognisable by their unique pattern of ventral markings which remain unchanged throughout the animal’s life (Marshall & Pierce 2012), and thus individuals can be repeatedly identified in the wild throughout their lives via photo-ID (Couturier et al.

2014). This study used the black spots between animal's gill slits and upon its lower abdomen to identify and catalogue each individual encountered in surveys (Fig. 2.3).

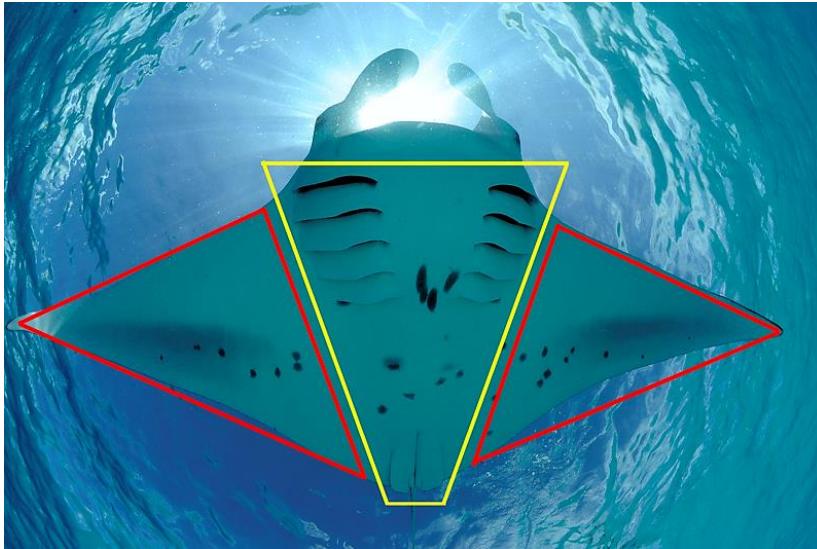


Figure 2.3 Variations in the unique pattern of black spots on the ventral surface of each *Manta alfredi* are used to identify individuals using the primary (yellow) and secondary (red) areas.

Using a combination of manual and automated matching systems, all photo-IDs collected during study surveys, or submitted to the Manta Trust by the general public, were compared against a database of identified individuals by one of the Maldives Manta Trust staff (www.mantatrust.org/about-us/the-manta-team/). To confirm a match, or record a new individual, every photo-ID (>68,000 images of 4,000 individuals) was double checked by the Maldives Manta Trust Project Leader, then triple checked by myself. Each manta ray was assigned a unique ID-code, and every sighting logged and the corresponding photo-ID image archived. Repeat sightings of the same individual on the same day were logged as a single sighting event.

Although every manta ray has a unique pattern of ventral spots and shading, these patterns cannot be categorised accurately enough using binary vectors, although attempts have been made (Kitchen-Wheeler 2010), and the current automated manta recognition technologies are still not good enough to be solely relied upon (Town et al. 2013). Therefore, during this study a linearly continuous display of the ventral spot patterning of every individual manta ray in the database was developed to manually compare each photo-ID image against for a match (Fig. 2.4). This continuous cross-checking process reduced the chances of mis-identifications occurring (i.e. duplicates), and increased the likelihood of them being discovered when they did occur. During this study, 60 duplicated individuals (1.5% of the total individuals identified) were discovered through this cross-checking process, and the database corrected.

The automated recognition technology software used to compliment the manual search process is called *IDtheManta*, developed by the University of Bristol in collaboration with the Manta Trust (Hughes & Burghardt 2015). *IDtheManta* addresses the task of automatic visual identification of individual manta rays from images of their ventral surface. The approach appreciates these surfaces as highly flexible, partially occluded objects with sparse, individually characteristic spot patterns. Building on the work of Town et al. (2013), the approach enables accurate, scalable and robust recovery of animal identities from images captured in challenging underwater environments.

In this study, 75% of the identification pictures used were taken with either a Nikon D700 SLR with a 16mm wide angle lens, or a Sea & Sea DX1G compact underwater camera with wide angle converter lens. The rest were taken on a variety of other compact underwater camera and video equipment.

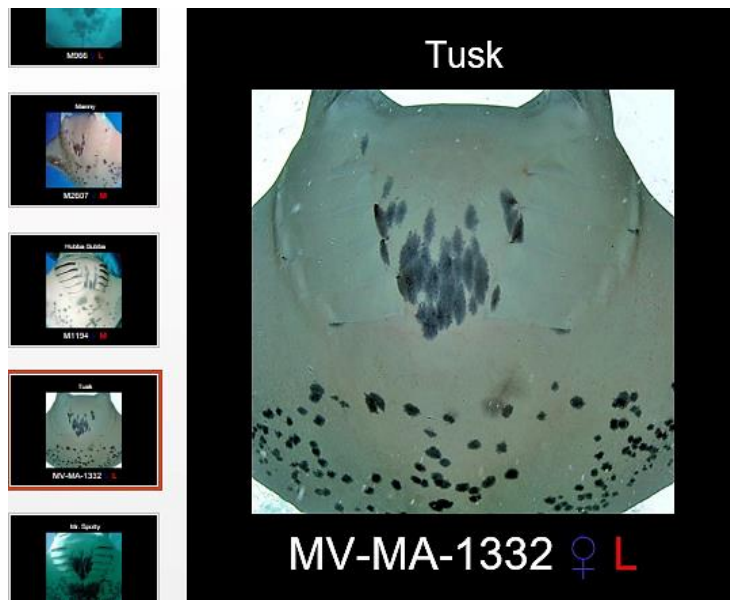


Figure 2.4 Identification gallery containing the master image of each of the 4,000 *M. alfredi* in the database, arranged continuously by spot number and position.

2.3.3 Size, sex and maturity status

The disc width of a manta was estimated against known lengths of divers or snorkelers swimming immediately above or below the animal. Throughout the study a technique was periodically employed whereby two parallel green laser pointers mounted 50 cm apart using a custom-made bracket, which also supported an underwater camera and housing, were used to validate size estimations by projecting the laser beams onto the body of the photographed manta ray, allowing extrapolation of its size as per Deakos (2010). However, because of the limited accuracy of the disc width estimation used for the majority of

individuals not measured with lasers (>90%), the four size class categories shown in table 2.1 have been used to represent the information.

Table 2.1 Description of size classes and indicators of maturity status used to categorise *Manta alfredi* during the study period of January 2005 through to December 2015.

| Size class | Disc width (cm) | Female | Indicators of maturity status | Male | Indicators of maturity status |
|------------|-----------------|-------------------|--|-------------------------------|---|
| 1 | < 210 | Immature juvenile | None | Immature juvenile | Very small uncalcified claspers that do not extend past the posterior edge of pelvic fins |
| 2 | 210 - 270 | Immature juvenile | None | Immature juvenile or subadult | Claspers beginning to calcify and enlarge and may extend past the posterior edge of pelvic fins |
| 3 | 271 - 320 | Immature juvenile | None | Mature adult | Claspers fully enlarged and calcified, engorged clasper glands |
| 4 | > 320 | Mature adult | Mating scars, fresh mating wounds, pregnancy bulge | N/A | N/A |

Methodologies developed from those of Marshall & Bennett (2010) and Deakos (2010) were used to determine the sex and maturity of mantas, and if a female was pregnant. Males have claspers (Fig. 2.5C – H), which are absent in females (Fig. 2.5A – B), while the claspers of immature juvenile males are small, un-calcified and don't extend past the posterior edge of the pelvic fin (Fig. 2.5C). In immature subadult males (Fig. 2.5D) the claspers have begun to enlarge and calcify, but no clasper glands are visible (circled on mature males Fig. 2.5E – F). Enlargement and calcification of claspers in *M. alfredi* occurs over a relatively narrow body size range (Table 2.1) (Coelho & Erzini 2006; Gelsleichter et al. 2002; Powter & Gladstone 2008), with the majority of calcification occurring only once the claspers have extended well beyond the posterior edge of the pelvic fins (Deakos 2010; Marshall & Bennett 2010a; White et al. 2006). As gonadal maturation in many elasmobranchs coincides with clasper calcification (Jones et al. 2008), a male *M. alfredi* in this study was considered mature only when his claspers were fully calcified and extended well past the posterior of the pelvic fins (Fig 2.5E – H). In mature males, wounds may occur on the clasper tips (Fig. 2.5G), which Marshall & Bennett (2010) attributed to copulation. However, similar wounds inflicted at cleaning stations by cleaner wrasse (Labridae) and triggerfish (Balistidae) also occur on the tail tips (Fig. 2.5G) of both sexes, and appear to result from instances of behavioural parasitism and opportunistic predation (Cheney & Côté 2005), and therefore were not used as an indicator of maturity in this study.

Female *M. alfredi* were considered mature if: (1) they were visibly pregnant, based on an extreme distention of the abdomen and back region (Fig. 2.6) (Deakos 2011; Marshall & Bennett 2010a), (2) they possessed dorsal mating scars (Fig. 2.7), or ventral mating wounds or scars (Fig. 2.8), or (3) the animal was estimated to be > 320 cm in disc width. During this study no female smaller than this size was recorded pregnant or seen to possess mating scars.

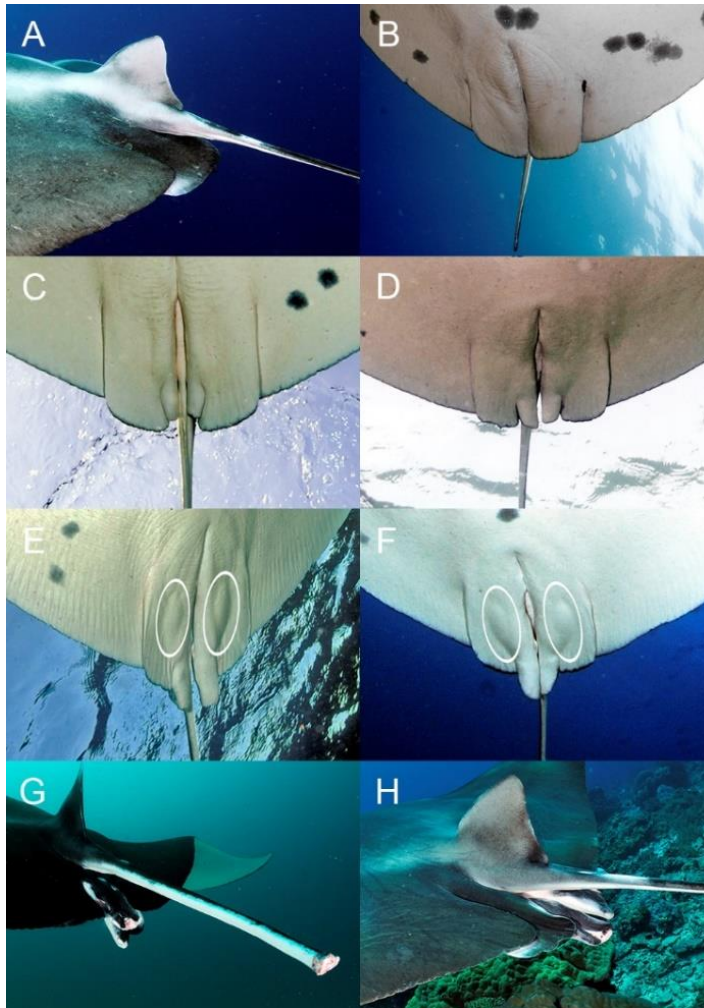


Figure 2.5 Sexual dimorphism between female (A – B) and male (C – H) *Manta alfredi* in the Maldives and the different maturity stages of males within the population: (C) immature, (D) subadult, and (E – H) mature, with visible clasper glands (circled). Wounds to claspers and tail tip (G) are inflicted by fish at cleaning stations.

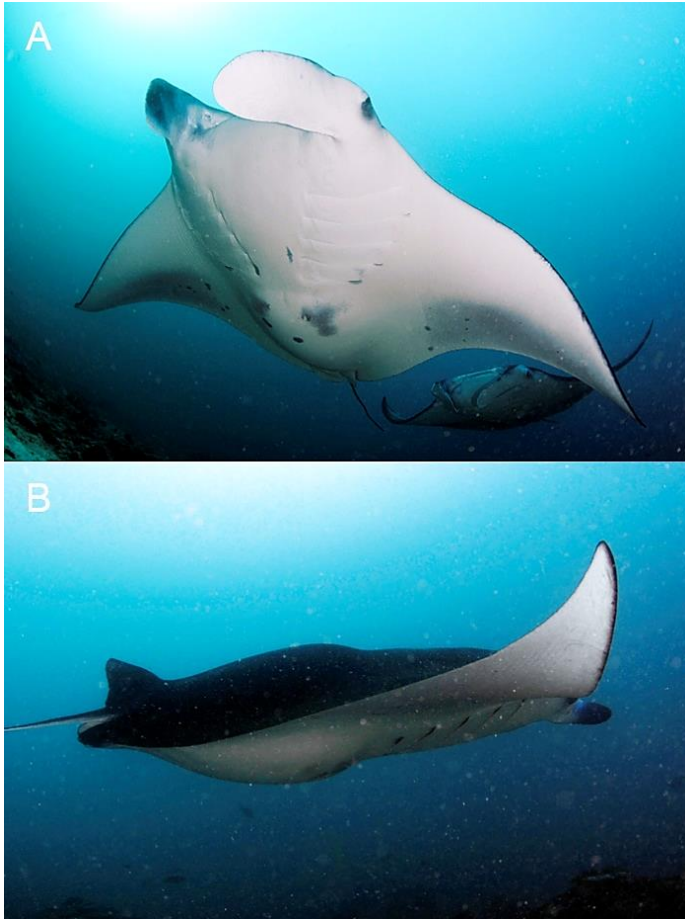


Figure 2.6 The distention of the abdominal (A) and back (B) region of pregnant female *Manta alfredi* becomes visibly apparent at around 5 – 6 months into the yearlong gestation.

The permanent dorsal mating scars are usually oval or circular in shape, ranging in size from 1 – 20 cm in diameter, they are coloured either black or white and inflicted during copulation by the hard cartilaginous ridges which protrude from the roof of the male's buccal cavity (Fig. 2.7B). Ventral mating wounds are also permanent, although the initial red colouration of these abrasions (Fig. 2.8A), caused by the rows of small teeth in the male's lower jaw (Fig. 2.8B), fade to pale grey and creamy white (Fig. 2.8C – D) within a month of copulation (Marshall & Bennett 2010a). Following Kajjura et al. (2000) and Marshall & Bennett (2010a), mating scars were classified in my records as 'fresh mating wounds' if the scars were reddish in colour, or simply 'mating scars' if they were pale grey to creamy white, or were on the dorsal surface of the pectoral fins.

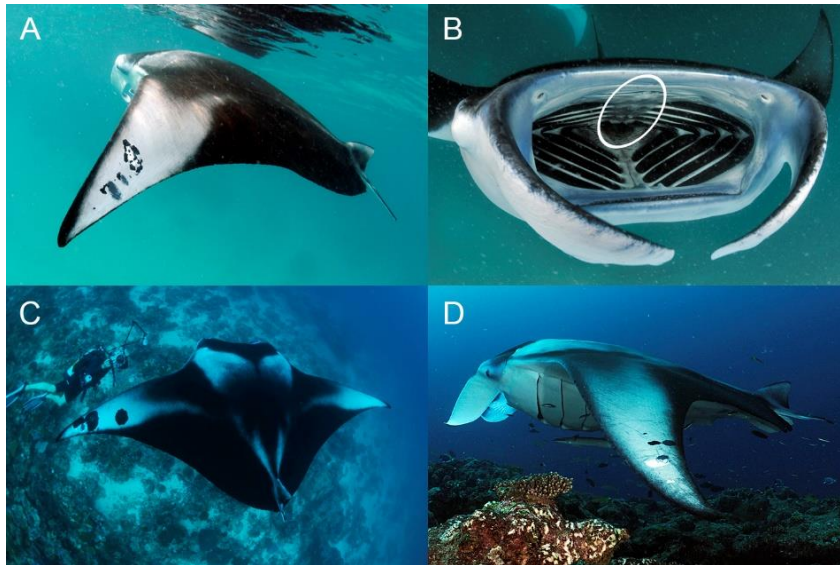


Figure 2.7 Dorsal mating scars on the tip of the left pectoral fin (A, C, D) of female *Manta alfredi* caused by hard cartilaginous ridges in the roof of the male's buccal cavity (B circled) inflicted from a bite during copulation.

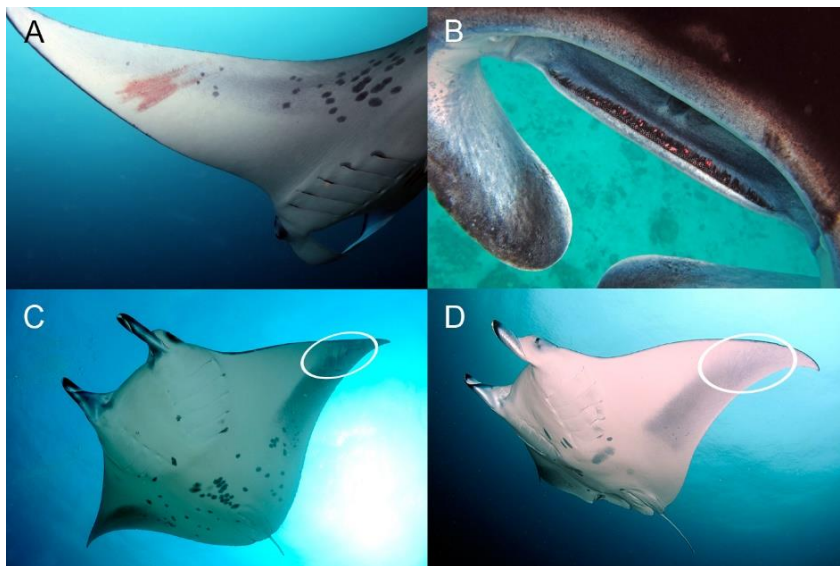


Figure 2.8 Fresh mating wounds (A) and mating scars (C – D circled) on the ventral tip of the left pectoral fin of female *Manta alfredi* caused by teeth in the male's lower jaw (B) inflicted from a bite during copulation.

2.3.4 Sampling protocol

In the Maldives, manta rays are accustomed to interacting with tourist divers and snorkellers at aggregation sites where the rays predictably gather at certain times of the year to feed, clean and socialise (Anderson et al. 2011a). Manta rays are host to a wide variety of ectoparasites which live and feed on their bodies, hiding themselves inside mouths, spiracles, gills and anywhere else they can secure a firm grip out of harm's way (Marshall 2009; pers. obs.). To rid themselves of these parasites, manta rays visit specialised 'cleaner fish' at sites called 'cleaning stations', usually a prominent reef outcrop or a coral bommie (Côté 2000; Feder 1966; Losey Jr 1972; pers. obs.). The most active cleaning sites in the Maldives are often those situated close to the atoll channels, the mantas' favoured feeding areas (pers. obs.). The prevalent cleaner fish species in the Maldives are the blue-streaked cleaner wrasse

(*Labroides dimidiatus*), the bicour cleaner wrasse (*Labroides bicour*), the moon wrasse (*Thallasoma lunare*) and the two-tone wrasse (*Thallasoma amblycephalum*) (pers. obs.).

A typical survey during this study entailed diving or freediving at one of these aggregation sites, where close encounters with the unperturbed rays easily allowed photo-ID images to be taken and observations recorded of the individuals present. Manta surveys were performed on SCUBA or while freediving from either a dedicated research vessel or commercial diving vessels. Surveys were performed at different times of day in all months of the year throughout the month. SCUBA surveys lasted on average 60 minutes and ranged to a maximum depth of 30 metres. Freediving surveys lasted on average 120 minutes. Myself, or a trained staff member or volunteer from the Manta Trust, conducted the surveys (www.mantatrust.org/in-the-field/maldives/).

When manta rays were encountered, where possible the following information was recorded for each individual: (1) species, (2) photo-ID, (3) sex, (4) size class, (5) maturity status, and (6) behavioural activity. Behavioural activity was also further broken down into four major groups (feeding, cleaning, courtship, cruising) for use on other parts of this research.

2.3.5 Data analysis

After a survey, video and photographic material was collated and edited, and individual manta rays identified and logged. Data were entered in Microsoft Excel and analysed using SPSS version 19.0 (SPSS, Inc. 2011). Pearson's Chi-Square was used to examine if differences occurred between sex, maturity status and size class at cleaning vs. feeding sites, given data did not meet the assumptions of normality and expected values were greater than five for all instances (Dytham 2010). I also used the same test to analyse sighting frequencies between the sexes and individuals of different maturity status at feeding locations in channels vs. lagoons. Significance for all tests was set at $p < 0.05$.

2.4 Results

2.4.1 Population demographics

From January 2005 through to December 2015 and sporadically during the two previous decades, 38,804 photo-ID sightings of 4,000 individual *M. alfredi* were recorded in 21 of the Maldives' 26 atolls (Table 2.2) (Figs. 2.9 & 2.10) at 239 different sites. 33,288 (86%) of these sightings were made at 20 sites (Table 2.3). The sporadic sampling prior to 2005 resulted in

158 photo-ID sighting records of 123 individual *M. alfredi* during the period of August 1987 to December 2004.

Table 2.2 Distribution of individual *Manta alfredi* sightings throughout the 26 atolls of the Maldives during the study period of January 2005 through to December 2015 and sporadically in the two preceding decades.

| Common or political name | Geographical atolls (north to south) | Atoll size class | Atoll number | Size (km ²) | No. individuals | No. sightings | No. surveys |
|--------------------------|--------------------------------------|------------------|--------------|-------------------------|-----------------|---------------|-------------|
| Ihavandhippolhu Atoll | Ihavandhippolhu Atoll | Small | 1 | 292 | 41 | 47 | 35 |
| Thiladhunmathi Atoll | Thiladhunmathi Atoll | X-Large | 2 | 4108 | 228 | 267 | 105 |
| Makunudhoo Atoll | Māmakunudhoo Atoll | Small | 3 | 139 | 0 | 0 | 0 |
| Kalhifushi Atoll | Etthingili Alifushi Atoll | V-Small | 4 | 4 | 0 | 0 | 0 |
| Raa Atoll | Maalhosmadulu Uthuruburi Atoll | Large | 5 | 1180 | 199 | 256 | 79 |
| Fasdhū Atoll | Fasdhūetherē Atoll | Small | 6 | 134 | 204 | 791 | 111 |
| Baa Atoll | Maalhosmadulu Dhekunuburi Atoll | Large | 7 | 943 | 1860 | 24902 | 3786 |
| Goidhu Atoll | Goidhu Atoll | Small | 8 | 111 | 18 | 18 | 3 |
| Lhaviyani Atoll | Faadhippolhu Atoll | Medium | 9 | 699 | 302 | 1077 | 344 |
| Kaashidhu Atoll | Kaashidhu Atoll | V-Small | 10 | 9 | 0 | 0 | 0 |
| Gaafaru Atoll | Gahaafaru Atoll | Small | 11 | 87 | 0 | 0 | 0 |
| North Malé Atoll | Male'atholhu Uthuruburi | Large | 12 | 1565 | 599 | 4765 | 1676 |
| South Malé Atoll | Male'atholhu Dhekunuburi | Medium | 13 | 530 | 87 | 198 | 89 |
| Thoddu Atoll | Thoddu Atoll | V-Small | 14 | 5 | 4 | 5 | 5 |
| Rasdhu Atoll | Rasdhu Atoll | Small | 15 | 62 | 75 | 131 | 61 |
| Ari Atoll | Ari Atoll | X-Large | 16 | 2259 | 1009 | 4364 | 1389 |
| Vaavu Atoll | Felidhe Atoll | Large | 17 | 1092 | 60 | 108 | 47 |
| Vattaru Atoll | Vattaru Atoll | Small | 18 | 47 | 11 | 12 | 10 |
| Meemu Atoll | Mulaku Atoll | Large | 19 | 981 | 58 | 140 | 74 |
| Faafu Atoll | Nilandhe Atholhu Uthuruburi | Medium | 20 | 596 | 37 | 69 | 31 |
| Dhaalu Atoll | Nilandhe Atholhu Dhekunuburi | Medium | 21 | 734 | 5 | 7 | 4 |
| Thaa Atoll | Kolhumadulu Atoll | Large | 22 | 1695 | 8 | 9 | 9 |
| Laamu Atoll | Haddhunmathi Atoll | Medium | 23 | 880 | 104 | 1279 | 566 |
| Gaafu Atoll | Huvadhoo Atoll | X-Large | 24 | 3279 | 2 | 2 | 2 |
| Fuvahmulah Atoll | Fuvahmulah Atoll | V-Small | 25 | 9 | 0 | 0 | 0 |
| Addu Atoll | Addu Atoll | Small | 26 | 156 | 66 | 357 | 129 |
| Total | | | | 21596 | | 38804 | 8555 |

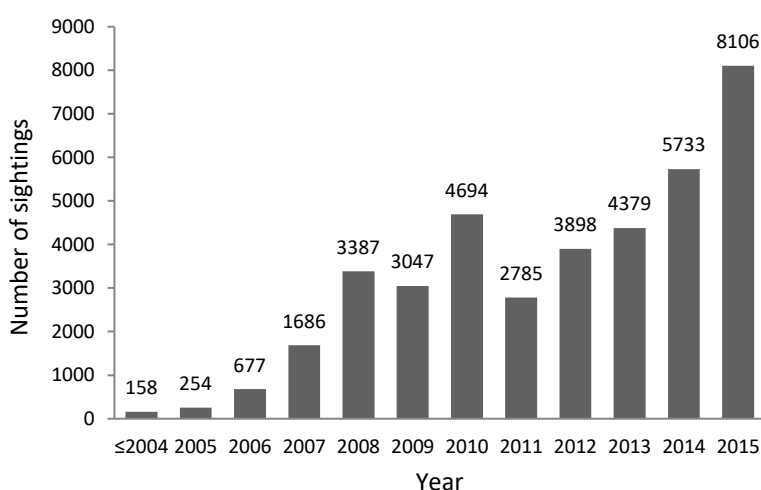


Figure 2.9 Annual distribution of *Manta alfredi* sightings over the study duration.

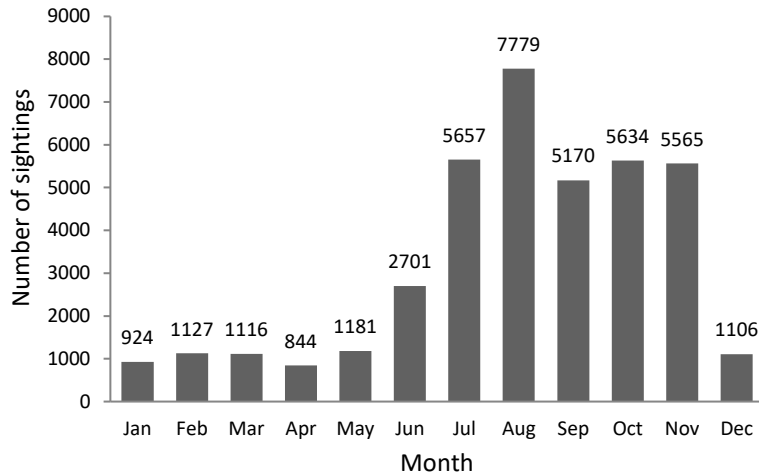


Figure 2.10 Monthly distribution of *Manta alfredi* sightings over the study duration.

Table 2.3 The twenty *Manta alfredi* aggregation sites surveyed with the highest number of sightings recorded over the study period. Demographic predominance was defined as the maturity status group which comprised the majority of individuals and sightings at each site.

| Site name | Atoll | Primary site function | Predominant demographic | Site location | No. of surveys | No. of individuals | No. of sightings | Av. no. mantas / survey | Standard Error |
|--------------------|-----------|-----------------------|-------------------------|---------------|----------------|--------------------|------------------|-------------------------|----------------|
| Huravalhi Falhu | Ari | Feeding | Adults | Channel Reef | 61 | 161 | 299 | 4.9 | 0.63 |
| Maaneigaa | Baa | Feeding | Juveniles | Lagoonal Reef | 75 | 169 | 309 | 4.1 | 0.54 |
| Dhonkalo Thila | Ari | Cleaning | Adults | Channel Reef | 82 | 208 | 340 | 4.1 | 0.54 |
| Mudakan | Addu | Cleaning | Adults | Channel Reef | 112 | 62 | 343 | 3.1 | 0.27 |
| Maavaru Falhu | Ari | Feeding | Juveniles | Lagoonal Reef | 134 | 149 | 358 | 2.7 | 0.19 |
| Moofushi Beyru | Ari | Cleaning | Adults | Outer Reef | 147 | 151 | 387 | 2.6 | 0.16 |
| Dhanifaru | Lhaviyani | Feeding | Juveniles | Lagoonal Reef | 105 | 155 | 404 | 3.8 | 0.35 |
| Sunlight Faru | N-Malé | Cleaning | Adults | Inner Reef | 154 | 168 | 436 | 2.8 | 0.24 |
| Rasfari North | N-Malé | Cleaning | Adults | Outer Reef | 167 | 221 | 531 | 3.2 | 0.30 |
| Dhigu Thila | Baa | Cleaning | Adults | Inner Reef | 142 | 407 | 579 | 4.1 | 0.59 |
| Rangali Madivaru | Ari | Cleaning | Adults | Outer Reef | 253 | 175 | 666 | 2.6 | 0.18 |
| Bathalaa Kandu | Fasdhu | Feeding | Juveniles | Channel Reef | 101 | 182 | 732 | 7.2 | 0.88 |
| Dhigurah Falhu | Ari | Feeding | Juveniles | Lagoonal Reef | 203 | 193 | 832 | 4.1 | 0.33 |
| Dharavandhoo Beyru | Baa | Cleaning | Adults | Outer Reef | 211 | 382 | 1091 | 5.2 | 0.65 |
| Hurai Faru | Baa | Feeding | Adults | Channel Reef | 616 | 426 | 1189 | 1.9 | 0.25 |
| Hithadhoo Beyru | Laamu | Cleaning | Adults | Outer Reef | 468 | 103 | 1262 | 2.7 | 0.16 |
| Reethi Falhu | Baa | Feeding | Juveniles | Lagoonal Reef | 432 | 510 | 1484 | 3.4 | 0.43 |
| Veyofushi Falhu | Baa | Feeding | Juveniles | Lagoonal Reef | 731 | 448 | 2063 | 2.8 | 0.22 |
| Lankan Beyru | N-Malé | Cleaning | Adults | Outer Reef | 1166 | 429 | 3488 | 3.0 | 0.14 |
| Hanifaru Bay | Baa | Feeding | Adults | Channel Reef | 1107 | 1550 | 16495 | 14.9 | 0.82 |

Of the animals seen, 50% (N = 2,010) were female, 49% (N = 1,945) male, and the sex of the remaining 45 could not be determined. 55% (N = 2,217) were adult, of which 38% (N = 845) were female and 62% (N = 1,372) male. Of the 1,738 juveniles, 67% (N = 1,165) were female and the rest (N = 573) male (Table 2.4).

Table 2.4 Distribution of sex and size classes recorded within the *Manta alfredi* population over the full study period.

| Sex | Size class | No. individuals | Percentage | No. sightings | Percentage |
|---------|------------|-----------------|------------|---------------|------------|
| Females | 1 | 79 | 2.0% | 256 | 0.7% |
| | 2 | 492 | 12.3% | 4561 | 11.8% |
| | 3 | 594 | 14.9% | 6700 | 17.3% |
| | 4 | 845 | 21.1% | 12015 | 31.0% |
| | Sub-total | 2010 | 50.3% | 23532 | 60.6% |
| Males | 1 | 87 | 2.2% | 241 | 0.6% |
| | 2 | 486 | 12.2% | 3221 | 8.3% |
| | 3 | 1372 | 34.3% | 11760 | 30.3% |
| | Sub-total | 1945 | 48.6% | 15222 | 39.2% |
| Unknown | 1 | 7 | 0.2% | 12 | 0.0% |
| | 2 | 17 | 0.4% | 17 | 0.0% |
| | 3 | 21 | 0.5% | 21 | 0.1% |
| | Sub-total | 45 | 1.1% | 50 | 0.1% |
| Total | | 4000 | 100% | 38804 | 100% |

2.4.2 Habitat use

Population demographics of *M. alfredi* varied significantly from the expected values (calculated from the total population ratios) when grouped by maturity status, sex and size class at cleaning stations and feeding sites. Significantly more adults than expected were observed at cleaning stations ($\chi^2 = 194.26$, $df = 1$, $N = 2262$, $p < 0.001$), and although the number of adult and juvenile individuals at feeding sites were evenly split, the proportion of juveniles was significantly higher than expected ($\chi^2 = 42.90$, $df = 1$, $N = 2898$, $p < 0.001$) (Fig 2.11).

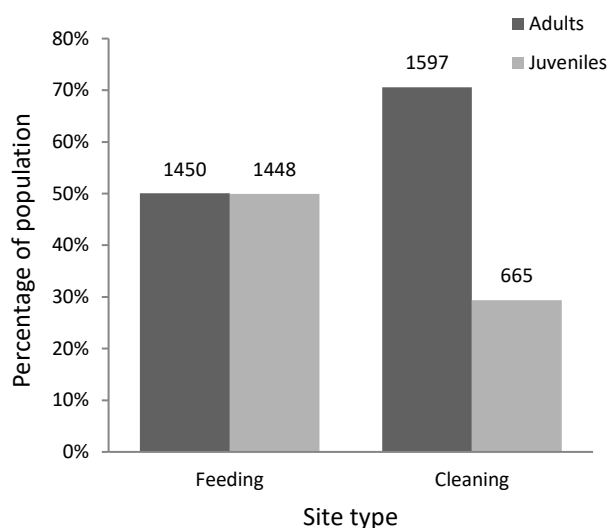


Figure 2.11 Use of cleaning and feeding sites by adult and juvenile *Manta alfredi* across the full sample. Actual numbers presented at the top of bars.

Significantly more females than expected were observed at cleaning stations ($\chi^2 = 10.76$, $df = 1$, $N = 2262$, $p < 0.001$), but not at feeding sites ($\chi^2 = 0.55$, $df = 1$, $N = 2898$, $p = 0.457$) (Fig. 2.12). Smaller individuals were significantly more likely to be observed at feeding sites ($\chi^2 =$

52.80, $df = 3$, $N = 2898$, $p < 0.001$), and larger ones at cleaning stations ($\chi^2 = 294.31$, $df = 3$, $N = 2262$, $p < 0.001$) (Fig. 2.13).

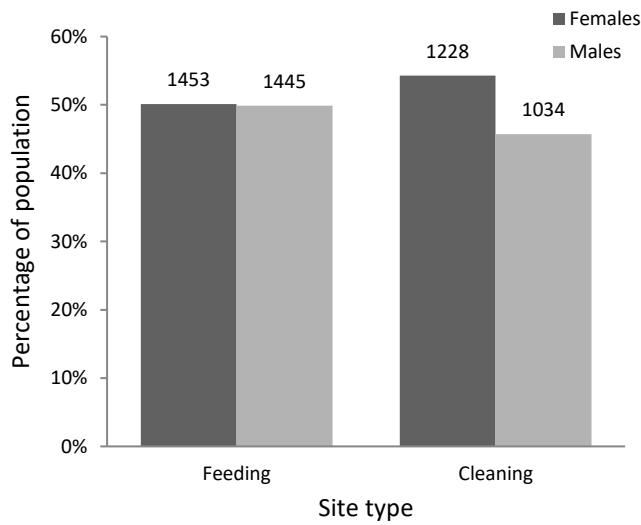


Figure 2.12 Use of cleaning and feeding sites by sex of *Manta alfredi* across the full sample. Actual numbers presented at the top of bars.

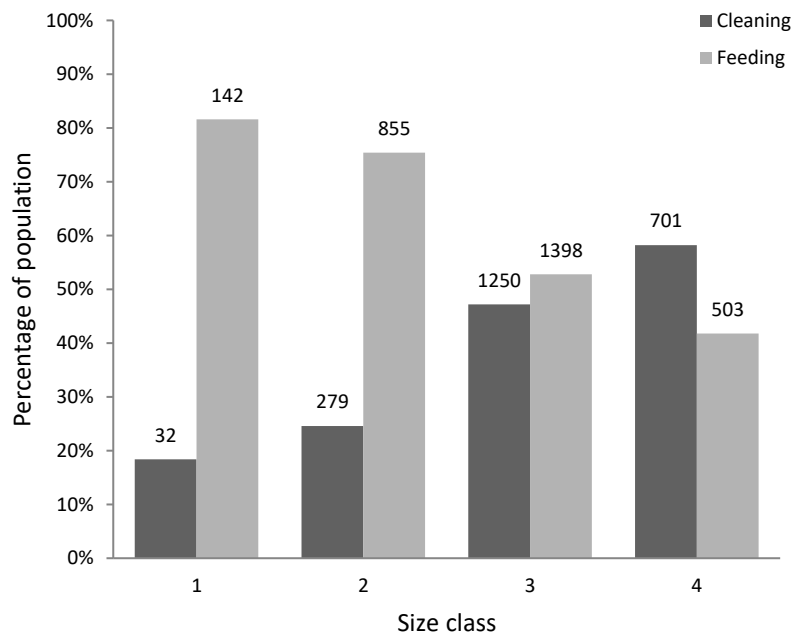


Figure 2.13 Use of feeding and cleaning sites by size class of *Manta alfredi* across the full sample. Actual numbers presented at the top of bars.

The sighting frequency of the population demographics also varied significantly from the expected values (based on the population ratios reported for each site type; i.e. cleaning or feeding) when grouped by maturity status and sex. Significantly more sightings of adults than expected were observed at cleaning stations ($\chi^2 = 323.76$, $df = 1$, $N = 10701$, $p < 0.001$) and at feeding sites ($\chi^2 = 256.66$, $df = 1$, $N = 27901$, $p < 0.001$) (Fig 2.14), and significantly more sightings of females than expected were observed at cleaning stations ($\chi^2 = 332.74$, $df = 1$, $N = 10701$, $p < 0.001$), and at feeding sites ($\chi^2 = 1057.56$, $df = 1$, $N = 27901$, $p < 0.001$) (Fig. 2.15).

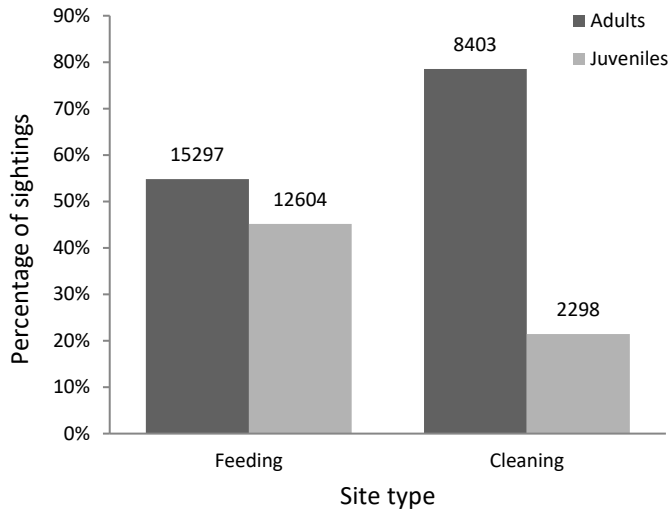


Figure 2.14 Sightings of *Manta alfredi*, grouped by maturity status and site type, across the full sample. Actual numbers presented at the top of bars.

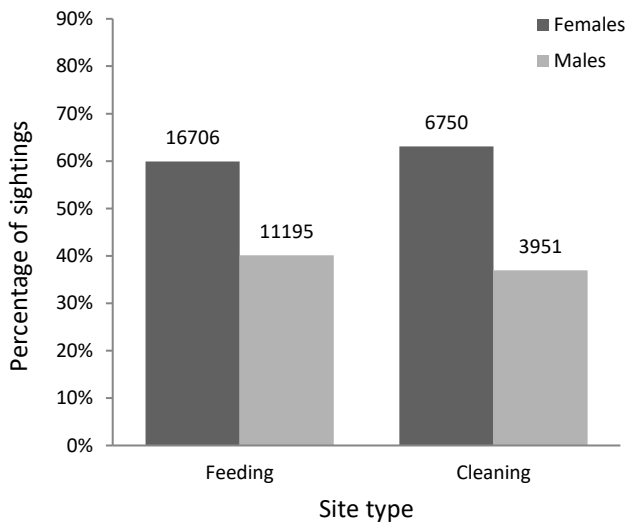


Figure 2.15 Sightings of *Manta alfredi*, grouped by sex and site type, across the full sample. Actual numbers presented at the top of bars.

The sighting frequency of the population demographics between the different feeding site types also varied significantly from the expected values (based on the population ratios reported for each feeding site type; i.e. lagoon or channel) when grouped by maturity status and sex. Significantly more sightings of adults than expected occurred at channel sites ($\chi^2 = 295.19$, $df = 1$, $N = 20270$, $p < 0.001$), and significantly more sightings of juveniles than expected occurred at lagoon sites ($\chi^2 = 89.54$, $df = 1$, $N = 7631$, $p < 0.001$) (Fig. 2.16). Significantly more sightings of females than expected occurred at channel sites ($\chi^2 = 1404.04$, $df = 1$, $N = 20270$, $p < 0.001$), and significantly more sightings of males than expected occurred at lagoon sites ($\chi^2 = 25.44$, $df = 1$, $N = 7631$, $p < 0.001$) (Fig. 2.17).

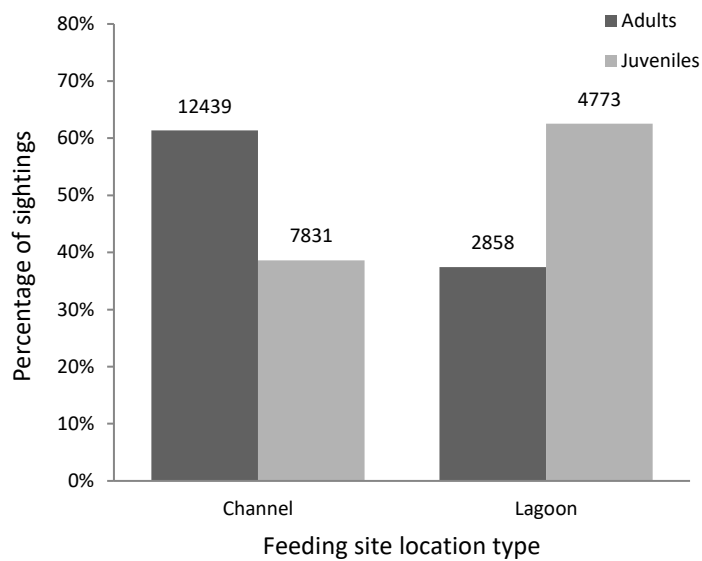


Figure 2.16 Sightings of *Manta alfredi*, grouped by maturity status and different feeding location types, across the full study. Actual numbers presented at the top of bars.

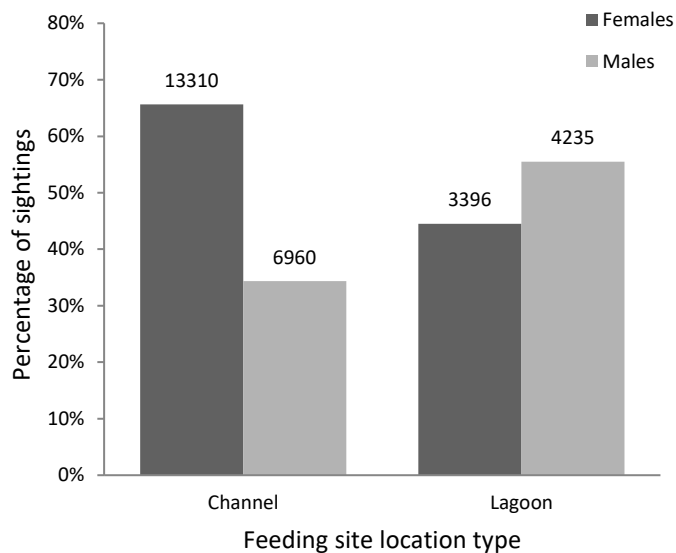


Figure 2.17 Sightings of *Manta alfredi*, grouped by sex and feeding location type, across the full study. Actual numbers presented at the top of bars.

2.4.3 Young of the year

Each year, among the *M. alfredi* individuals recorded as new to the study, the proportion which were recorded as size class 1 fluctuated, peaking in 2009 and 2015, with a trough in 2012 and 2013 (Fig. 2.18). These patterns match the records for individuals recorded as young of the year (YoY) (N = 41) (individuals upon first sighting considered to be born within the year based on their very small disc width, which was on average 160 cm) (Fig. 2.19). All YoY individuals were still juvenile when last seen, including the 9 with the longest sighting spans (period from first to last sighting in years) of 5 – 10 years, and were each on average estimated to have grown by 11 cm per year (Table 2.5).

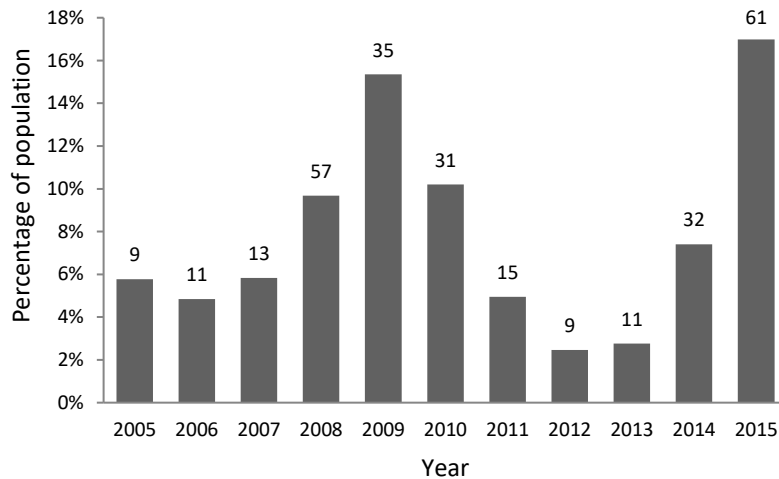


Figure 2.18 Figures for size class 1 *Manta alfredi* individuals sighted for the first time annually, as a proportion of all sizes of individuals seen in a year. Actual numbers presented at the top of bars.

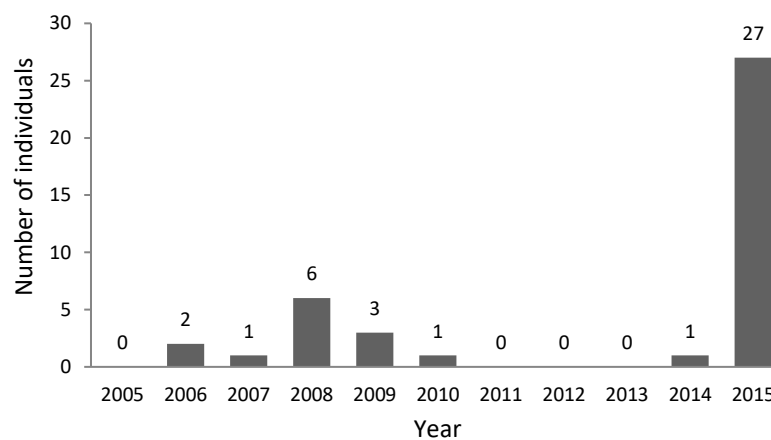


Figure 2.19 Number of young of the year *Manta alfredi* observed in each year of the study.

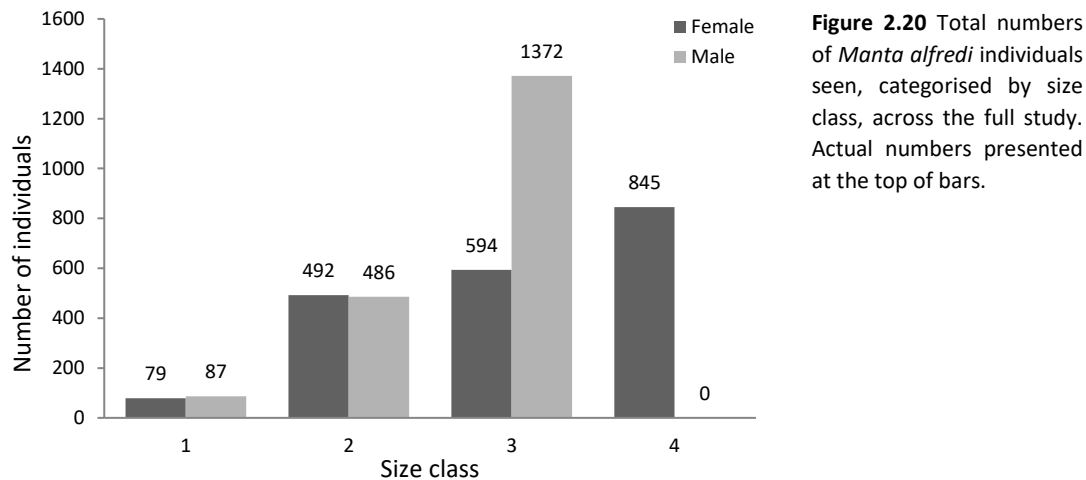
Table 2.5 Young of the year *Manta alfredi* individuals with the longest sighting spans.

| ID number | Sex | Maturity status | Sighting atoll/s | First sightings | Last sighting | Total sight. | Size at 1st sight. | Size at last sight. | Size increase (cm) | Sighting span (years) | Growth (cm/year) | Size class |
|------------|--------|-----------------|------------------|-----------------|---------------|--------------|--------------------|---------------------|--------------------|-----------------------|------------------|------------|
| MV-MA-0159 | Female | Juvenile | N-Malé | 08/07/2006 | 15/10/2015 | 14 | 170 | 270 | 100 | 10 | 10.0 | 3 |
| MV-MA-0307 | Female | Juvenile | Baa | 21/01/2007 | 24/07/2014 | 23 | 160 | 260 | 100 | 8 | 12.5 | 2 |
| MV-MA-0627 | Male | Juvenile | Lhaviyani | 22/07/2006 | 08/12/2013 | 5 | 150 | 240 | 90 | 8 | 11.3 | 2 |
| MV-MA-1231 | Male | Juvenile | Lhaviyani | 12/11/2008 | 14/12/2014 | 7 | 150 | 230 | 80 | 7 | 11.4 | 2 |
| MV-MA-1432 | Male | Juvenile | Ari & Vaavu | 10/06/2009 | 20/10/2015 | 10 | 160 | 230 | 70 | 7 | 10.0 | 2 |
| MV-MA-1730 | Male | Juvenile | Baa & Fasdhu | 22/09/2008 | 25/09/2014 | 34 | 150 | 220 | 70 | 7 | 10.0 | 2 |
| MV-MA-1774 | Female | Juvenile | Baa | 30/12/2009 | 25/11/2015 | 41 | 150 | 230 | 80 | 7 | 11.4 | 2 |
| MV-MA-0964 | Female | Juvenile | Baa | 24/05/2008 | 17/11/2013 | 6 | 160 | 220 | 60 | 6 | 10.0 | 2 |
| MV-MA-1263 | Female | Juvenile | Baa & Fasdhu | 03/08/2008 | 17/11/2012 | 26 | 150 | 210 | 60 | 5 | 12.0 | 1 |

2.4.4 Size and age at maturity

The smallest individuals recorded in the study were estimated to be 140 cm in disc width (DW) and the largest 360 cm DW. No male larger than 310 cm DW was recorded and no mature female smaller than 320 cm DW. Males were estimated to undergo maturation (subadult stage) between a DW of ~260 – 280 cm. 4% (N = 166) of individuals were categorised as size class 1 upon their last sighting record, 25% (N = 978) fell into class 2, 50%

(N = 1,966) in class 3, and 21% (N = 845) in class 4. For both sexes, the size class containing the adults held the highest number of individuals (Fig. 2.20).



Of the 4,000 individuals observed in this study, 49% (N = 1,946) were categorised as juveniles on their first sighting, excluding the 45 of unknown sex. By their last sighting, 11% (N = 208) of these had matured, whereby 76% (N = 158) were adult males and 24% (N = 50) adult females. Of these 158 males, 42 were observed every year as they progressed from immature juveniles, to subadults, then mature adults. 71% (N = 30) of these males transitioned through the subadult stage in two years, and the others in one (N = 9) or three (N = 3).

Although the age of each individual upon first sighting could not be determined precisely, the sighting span, calculated as the total span in years from the first to last sighting, for each individual was recorded. Age at maturity for males was estimated to be 9 – 10 years, based on the fact that all individuals which were juvenile when first observed (N = 731) had become mature over this timeframe (Table 2.6) (Fig. 2.21). For females, age at maturity could not be accurately estimated because only 4% of the individuals which were juvenile when first observed (N = 1,215) attained maturity over the 11 year study period (Table 2.6). However, a polynomial regression ($y = \beta_0 + \beta_1 x + \beta_2 x^2 + \dots + \beta_n x^n + \epsilon$) curve fitted to the ratio shift of immature to mature females (y) over the 11 year study (x), suggests that it will take 16 – 17 years for all of the females which were juvenile when first observed to attain maturity (Fig. 2.21).

Table 2.6 Change in maturity status for *Manta alfredi* which were juveniles when first sighted (N = 1,946), grouped by sighting span in years.

| Sex | Sighting span (years) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Total |
|---------|-----------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|------|------|-------|
| Males | No. juveniles | 277 | 83 | 66 | 51 | 54 | 20 | 13 | 7 | 2 | 0 | 0 | 573 |
| | % | 100% | 93% | 90% | 74% | 69% | 54% | 42% | 19% | 7% | 0% | 0% | |
| | No. adults | 0 | 6 | 7 | 18 | 24 | 17 | 18 | 29 | 25 | 8 | 6 | 158 |
| | % | 0% | 7% | 10% | 26% | 31% | 46% | 58% | 81% | 93% | 100% | 100% | |
| | Sub-total | 277 | 89 | 73 | 69 | 78 | 37 | 31 | 36 | 27 | 8 | 6 | 731 |
| Females | No. juveniles | 462 | 155 | 146 | 113 | 66 | 49 | 54 | 68 | 36 | 10 | 6 | 1165 |
| | % | 100% | 99% | 99% | 97% | 94% | 92% | 92% | 84% | 77% | 71% | 60% | |
| | No. adults | 0 | 1 | 1 | 3 | 4 | 4 | 5 | 13 | 11 | 4 | 4 | 50 |
| | % | 0% | 1% | 1% | 3% | 6% | 8% | 8% | 16% | 23% | 29% | 40% | |
| | Sub-total | 462 | 156 | 147 | 116 | 70 | 53 | 59 | 81 | 47 | 14 | 10 | 1215 |

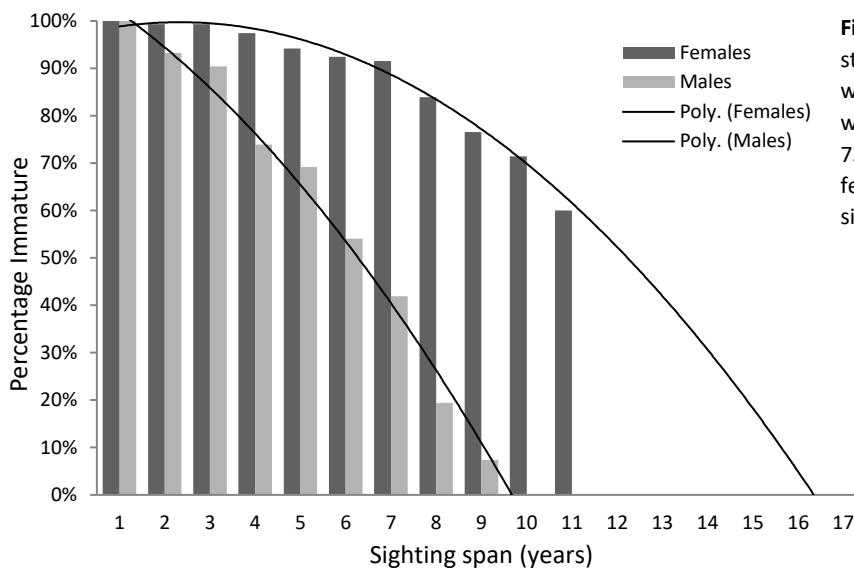


Figure 2.21 Maturity status for *Manta alfredi* which were juveniles when first sighted (N = 731 males, N = 1,214 females), grouped by sighting span.

To provide a comparative approach, age at maturity (α) was also calculated using the estimated range of; size at birth (β) (140 – 170 cm disc width), size at maturity (m) for males (260 – 280 cm disc width) and females (320 – 330 cm disc width), and average growth (g) per year of the recorded YoY (11 cm):

$$\alpha = m - \beta / g$$

Estimates ranged from 9 – 13 years for males and 13 – 17 years for females, consistent with the sighting span estimates of age at maturity.

Figs. 2.22 – 2.30 and table 2.7 provide photographic documentation of maturation in twelve *M. alfredi* (N = 6 male, N = 6 female) over 12 years. Each male was estimated as a size class 1 juvenile (<210 cm disc width) when first encountered, while the females size estimates ranged from class 1 to 3 (class two 220 – 270 cm, class three 271–320 cm disc width), although their exact age and size could not be determined. By the end of the study two of

the males had matured (MV-MA-0427 and MV-MA-1736), two were subadults (MV-MA-0641 and MV-MA-0920) and two (MV-MA-0627 and MV-MA-1730) still showed no sign of maturation when last sighted. By the end of the study five of the females were still juveniles, with no evidence of pregnancies, ventral or dorsal mating scars. The remaining female (MV-MA-0019) was observed with ventral mating scars for the first time in 2014, and was recorded visibly pregnant later that year. MV-MA-0019 was the only female estimated to be size class 3 when first sighted.

Table 2.7 Sighting spans and maturity status of twelve *Manta alfredi*, which were juveniles when first sighted. (-) signifies no sightings, (J) signifies juvenile when sighted, (S) signifies subadult, and (A) signifies adult.

| Manta ID number | Sex | Maturity status | Size class | | Sightings | | | | | | | | | | | Sighting span (years) | | | | | |
|-----------------|--------|-----------------|---------------|--------------|-----------|------|------|------|------|------|------|------|------|------|------|-----------------------|-----------------|-----------------|---------------|------------|---|
| | | | First sighted | Last sighted | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | Total sightings | Immaturity span | Maturity span | Total span | |
| MV-MA-0427 | Male | Adult | 1 | 3 | J | - | - | - | J | - | J | - | S | A | - | A | 15 | 9 | 3 | 12 | |
| MV-MA-0627 | Male | Juvenile | 1 | 2 | - | - | J | - | J | - | J | - | - | J | - | - | 5 | 8 | 0 | 8 | |
| MV-MA-0641 | Male | Subadult | 1 | 2 | - | - | - | J | - | - | J | J | J | J | S | S | 17 | 9 | 0 | 9 | |
| MV-MA-0920 | Male | Subadult | 1 | 2 | - | - | - | J | J | J | J | J | J | - | S | S | 27 | 9 | 0 | 9 | |
| MV-MA-1730 | Male | Juvenile | 1 | 2 | - | - | - | J | - | - | J | J | J | J | - | - | 34 | 7 | 0 | 7 | |
| MV-MA-1736 | Male | Adult | 1 | 3 | - | - | - | - | J | J | J | J | J | J | S | S | A | 87 | 7 | 1 | 8 |
| MV-MA-0019 | Female | Adult | 3 | 4 | - | J | - | J | J | J | J | - | J | J | A | A | 47 | 9 | 2 | 11 | |
| MV-MA-0057 | Female | Juvenile | 2 | 3 | - | J | - | J | J | J | J | J | J | J | - | - | 45 | 9 | 0 | 9 | |
| MV-MA-0217 | Female | Juvenile | 1 | 3 | - | - | J | J | J | J | J | J | J | J | J | - | 159 | 9 | 0 | 9 | |
| MV-MA-0486 | Female | Juvenile | 2 | 3 | - | - | - | J | J | J | J | J | J | J | J | J | 101 | 9 | 0 | 9 | |
| MV-MA-0780 | Female | Juvenile | 2 | 3 | - | - | - | J | J | J | J | J | J | J | J | J | 87 | 9 | 0 | 9 | |
| MV-MA-0870 | Female | Juvenile | 1 | 3 | - | - | - | J | J | J | J | J | J | J | J | J | 130 | 9 | 0 | 9 | |

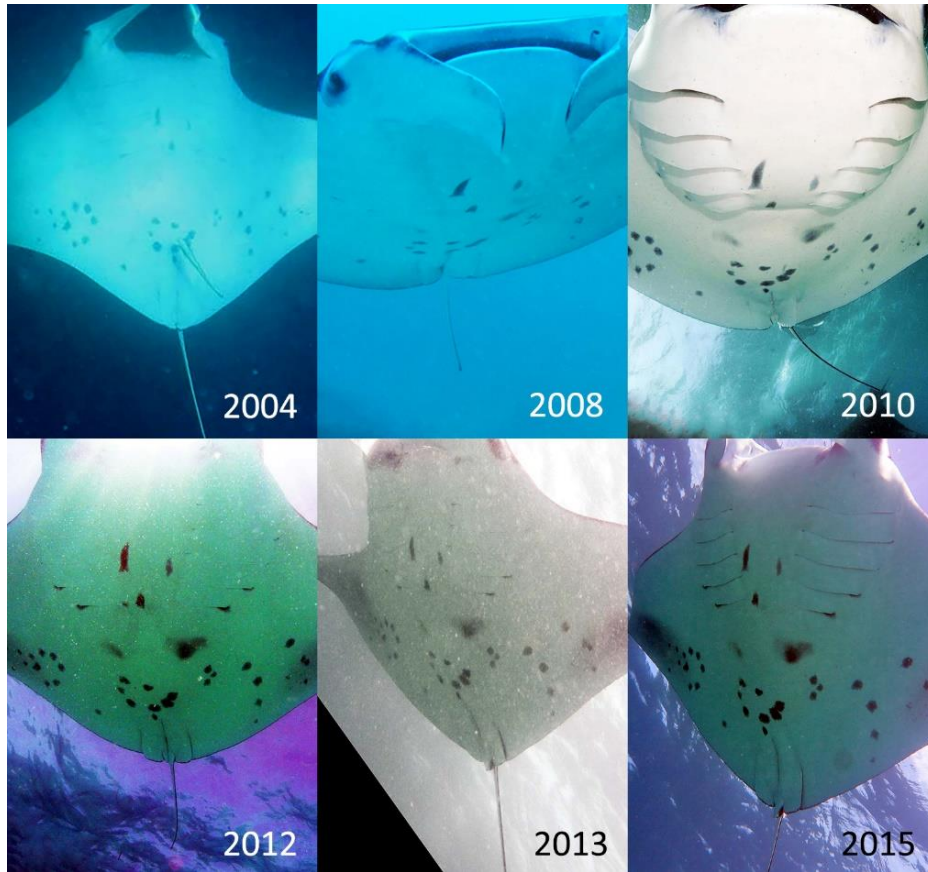


Figure 2.22 MV-MA-0427 was first sighted in 2004 as a size class 1 juvenile male *Manta alfredi*. In 2012 he was classified as a size class 2 subadult and by 2013, nine years later, a size class 3 male mature.

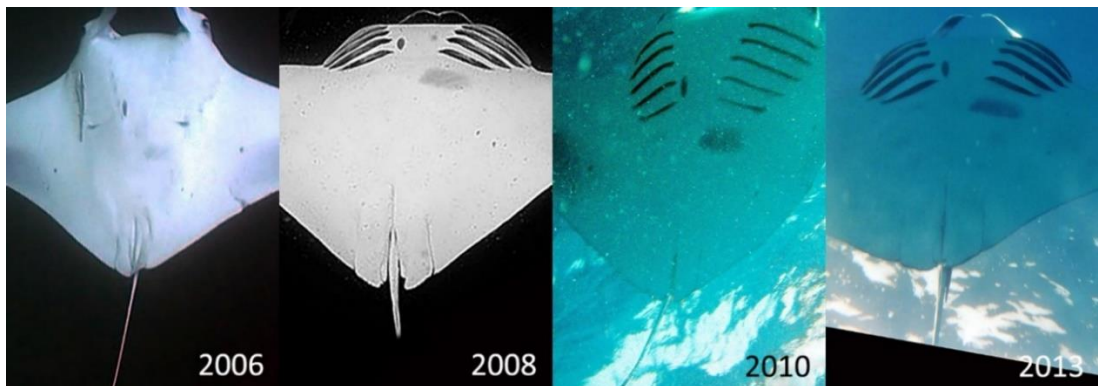


Figure 2.23 MV-MA-0627 was first sighted in 2006 as a size class 1 juvenile male *Manta alfredi*. In 2013, seven years later, he was classified as a size class 2 juvenile, with no signs of clasper enlargement or calcification.

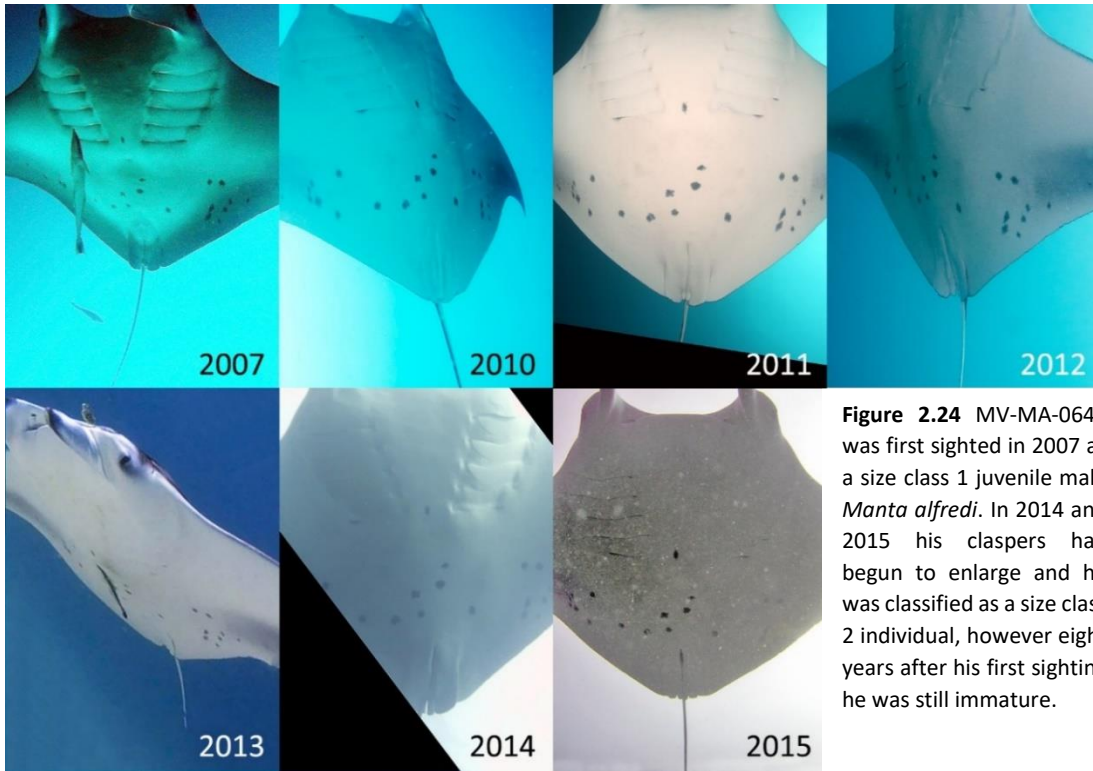


Figure 2.24 MV-MA-0641 was first sighted in 2007 as a size class 1 juvenile male *Manta alfredi*. In 2014 and 2015 his claspers had begun to enlarge and he was classified as a size class 2 individual, however eight years after his first sighting he was still immature.

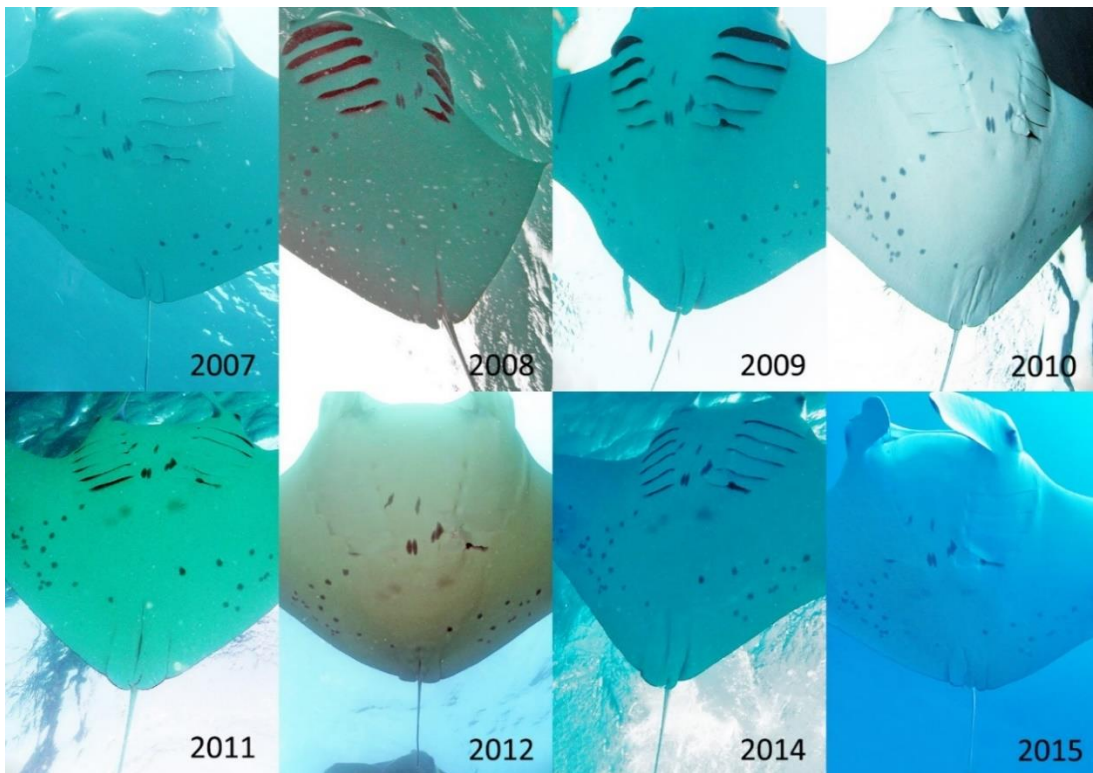


Figure 2.25 MV-MA-0920 was first sighted in 2007 as a size class 1 juvenile male *Manta alfredi*. In 2014 and 2015 his claspers had begun to enlarge and he was classified as a size class 2 individual, however eight years after his first sighting he was still immature.

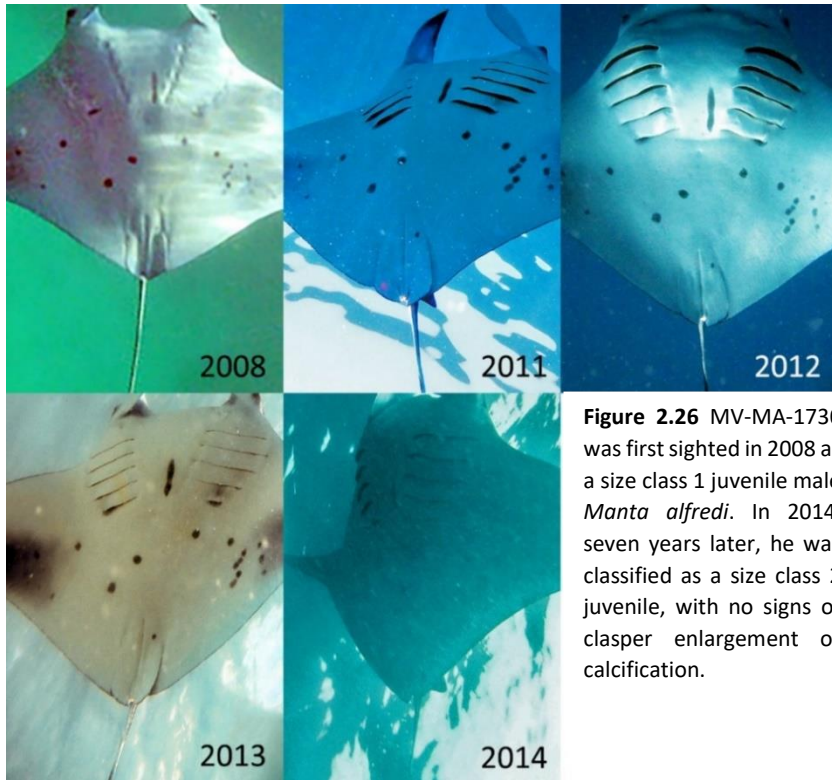


Figure 2.26 MV-MA-1730 was first sighted in 2008 as a size class 1 juvenile male *Manta alfredi*. In 2014, seven years later, he was classified as a size class 2 juvenile, with no signs of clasper enlargement or calcification.

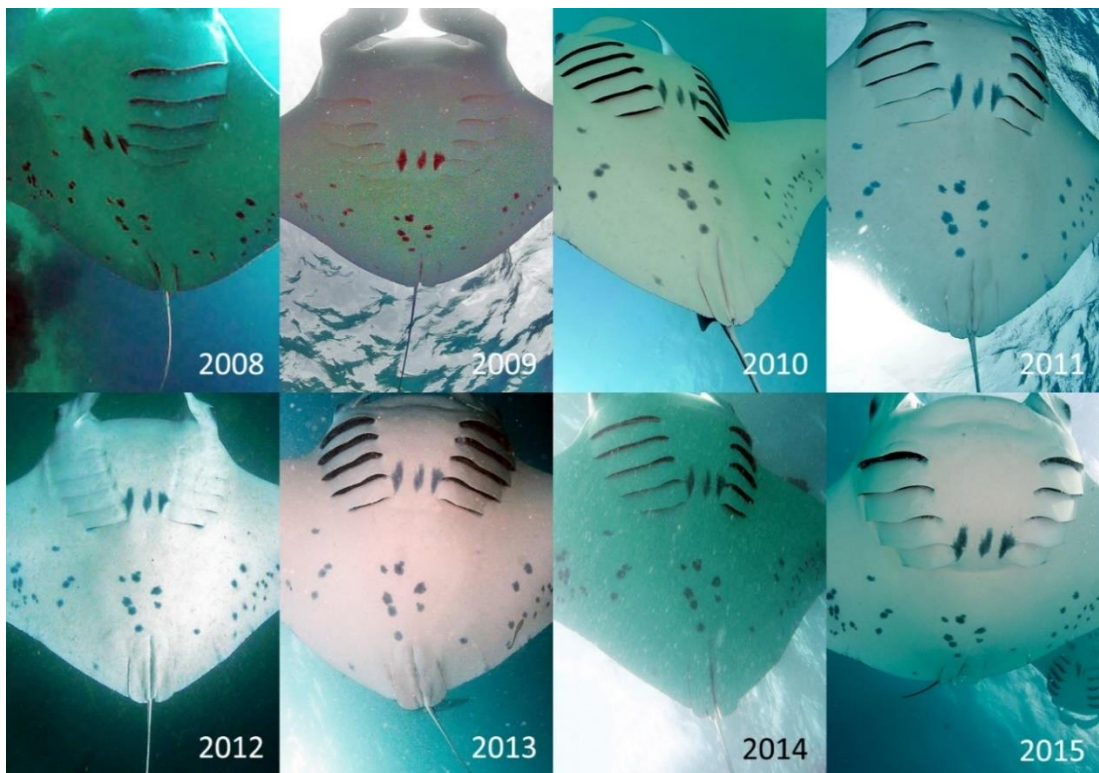


Figure 2.27 MV-MA-1736 was first sighted in 2008 as a size class 1 juvenile male *Manta alfredi*. In 2013 and 2014 he was classified as a size class 2 subadult and by 2015, seven years after the first sighting, a size class 3 male mature.



Figure 2.28 MV-MA-0019 was first sighted in 2005 as a size class 3 juvenile female *Manta alfredi*. In 2014 ventral mating scars were recorded for the first time. Later the same year she was observed visibly pregnant and classified as a size class 4 adult, nine years after the first sighting. When last sighted in 2015, no dorsal mating scars were present.

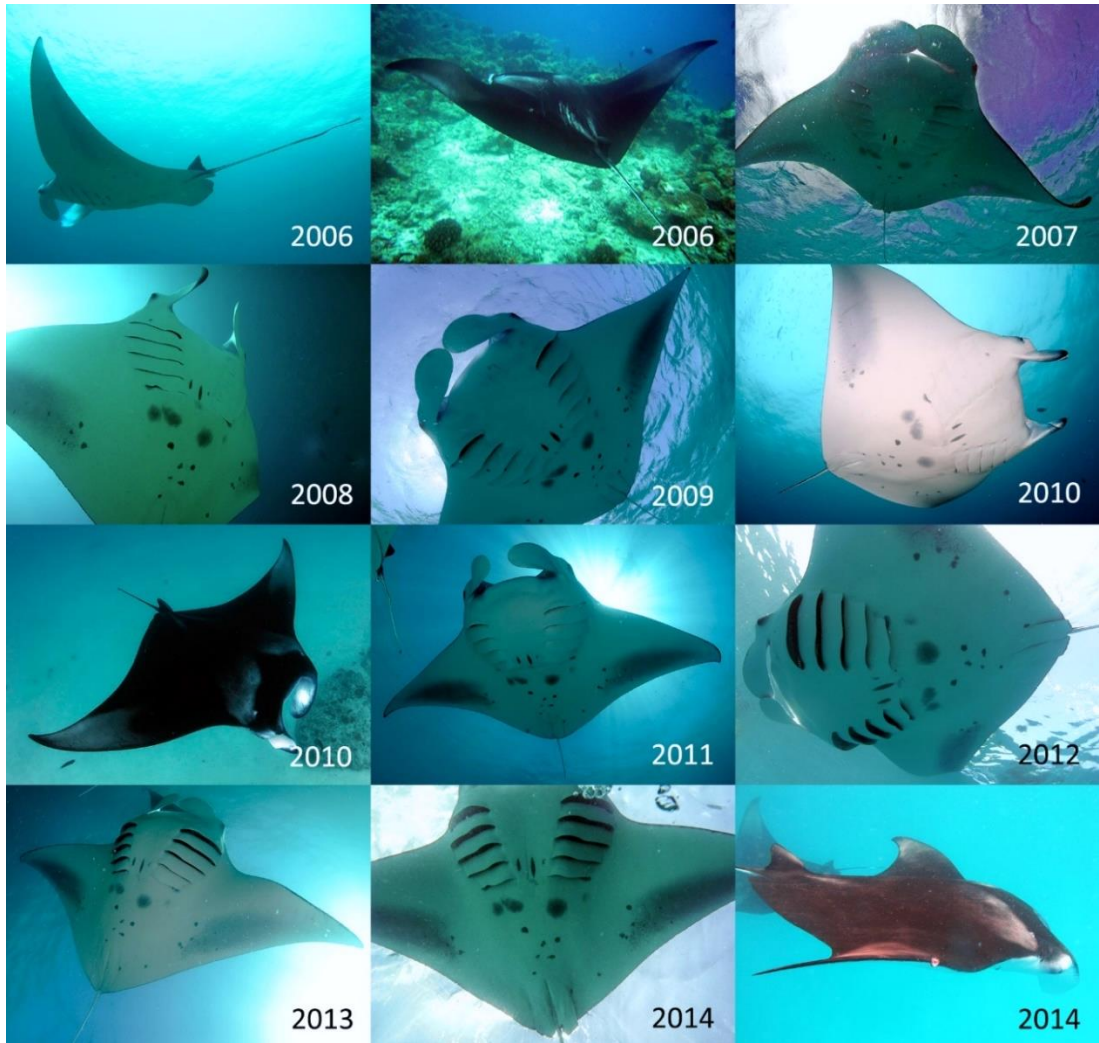


Figure 2.29 MV-MA-0217 was first sighted in 2006 as a size class 1 juvenile female *Manta alfredi*. In 2014, eight years later, she was classified as a size class 3 juvenile, with no signs of maturity.



Figure 2.30 MV-MA-0870 was first sighted in 2007 as a size class 1 juvenile female *Manta alfredi*. In 2015, eight years later, she was classified as a size class 3 juvenile, with no signs of maturity.

2.4.5 Longevity

The average sighting span recorded for each of the 4,000 individuals was 3.75 years. 37% (N = 1,480) of individuals were only seen in one year, 37% (N = 1,457) between 2 – 5 years, 24% (N = 969) between 6 – 10 years, and 2% (N = 94) between 11-24 years (Fig. 2.31). However, given the paucity of records preceding the onset of the study, the 2% fraction of individuals recorded with sighting spans exceeding 10 years is not a reliable indicator of longevity. 15 individuals were recorded with a sightings span of 15 years or more, of which 3 exceeded 20 years. Fig. 2.32A – B shows an adult female (MV-MA-0012) that was sighted 36 times at multiple locations in North Malé Atoll across a span of 23 years, Fig. 2.32C – D an adult male (MV-MA-0391) sighted 6 times at multiple locations in Ari Atoll across 21 years, and Fig. 2.32E – F an adult female (MV-MA-0771) sighted 101 times in Baa and Ari Atolls across 24 years.

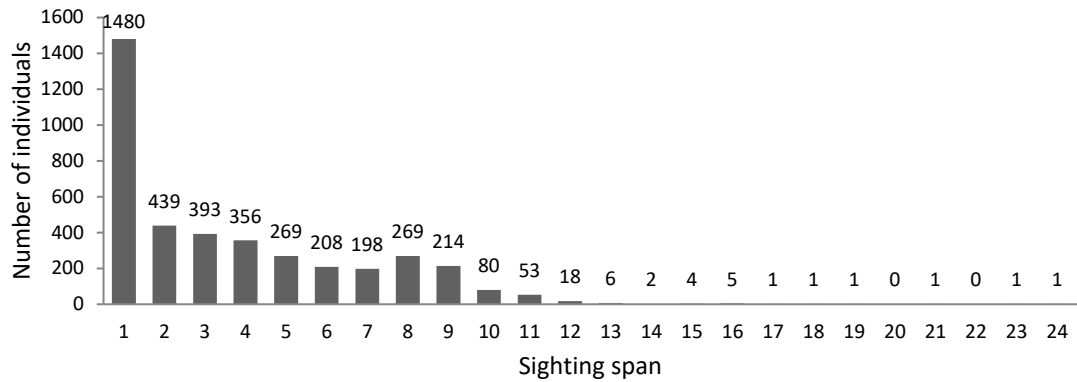


Figure 2.31 Sighting span (years) for each *Manta alfredi* (N = 4,000) in the full sample.

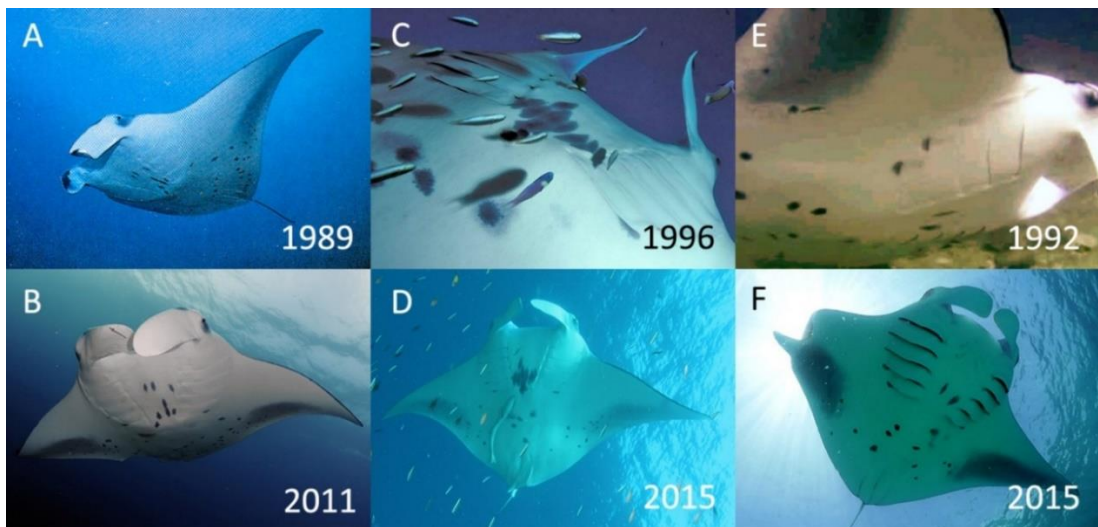


Figure 2.32 Three *Manta alfredi* had sightings spans exceeding 20 years (Image A © Mustag Hussain, C © Steve Jones, D © Richard Rees, and E © Raffaele Mattu).

2.5 Discussion

2.5.1 Population demographics and habitat use

The application of Photo-ID techniques on *M. alfredi* at several key aggregation sites throughout their range has already provided some critical life history information on the species' population size, structure and ecology (e.g., Couturier et al. 2014; Deakos 2011; Kashiwagi 2014; Marshall & Bennett 2010a). However, all previous studies have focused predominantly on data collection at a small number of cleaning stations, potentially sampling only a section of the population demographic. Sampling Maldives wide from January 2005 through to December 2015 and sporadically in the two preceding decades, this study encompassed over 150 *M. alfredi* cleaning and feeding aggregations sites for both adults and juveniles, collecting data across the majority of the coastal population demographic.

The 38,804 sightings of 4,000 individuals resulted in a 'recapture' rate of 72%, suggesting a large proportion of the remote archipelago's total *M. alfredi* population was sampled during this study. Although 51% (19,983) of the total sightings were recorded at just two primary study sites (Hanifaru Bay and Lankan Beyru), the demographic composition of *M. alfredi* at these two sites were consistent with others of a similar primary site function and location (i.e. feeding vs cleaning, or channel vs lagoon). Furthermore, 76% (1,454) of the total individuals' record at these two primary sites (1,907) were also recorded at other locations during the study. Similarly, demographic composition remained consistent spatially throughout the archipelago between the different atolls. Indeed, 21% (847 individuals) of the total sampled population were recorded in multiple atolls, often hundreds of kilometres apart, suggesting migrations throughout the archipelago is a common occurrence. These cross atoll movements were also unbiased towards either sex or size class of the individual.

All previous *M. alfredi* population studies have recorded far fewer individuals: Mozambique (N = 449) (Marshall et al. 2011a), eastern Australia (N = 716) (Couturier et al. 2014), Hawaii (N = 290) (Deakos et al. 2011), Japan (N = 305) (Kashiwagi 2014), and the Maldives, where Kitchen-Wheeler (2011) documented 1,835 individuals from the archipelago's central atolls between 2000 and 2009. Although no sightings data from the aforementioned Kitchen-Wheeler study were included in this study, the majority of the individual *M. alfredi* identified in this earlier study were also recorded here.

The total *M. alfredi* population sex ratio in this study was split evenly, with a 1:1.03 male-to-female ratio, conforming to the Hawaii and Japan studies referred to above, while in the Mozambique (1:3.5), eastern Australia (1:1.3) and the previous Maldives (1:1.8) studies the ratio was significantly biased towards females. However, when the sex ratio of the population recorded only at cleaning stations is considered in this study, there was also a significant bias towards females (1:1.2), although less than the previous Maldives study (Kitchen-Wheeler et al. 2011). This discrepancy between my study and Kitchen-Wheeler et al's may be due to variation in the total number and geographical spread of sites sampled. In my study, as in that from eastern Australia (Couturier et al. 2014), females were observed at cleaning stations significantly more often than males, with an average of 5.5 sightings per female, compared to just 3.8 per male. With fewer visitations per male, which are smaller and often less bold than the females (pers. obs.), the likelihood of missed recaptures are increased. Therefore a longer study period, sampling continuously throughout the years and across a much greater number of sites, is more likely to provide a true representation of the demographic ratio.

Despite 44% of the sampled population (N = 1,738) in my study being comprised of juveniles, 2.4 times as many adults were observed at cleaning stations, significantly more than expected by chance. Furthermore, each adult was observed frequenting cleaning stations significantly more often than juveniles, with on average 5.3 sightings per adult, compared to just 3.5 per juvenile. Given the greater number of adults visiting cleaning stations than juveniles, it therefore was to be expected that larger *M. alfredi* were also significantly more likely to be observed there. However, even between juvenile size classes, larger individuals were significantly more prevalent at cleaning stations, suggesting both size and maturity status influence visitations.

Given the variation in the extent to which different sexes, maturity status and size classes of *M. alfredi* visit cleaning stations beyond what could reasonably be explained by differences in cleaning requirements, it is likely other drivers are influencing the use of these sites. *M. alfredi* observed at cleaning stations in this study would regularly spend many hours a day at these reefs (pers. obs.). However, many of the individuals were only engaged in cleaning activity for some of the time, interacting instead with other manta rays, or individually cruising around the reef in the vicinity of the cleaning station. The majority of *M. alfredi* cleaning stations in the Maldives are situated on shallow reefs, either outside an atoll close to a channel, or just inside a channel passage (pers. obs.). Strong currents suck plankton rich water from ocean depths into the shallows through these channels (P. Hosegood et al., unpublished data), creating feeding opportunities. These shallow feeding sites are adjacent to productive deep water areas (Sasamal 2006; Sasamal 2007), where Anderson et al. (2011b) have speculated that *M. alfredi* may regularly feed upon zooplankton in the 'deep scattering layer'. Using acoustic telemetry, actively tracked *M. alfredi* in Hawaii (Clark 2010) regularly moved offshore, possibly to exploit rising layers of deep-water zooplankton (Lampert 1989; Lo et al. 2004) and satellite tagging studies elsewhere also suggest *M. alfredi* make regular forays into the mesopelagic zone, undertaking deep dives, most likely in search of food, to depths where the water temperature is significantly lower than in inshore reefs (Braun et al. 2014; Braun et al. 2015; Jaine et al. 2014). Temperature is recognised as the most important environmental factor affecting feeding and reproduction of elasmobranchs (Fangue & Bennett 2003; Wallman & Bennett 2006). Therefore, after deep water feeding forays by *M. alfredi*, returning to the warmer inshore habitat of the cleaning station may augment metabolic and physiological functions, such as digestion and gestation (Hight & Lowe 2007; Jirik & Lowe 2012). For example, in an experiment on captive Atlantic stingrays (*Dasyatis sabina*) by Wallman & Bennett (2006), individuals of both sexes preferred significantly

warmer water temperatures after being fed, and pregnant females preferred significantly warmer water than non-pregnant females, potentially reducing gestation time by as much as two weeks. Basking behaviour has also been documented in satellite tagged sickle-fin devil rays (*Mobula tarapacana*), which dived to 1,800 m where temperatures were around 5 °C (Thorrold et al. 2014), and also in giant devil rays (*Mobula mobular*) (Canese et al. 2011). These close relatives of *M. alfredi* frequently basked at the surface for extended periods after deep dives, presumably to recover body temperatures after long periods in cold water. Whale sharks (*Rhincodon typus*) and blue sharks (*Prionace glauca*) also exhibit similar behavioural thermoregulation, returning to the shallow warmer waters after deep-water forays (Carey & Scharold 1990; Thums et al. 2012).

The majority of *M. alfredi* courtship and mating behaviour has been reported at cleaning stations (Deakos 2011; Marshall & Bennett 2010a; Chapter 3), therefore females aggregating at these sites to be cleaned, and possibly to aid digestion and speed up gestation, are thus likely to provide a good opportunity for males to find a mate. Indeed, during periods of courtship and mating in the Maldives, which are also the peak pupping times, the sex ratio at cleaning stations shifts from a female to male bias, suggesting these sites may also function as leks (Chapter 4). If the drive to find a mate influences the visitation of *M. alfredi* to cleaning stations, this would also explain why juveniles visit these sites less frequently than adults and why it is important to sample throughout the year to avoid sample bias.

Cleaning stations may also act as a refuge site for the mantas, where predation risk from pelagic sharks (Marshall & Bennett 2010b) and cetaceans (Alava & Merlen 2009; Anderson 2005; Visser & Bonoccorso 2003) is reduced because manta rays are more vulnerable to attacks from below when away from the reef in open water (pers. obs.). Predator avoidance may also explain why small juvenile mantas in this study were significantly more prevalent in shallow lagoons compared to larger adults. This was also noted by McCauley et al. (2014) on a study of *M. alfredi* at Palmyra Atoll. In my study, male *M. alfredi* were also significantly more likely to be observed feeding in lagoons than females, possibly because their smaller maximum disc width makes them more vulnerable to predation than females. Conversely, sightings of females were significantly more frequent than males at feeding sites located in channels. Indeed, although there was no sex bias overall at feeding sites, each female was observed on average 11.5 times compared to just 7.7 per male (Chapter 5).

Female *M. alfredi* exhibited higher fidelity to the sites surveyed in this study than males, with significantly higher than the average rate of repeat sightings at both feeding and cleaning

sites overall. Philopatric behaviour in pelagic species is often sex-specific, with females remaining near, or repeatedly returning to, a natal site or region, while males roam more widely (Engelhaupt et al. 2009; Hueter et al. 2005; Lee et al. 2007). Genetic, photo-ID studies and satellite and acoustic tagging suggest many species of elasmobranchs, such as the white shark (*Carcharodon carcharias*), sand tiger shark (*Carcharias taurus*), scalloped hammerhead shark (*Sphyrna lewini*), lemon shark (*Negaprion brevirostris*), blacktip reef shark (*Carcharhinus melanopterus*), and thornback ray (*Raja clavata*), also exhibit female philopatry and male-biased dispersal (Anderson et al. 2011c; Bansemer & Bennett 2009; Chevolut et al. 2006; Daly-Engel et al. 2012; Feldheim et al. 2004; Feldheim et al. 2014; Mourier & Planes 2013). However, to test whether male *M. alfredi* in the Maldives roam more widely, or if sexual segregation explains the differences in the observed visitation rates between the sexes, a combination of the aforementioned methodologies are required if a complete picture of the habitat utilisation of this species is to be attained.

2.5.2 Young of the year

The smallest individual recorded in the study was estimated to be 140 cm in disc width and the largest young of the year (YoY) was 170 cm. The average size of all YoY (N = 41) in my study was consistent with the current literature (Kashiwagi 2014; Marshall & Bennett 2010a) at 160 cm in disc width, and juveniles were estimated to grow about 11 cm a year. However, growth rates for *M. alfredi*, as is the case for the majority of other elasmobranchs, are unlikely to be linear from birth to death. Indeed, growth curves fitted to other elasmobranch species usually produce a reverse exponential shape, producing growth rates declining linearly with age (Smart et al. 2016).

On average, only four YoY were recorded annually throughout the study period, however the distribution of these records was highly variable, with 66% (N = 27) of all the YoY recorded in 2015. It is possible some YoY were misidentified as older individuals when first sighted, therefore a truer representation of the inter-annual variation in the number of newborn entering the population is likely to be the number of newly sighted size class one individuals, which was on average 27 individuals annually. Each year, among the *M. alfredi* individuals recorded as new to the study, the proportion which were recorded as size class one fluctuated, following the same pattern as the YoY. Sightings peaked in 2009 and 2015, with a trough in 2012 and 2013. These fluctuations coincide with similar variations in reproductive activity (pregnancies and courtship behaviour) recorded during the same period, suggesting fecundity is highly variable inter-annually (Chapter 4).

2.5.3 Size and age at maturity

The maximum disc width estimates of 360 cm for females and 310 cm for males were consistent with the Deakos (2010) study in Hawaii, although smaller than elsewhere; Japan 420 cm (Kashiwagi et al. 2010), eastern Australia 450 cm (Couturier et al. 2014) and Mozambique ~500cm (Marshall & Bennett 2010a). Although differences in environmental conditions between these countries may account for some variation in manta size, the different methods used to estimate disc widths are also likely to affect findings. Indeed, the previous Maldives study by Kitchen-Wheeler estimated maximum disc widths for *M. alfredi* of 450 cm (2011), 25% larger than the maximum estimated in this study. Future efforts to estimate *M. alfredi* size should therefore employ more accurate methodologies, such as the use of stereo-video techniques (Watson et al. 2010), especially when attempting to measure growth.

Males were estimated to undergo maturation between disc widths of ~260 – 280 cm based on clasper morphology. This subadult stage for the majority of individuals took two years, ranging from one to three. All males exceeding 280 cm in disc width were determined to be mature and size at maturity was estimated to be ~270 – 280 cm in disc, consistent with Deakos (2010). However, intense mating competition between male mantas means only a small proportion of the morphologically mature individuals are likely to be sexually successful (Chapters 3 and 4). Female sexual maturity can only be determined externally by indicators of mating activity. In this study, no female smaller than 320 cm in disc width was observed pregnant, or having mating scars or wounds. Size at maturity was therefore estimated to be ~320 – 330 cm in disc width, also consistent with Deakos (2010), but smaller than in other studies (Couturier et al. 2014; Kashiwagi 2014; Marshall & Bennett 2010a).

Sexual maturity for *M. alfredi* in the Maldives, as in Hawaii (Deakos 2011) and Japan (Kashiwagi 2014), appears delayed until growth exceeds 90% of maximum size for both sexes, indicating that a large body provides a reproductive advantage at the expense of a shorter reproductive time period. Indeed, Deakos (2010) found that larger females were pregnant more often, and suggested that larger males may benefit from greater energy reserves while competing with other males for a mate during courtship. Female *M. alfredi* invest heavily in reproduction and the larger disc widths attained (16% larger than males in this study) throughout the range of this species are likely an adaptation to increase physiological

resources for reproduction, resulting in the production of larger and healthier offspring (Cortés 2000; Hussey et al. 2010).

The number of individuals within each size class increased with size for both sexes, with the majority of all individuals recorded as mature. Indeed, only 4% (N = 166) of the recorded population were classified as size class one individuals, suggesting relatively few *M. alfredi* are born each year and they grow rapidly during the first few years, consistent with the findings of Kashiwagi (2014). A study by Fisher et al. (2013) in Chesapeake Bay upon a close relative of mantas, the cownose ray (*Rhinoptera bonasus*), found that juveniles grew considerably faster during the first few years, a common strategy in many elasmobranchs to reduce risk of predation (e.g., Kusher et al. 1992; White et al. 2001). The Chesapeake Bay study also found that juvenile males grew faster than females, reaching sexual maturity earlier and at a smaller size, as did Kashiwagi (2014) in Japan.

Despite faster growth in the first few years after birth, *M. alfredi* appear to have among the slowest maturation periods of any elasmobranch species, with only 11% (N = 208) of individuals (N = 1,946) which were categorised as juveniles on their first sighting maturing during the study. Age at maturity was estimated to be 9 – 10 years for males and 16 – 17 years for females based on photo-ID recapture sighting spans and external indicators of maturation, and 9 – 13 years for males and 13 – 17 for females based on growth rates.

Estimates of age at maturity for *M. alfredi* are currently based on sightings of just a handful of individuals. A study in Hawaii (Clark 2010) estimated age at maturity for males to be 3 – 6 years based on the sighting spans and external indicators of maturation of two juveniles, assumed to be young of the year (YoY) when first sighted with estimated disc widths of 150 and 180 cm. A study in Japan (Kashiwagi 2014) estimated age at first reproduction for males to be ~10 years, where two juveniles, estimated to be YoY when first sighted, attained maturity between 4 – 9 years. The same study also estimated age at first reproduction for females to be ~10 years, although estimates for age at maturity ranged from 8 – 15 years from a sample of nine individuals.

Despite the methodological restrictions in this study, whereby age at maturity estimates may be biased by the assumption that all individuals grow at the same rate and mature at approximately the same age, all YoY individuals (N = 41) observed during the study were still juvenile when last seen, including the 9 with the longest sighting spans of 5 – 10 years. Furthermore, of the 71 females which were juveniles when first sighted and have sighting

spans of ≥ 9 years, only 27% (N = 19) were observed to have matured by the end of the study. As the sighting span represents the minimum possible age for all of these individuals, only one of which was considered to be YoY when first sighted, the estimate of 16 – 17 years for the average age at maturity for female *M. alfredi* is unlikely to be an overestimate for the majority of individuals. Likewise, of the 41 males which were juveniles when first sighted and have sighting spans of ≥ 9 years, none were considered to be YoY when first sighted, including the two individuals which were still subadults at the end of the study after sightings spans of 9 years. The sighting span data is further supported by the growth estimates. If male and female *M. alfredi* grow at the same rate (or males slightly faster); do not reach maturity until 90% of their maximum disc width has been attained; and females grow 16% larger than males, the estimated ages at maturity of 9 – 13 years for males and 13 – 17 for females would be expected.

It is therefore likely that the Hawaii study by Clark (2010) underestimated male age at maturity and the Japan study by Kashiwagi (2014) underestimated female age at first reproduction. Increasing maximum size is often correlated with increasing age of maturity in elasmobranchs (Dulvy et al. 2008). Manta rays are the largest of all batoids, therefore the size and age at maturity estimates from this study are further supported when data from a much smaller cousin of the manta rays is considered. Male *M. alfredi* mature at a disc width of ~ 270 – 280 cm, and females at ~ 320 – 330 cm, yet even in *R. bonasus*, which attain maturity at a disc width of 85 – 86 cm in both sexes, individuals still take ~ 6 – 7 and ~ 7 – 8 years to mature for males and females respectively (Fisher et al. 2013). Indeed, on average across a sample of 52 species, male batoids mature at an age of 6.9 years, and female slightly older at 8.6 years (Frisk 2010).

The longer time (~ 5 years) which it takes female *M. alfredi* to attain maturity would also explain why there is a shift in the demographic sex ratio between the maturity statuses of the population. Despite an evenly split sex ratio in the total study population, there are twice as many juvenile females as males (N = 1,165 females, N = 573 males), while conversely there are 1.6 adult males (N = 1,372) for every adult female (N = 845). For both sexes there is a trade-off between maximising reproductive time and reproductive success. These trade-offs influence the operational sex ratio and fecundity of *M. alfredi* populations, the species' reproductive strategies and courtship, and mating behaviours, which are all explored in Chapters 3 and 4.

2.5.4 Longevity

The longest sighting span recorded in this study was 24 years, although very little sighting data was available from the study population prior to 2005, making it hard to accurately document longevity. However, 83% (N = 78) of individuals with sighting spans greater than 10 years were adults when first observed, supporting conclusions that *M. alfredi* live for at least 30 years (Clark 2010; Couturier et al. 2014; Homma et al. 1999; Kashiwagi et al. 2010). Indeed, a mature *M. alfredi* recorded with a 30 year sighting span in Australia was mature when first observed, suggesting maximum longevity for this species is likely to be at least 40 years (Couturier et al. 2012), higher than the average for other batoids of 17 and 15 years for females and males respectively (Frisk 2010). The three individuals in this study with sighting spans of ≥ 20 years are among only ~ 30 *M. alfredi* globally recorded with longevity estimates exceeding 20 years, the majority of which come from one study in Japan (Kashiwagi 2014). However, this study is just one of half a dozen studies globally which have been consistently collecting photo-ID data on manta rays for more than a decade.

Comparative analyses of the life histories of batoid species by Frisk (2010) found the ratio of age at maturity (α) and longevity (ω) to be $\alpha/\omega = \sim 0.5$ for batoids (0.5 females, 0.45 males), above those of teleost fishes (0.2 – 0.4) (Winemiller & Rose 1992), suggesting batoids reach maturity later and have fewer reproductive years (relative to the length of their juvenile stage), compared to most teleosts. More widely, Beverton and Holt (1957) noted the Length relationship to be $L\alpha/L^\infty = \sim 0.7$ as a central tendency for teleost fish ($\infty = \text{maximum}$). The age ratio for female *M. alfredi* in this study was 0.38 (= 15/40) and 0.28 (= 11/40) for males (using the mean age at maturity estimate across both methodologies for each sex), and 0.90 (= 3.25/3.6) for female and 0.89 (= 2.75/3.1) for male length (mean size at maturity disc width) relationships. Similar values of 0.25 and 0.90 for female *M. alfredi* were found in Japan (Kashiwagi 2014), suggesting these values may characterise the life history strategy of this species as; large, growing relatively quickly to full size, enabling long reproductive lifespans.

2.5.5 Conclusions

This multi-decade study on an isolated and unfished population of *M. alfredi* recorded the world's largest known population of this species; characterising habitat use, the population demographics, age and size at maturity, longevity, and YoY abundance. The findings of this study suggest that sampling over many years throughout the geographical range of a *M.*

alfredi population and across different habitat types used by this species is important to ensure a representative sample of the population demographics.

The use of shallow coral reefs by *M. alfredi* may be a function of behavioural thermoregulation and predator avoidance, with cleaning stations acting as focal gathering points where social behaviour is undertaken, such as courtship and mating. Indeed, the function of cleaning for parasite removal, or wound healing, may often be a secondary or tertiary driver of site use. The frequent use of shallow protected lagoons by juvenile *M. alfredi* suggests these sites may act as nursery areas, a common predator avoidance technique employed by a wide variety of juvenile shark and ray species (Heupel et al. 2007).

Maximum disc widths and size at maturity for males and females were consistent with findings from Hawaii, although smaller than in other studies. Age at maturity for both males and females may be higher than currently reported in the literature, although size at birth and maximum longevity estimates were consistent with previous studies. In general, the findings of this study support the trend that larger batoid species are longer-lived, slower growing and have a lower reproductive output than smaller species (Dulvy & Reynolds 2002; Frisk et al. 2005; Frisk et al. 2001).

M. alfredi have among the lowest fecundity of all elasmobranchs (Dulvy et al. 2014b; Chapter 4), with low natural mortality (Kashiwagi 2014). Current estimates of life history parameters have generation span at 11 years (Dulvy et al. 2014b), which this study suggests should be increased to 16 years (mean age at maturity for females of 15 years, plus one year gestation). This increased estimation of generation span, coupled with their low fecundity, has serious implications for the management and conservation of *M. alfredi*. Even small increases in mortality are likely to be unsustainable for a population, marking *M. alfredi* as highly vulnerable to human impacts in general and especially to over-fishing, which is a major threat given the lucrative market for manta gill plates in Asian medicine (O'Malley et al. 2016). This study validates the importance of undertaking long-term studies on species with conservative life history strategies if important ecological questions are to be answered (Clutton-Brock & Sheldon 2010) and the formulation of effective management plans are to be achieved (Cortés 1998; Cortés 2002).

Chapter 3

Courtship and mating behaviour of
manta rays (*Manta alfredi* and *M.*
birostris) in the Maldives

3.1 Abstract

Manta rays (*Manta* spp.) are among the most conspicuous and charismatic of the elasmobranchs, however their courtship and mating behaviour is rarely observed. Although the mating stages of manta reproduction have been described, the full detail of their elaborate courtship has not. The aim of this decade-long study was to elucidate the entire courtship and mating behaviour of both manta species (*M. alfredi* and *M. birostris*) using behavioural observations, video and photographic records. From November 2003 through 2013, over 4,000 surveys were undertaken at known manta ray aggregation sites in the Maldives to record any observed manta ray reproductive activity. A total of 24,565 photo-ID sightings of 3,186 individual *M. alfredi* and 70 photo-ID sightings of 68 individual *M. birostris* were recorded in 19 atolls at 171 different sites. Courtship activity was observed on 84 surveys at 17 different sites. A total of 101 courtship events were recorded, with 83% of them at cleaning sites. The observed courtship activity was categorised into seven distinct courtship stages which are described in detail: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5) copulation, (6) post-copulatory holding, and (7) separation. Photographs provide the first scientific record of the entirety of manta courtship and mating, thereby enhancing the knowledge of these events. Both *M. alfredi* and *M. birostris* appear to engage in the same elaborate courtship rituals, exhibiting the same behaviours during all stages of the courtship and mating process.

Keywords: courtship rituals, copulation, reproductive behaviour, cleaning stations, mate choice

3.2 Introduction

Although much is known about the reproductive anatomy and different modes of elasmobranch reproduction (Carrier et al. 2012; Dodd 1983; Gilmore 1993; Wourms 1977; Wourms & Demski 1993), shark and ray reproductive behaviour is poorly understood, mainly due to the difficulties of observing natural behaviour in free-living animals and the artificial nature of studies in captivity (Whitney et al. 2004). Most recently Pratt and Carrier (2005) summarised the published literature on elasmobranch reproductive behaviour, but their work represented just a small fraction of the total species pool. In their study, many reports came from captive animals and little photographic documentation was provided.

Manta rays are highly conspicuous and charismatic elasmobranchs. These zooplanktivorous rays belong to the Mobulidae family which comprises nine species in the Genus *Mobula* (Rafinesque 1810) and two in the Genus *Manta* (Bancroft 1829). The latter was re-classified in 2009 (Marshall et al. 2009) when the monospecific genus was split into the two species of manta currently recognized: the reef manta (*Manta alfredi*) (Krefft 1868) and the oceanic manta (*Manta birostris*) (Walbaum 1792). A third putative species, the Caribbean manta (*Manta c.f. birostris*), has also been proposed (Marshall et al. 2009). *M. alfredi* are widely distributed throughout the tropical and sub-tropical waters of the Pacific and Indian Oceans, although populations appear to be highly fragmented (Couturier et al. 2012; Kashiwagi et al. 2011), most likely due to resource and habitat requirements (Anderson et al. 2011b). *M. alfredi* frequent the coastal reefs of continents and remote oceanic islands (Kashiwagi et al. 2011; Marshall et al. 2011b), but also venture offshore into the mesopelagic zone (Braun et al. 2014; Jaime et al. 2014). Likewise, *M. birostris* is distributed throughout the tropics, but is also found in sub-tropical and temperate waters within 41° of latitude north and south (Kashiwagi et al. 2011). It is thought that *M. birostris* spend the majority of their time offshore, but come into shallower regions along productive coastlines with regular nutrient upwellings (Marshall et al. 2011c; Stewart et al. 2016a). *M. birostris* also aggregate around oceanic island groups and offshore pinnacles, seamounts and submarine ridge systems, where they are known to engage in courtship (Compagno & Last 1999; Marshall et al. 2011c; Rubin 2002; Stewart et al. 2016b; Yano et al. 1999).

Manta rays are ovoviviparous matrotrophs (Dulvy & Reynolds 1997; Wourms 1977) and like all elasmobranchs, employ internal fertilization (Conrath & Musick 2012). The gestation time of *M. alfredi* is reported to be one year (Deakos 2011; Marshall & Bennett 2010a; Okinawa Churaumi Aquarium 2010), but remains unknown for *M. birostris*. Reproductive cycles often include resting periods, with biennial reproduction reported as the norm for individual *M. alfredi* within populations in Hawaii and Mozambique (Deakos 2011; Marshall & Bennett 2010a). Inter-birth intervals of several years or more are common in matrotrophs which also invest heavily in post parturition parental care, such as Sumatran orangutans (*Pongo pygmaeus abelii*), capuchins (*Cebus capucinus*), African and Asian elephants (*Loxodonta africana* and *Elephas maximus*), and bottlenose dolphins (*Tursiops sp.*) (Fedigan & Rose 1995; Lee & Moss 1986; Mann et al. 2000; Robinson et al. 2012; van Noordwijk & van Schaik 2005), but are less common in species which do not. In species which do not undertake post parturition parental care, biennial and triennial reproductive cycles have been reported in reptiles (Cree & Guillette 1995; Ibarra-Guerrero & Cussac 1996; Sever et al. 2000) and

elasmobranchs (Castro 2009; Colonello et al. 2006; Whitney & Crow 2006). Rest periods are most likely a mechanism to allow females to recuperate energy reserves (Catry et al. 2006; Trinnie et al. 2012). Manta rays normally give birth to a single, large pup (Beebe & Tee-Van 1941a; Bigelow & Schroeder 1953; Coles 1916), although rare cases of twins have been recorded (Marshall & Bennett 2010a). Their size at birth ranges from 130 – 190 cm in disc width (Marshall & Bennett 2010a; Okinawa Churaumi Aquarium 2010; Chapter 2).

Manta ray reproductive behaviour in the wild has rarely been observed and virtually all observations of courtship and copulation have been of *M. alfredi*, with just one documented mating of *M. birostris*. This single observation was recorded off the Ogasawara Islands, Japan in 1997 and describes a female copulating with two different males on the same day (Yano et al. 1999). The majority of manta ray courtship and mating has been reported at feeding and cleaning aggregation sites (Deakos 2011; Marshall & Bennett 2010a). Observations of mating events for *M. alfredi* in the wild currently number just five (Marshall & Bennett 2010a) and all were of females mating once, with one male. However, observations in the Maldives suggest female *M. alfredi* mate multiple times with multiple males during each courtship period (Chapter 4).

Field observations in Japan, Mozambique and Hawaii indicate the same complex mating process is undertaken by both manta species, which involves a five-step sequence of (1) chasing, (2) biting, (3) copulating, (4) post-copulation holding and (5) separation (Deakos 2011; Marshall & Bennett 2010a; Yano et al. 1999). Copulation occurs when the female slows to allow a pursuing male to position himself directly on top of her dorsal surface. At this point the male slides his mouth down the side of the female's body, nearly always her left, to the tip of her pectoral fin, before taking about a metre of this fin into his mouth. The male then bites down hard to gain leverage on the female's body. Twisting underneath her the male positions himself so that the female's ventral surface is in alignment with his, enabling him to insert a clasper into her cloacal opening before releasing his seminal fluid (Marshall & Bennett 2010a; Yano et al. 1999).

Although observations of actual copulation are extremely rare, pre-copulatory chasing has been more commonly observed, especially of *M. alfredi*, in which multiple escorting males pursue a single, fast swimming female in what is commonly termed a 'mating train' (Deakos 2011; Marshall & Bennett 2010a). This behaviour appears to be the basis of pre-copulatory mate choice by the females (Deakos 2011; Whitney et al. 2004). Also known as 'female recruitment runs' (Deakos 2011), they can last for hours. Because this pre-copulatory

courtship behaviour does not always result in mating (pers. obs.), and appears to be driven primarily by female mate choice, this behaviour hereafter is referred to as a 'courtship train'.

During courtship trains the female initiates high speed flips, turns and somersaults, while pursuing males mimic her evasive manoeuvres (Deakos 2011; Marshall & Bennett 2010a). Because several different behaviours appear to take place within step one of the courtship classification described by Yano et al. (1999), Marshall and Bennett (2010) proposed this step should be broken down into three subdivisions namely: (1) following or chasing, (2) a complex series of interactive turning and flipping performed by both the female and her suitors, and (3) evasive or avoidance behaviour exhibited by the female.

Courtship trains have been observed in several close relatives of mantas, namely in: flapnose ray (*Rhinoptera javanica*), cownose ray (*Rhinoptera bonasus*), spotted eagle ray (*Aetobatus narinari*), spinetail devil ray (*Mobula japonica*) and sicklefin devil ray (*Mobula tarapacana*) (pers. obs.; Tricas 1980; Uchida et al. 1990). Whitetip reef sharks (*Triaenodon obesus*) and nurse sharks (*Ginglymostoma cirratum*), also display the 'mating avoidance' shown in mantas whereby a female 'arches' her body during attempted copulation by males to keep her cloaca out of their reach (Pratt & Carrier 2001; Whitney et al. 2004).

There are also parallels between the courtship trains of manta rays and the tending behaviour undertaken by ungulates, where a male will associate with an oestrous female until he either copulates with her or is displaced by another male (Byers et al. 2005; Kucera 1978; Mysterud et al. 2004; Vos et al. 1967; Wolff 1998). In marine mammals similar behaviour has been observed in humpback whales (*Megaptera novaeangliae*). The general structure of humpback whale competitive mating groups, known as 'heat runs', was first described by Tyack and Whitehead (1982), who noted that these groups typically contain a central, relatively passive 'Nuclear Animal', usually a female, and a 'Principal Escort', usually the largest male (Spitz et al. 2002), who maintains position close to the Nuclear Animal and frequently fends off challenges from other males. To ensure that he can mate with the female after she gives birth, the Principal Escort attempts to hold his position next to the female throughout her late pregnancy (Baker & Herman 1984; Herman et al. 2007).

While the major stages of manta mating have already been described, this study will provide more detail. Hence, the aim of this decade long study was to elucidate the entire courtship and mating behaviour of both manta species using behavioural observations backed up by video and photographic records. Field research was undertaken in the Maldives in the Indian

Ocean, home to the world's largest known population of *M. alfredi* (Kitchen-Wheeler et al. 2011; Chapter 2). *M. birostris* occurs there at several locations where remote seamounts rise deeply from extremely deep water (Kashiwagi et al. 2011).

3.3 Methods

3.3.1 Study area, duration and sampling protocol

The 26 coral atolls which form the Maldives archipelago extend from 7° north down 870 km to half a degree south of the equator in the Indian Ocean (Fig. 3.1). During a decade-long study from November 2003 through to the end of 2013 over 4,000 surveys throughout the Maldives were undertaken at known aggregation sites for the manta rays *M. alfredi* and *M. birostris* to observe and photographically record courtship and reproductive behaviour. In total 24,565 photo-ID sightings of 3,186 individual *M. alfredi* and 70 photo-ID sightings of 68 individual *M. birostris* were made from 19 atolls at 171 different sites. At each site surveyed, the predominant behaviour of the observed manta rays was either feeding, or cleaning (Côté 2000; Feder 1966; Losey Jr 1972). In total 65 (38%) of the sites surveyed were considered primarily as cleaning sites, 82 (48%) as feeding sites, while the remaining 24 (14%) were sites where sightings consisted mostly of animals travelling through the area (see Chapter 2 methodology for site type descriptions).

In the Maldives, manta rays are accustomed to interacting with tourist divers and snorkellers at aggregation sites where the rays predictably gather at certain times of the year to feed, clean and socialise (Anderson et al. 2011a). A typical survey during this study entailed diving or freediving at one of these aggregation sites, where close encounters with the unperturbed rays easily allowed photo-ID images to be taken and observations recorded of the individuals present. Manta surveys were performed on SCUBA or while freediving from either a dedicated research vessel or commercial diving vessels. Surveys were performed at different times of day in all months of the year throughout the month. SCUBA surveys lasted on average 60 minutes and ranged to a maximum depth of 30 metres. Freediving surveys lasted on average 120 minutes. Myself, or a trained staff member or volunteer from the Manta Trust, conducted the surveys (www.mantatrust.org/in-the-field/maldives/).

When manta rays were encountered, where possible the following information was recorded for each individual: (1) species, (2) photo-ID, (3) sex, (4) female pregnancy status, (5) maturity status, and (6) behavioural activity. Behavioural activity was further broken down into four major groups: (1) feeding, (2) cleaning, (3) cruising, and (4) courtship. During the course of

an encounter an individual may undertake several different activities. In these situations the activity which dominated the encounter was recorded as the primary behaviour. Only the courtship behaviour is considered here. For a detailed description of each of the criteria listed above and the complete methodology, see Chapter 2.

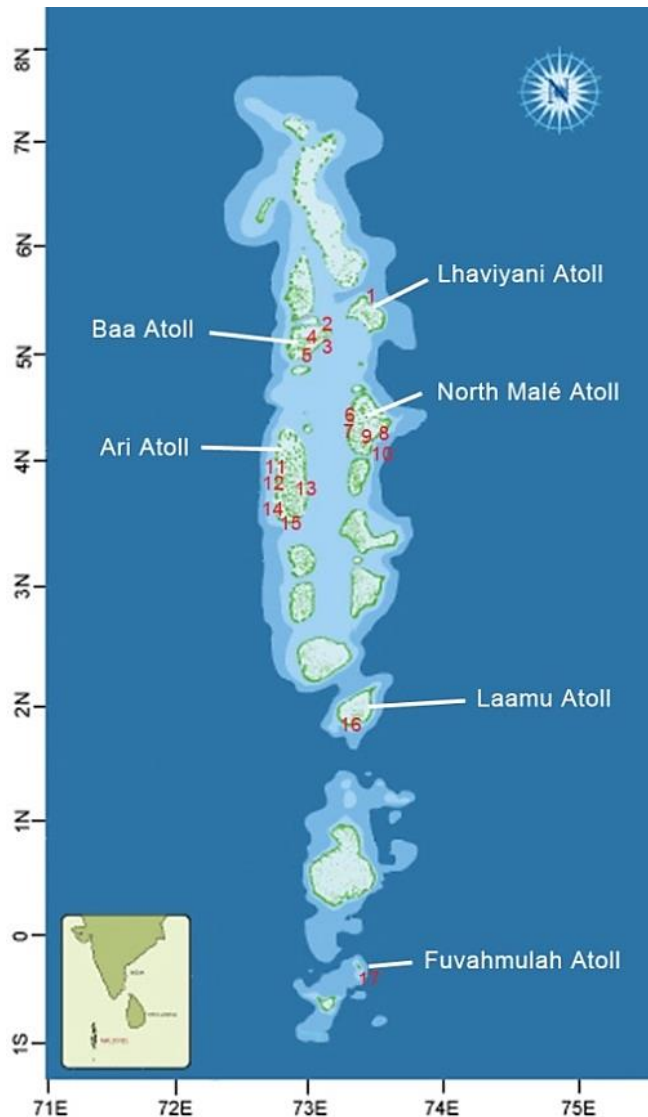


Figure 3.1 Map of the Maldives Archipelago showing the atolls, illustrated in green. Dark blue indicates where the sea floor exceeds 1,000 m; middle toned blue where it is between 500 – 1,000 m and the lightest blue where 500 m or shallower. The depth of the sea floor inside the lagoon of all the atolls is < 100 m. 1° latitude = 111 km. Red numbers indicate the location of the study sites where courtship and mating behaviour were recorded.

Notes on underwater slates were made of all courtships observed and where possible the individuals involved were photographed or videoed. Of these, 75% were captured as still photographs using a Nikon D700 SLR with a 16 mm wide angle lens, or a Sea & Sea DX1G compact underwater camera with wide angle converter lens. The remaining 25% were taken by a variety of other compact underwater cameras and video cameras. The observed courtship activity was categorised using a methodology developed during this study after initial observations found that the courtship and mating stages proposed by Yano et al (1999) and Marshall and Bennett (2010) did not accurately encompass, or define, all of the

behaviours observed. As a result, the following seven distinct courtship stages are identified: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5) copulation, (6) post-copulatory holding, and (7) separation (Table 3.1).

Given the scarcity of courtship or mating behaviour reported in the literature, and the rarity of observing these events in the wild, an extensive search of the on-line search engine *YouTube* (www.youtube.com) was performed in an attempt to gather further observational data to use in this study. Using different combinations of the key words ‘manta’, ‘mating’, ‘courtship’ and ‘copulation’ the search engine produced results for ~8,000 videos. All result videos containing relevant information were viewed (~150) and the manta behaviour exhibited was recorded. All information was collated and transferred to an Excel database for analysis.

Table 3.1 Description and observational notes on the courtship and mating stages of *Manta alfredi* and *Manta birostris*.

| Stage number | Stage name | Description of behaviour and observational notes |
|--------------|----------------------------|---|
| 1 | Initiation | Male/s 'shadow' the female's movements, following behind her; both the male/s and female's swimming speed remains consistent with normal manta ray cruising speed. A male may attempt to position his body on top of the female's back facing in the same direction. The male unfurls his cephalic fins and positions them so they are in contact with the dorsal head region of the female. |
| 2 | Endurance | Increased swimming speed, rapid 'chase' ensues where males (1- 26+) follow in single file 'courtship train' closely behind the tail of the female with their cephalic fins often unfurled, but mouths closed. The female's cephalic fins usually remain furled. Courtship trains can last for many hours at a time, periodically progressing to further stages of the courtship process and/or reverting back to earlier stages. |
| 3 | Evasion | Female makes erratic twists and turns at increased swimming speeds; targeting obstacles and other manta rays in the water column to manoeuvre around, they undertake backward somersaults, forward flips, and may also leap clear of the water. The males attempt to mirror the female's movements. |
| 4 | Pre-copulation positioning | Female reduces swimming speed, often rising in the water column close to the surface. The male positions himself on top of the female's back whereupon he uses his unfurled cephalic fins to guide his mouth down the leading edge of the female's pectoral fin (nearly always the left fin) until his open mouth engulfs the tip and grasps it firmly. Once the male has a firm grip he rotates his body underneath the female until the pair are positioned abdomen to abdomen. |
| 5 | Copulation | Copulation occurs near the surface, abdomen to abdomen, as the male continues to beat his pectoral fins while the female is usually motionless. Male makes rapid pelvic thrusts and the intertwined pair often spiral around in a clockwise motion while slowly sinking. Copulation lasts for approximately 30-40 seconds. |
| 6 | Post-copulation holding | Male may maintain grasp of the female's pectoral fin momentarily (several seconds only) after his clasper has been removed from her cloacal opening, although this stage may be skipped altogether with separation commencing simultaneously upon cessation of copulation. |
| 7 | Separation | The pair separate and swim off in different directions, although additional pursuing males may initiate another courtship and mating event with the same female. |

3.4 Results

Courtship activity was observed on 84 surveys at 17 different sites. Although it was not possible to identify every individual involved, 214 different *M. alfredi* (73 females and 141 males) and 4 *M. birostris* (1 female and 3 males) were individually identified by their unique ventral spot markings (Marshall & Pierce 2012). Over 10 years, a total of 101 courtship events

were recorded (Table 3.2), with 83% occurring at cleaning sites (70 surveys). All but one courtship event was by *M. alfredi*.

Using the seven stages of manta courtship behaviour described in the methodology, 37 instances of 'initiation' (stage 1) were observed, 71 of 'endurance' (stage 2), 20 of 'evasion' (stage 3), two of 'pre-copulation positioning' (stage 4), one of 'copulation' (stage 5), no 'post-copulation holding' (stage 6) and one of 'separation' (stage 7). Separation without post-copulation holding (stage 6) occurred because in the one case where copulation was observed the couple separated immediately.

Only one courtship event was observed for *M. birostris* which involved 8 males in a train behind one female. For *M. alfredi*, of the 37 initiation events instigated only 9 (24%) advanced to the endurance stage of a courtship train. The number of males involved in these trains varied greatly, with a minimum of one male chasing a single female, to a maximum of 26. The average ratio of males to females in a single train was 3:1 (SE \pm 0.3), although this rose to 5:1 (SE \pm 1.2) if the courtship escalated to the evasion stage. On four occasions, two females were involved in a single train (events 36, 62, 98 and 101 Table 3.2). During all of these events the second of the two females was directly behind the lead female and in event number 36 (Table 3.2) both females were pregnant and, based on their highly distended abdomens, were close to parturition. In total 16 near-term pregnant females (15% of the total number of females observed) were seen to engage in courtship during the study. 11 of the near-term pregnant females were engaged in 10 of the 71 observed courtship trains and/or evasive behaviour and the remaining 5 were involved in courtship initiation events which did not progress to courtship trains.

Table 3.2 Courtship and mating events of manta rays (*Manta alfredi* and *Manta birostris*) recorded throughout the Maldives from November 2003 through to December 2013. (-) signifies courtship and/or mating stage was not observed, (o) signifies stage was observed, (x) signifies stage did not occur, (P) signifies visible pregnancy.

| Event | Date | Aggregation site | Atoll | Site no. | Species | No. females | No. males | Stage 1 (initiation) | Stage 2 (endurance) | Stage 3 (evasion) | Stage 4 (pre-copulation positioning) | Stage 5 (copulation) | Stage 6 (post-copulation holding) | Stage 7 (separation) |
|-------|------------|-------------------|------------|----------|------------------------|-------------|-----------|----------------------|---------------------|-------------------|--------------------------------------|----------------------|-----------------------------------|----------------------|
| 1 | 19/11/2003 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | o | o | o | x | o |
| 2 | 06/01/2004 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 2 | - | o | o | o | - | - | - |
| 3 | 23/09/2005 | Fushifaru Kandu | Lhaviyani | 1 | <i>Manta alfredi</i> | 1-P | 1 | o | o | - | - | - | - | - |
| 4 | 07/04/2006 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 5 | 06/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 5 | - | o | - | - | - | - | - |
| 6 | 07/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 5 | - | o | - | - | - | - | - |
| 7 | 10/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | o | - | - | - | - |
| 8 | 10/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 4 | - | - | o | - | - | - | - |
| 9 | 10/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 6 | - | - | o | - | - | - | - |
| 10 | 13/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 4 | - | o | o | - | - | - | - |
| 11 | 13/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 7 | - | o | o | - | - | - | - |
| 12 | 13/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 6 | - | o | - | - | - | - | - |
| 13 | 13/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 14 | 15/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 15 | 15/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 16 | 18/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 17 | 18/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 2 | - | o | - | - | - | - | - |
| 18 | 21/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 19 | 21/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 20 | 22/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | o | - | - | - | - | - |
| 21 | 22/10/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 22 | 07/11/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 23 | 07/11/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 24 | 08/11/2006 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 25 | 14/01/2007 | Dhonkalo Thila | Ari | 11 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 26 | 27/01/2007 | Himendhoo Thila | Ari | 12 | <i>Manta alfredi</i> | 1-P | 1 | - | o | - | - | - | - | - |
| 27 | 08/02/2007 | Dhonkalo Thila | Ari | 11 | <i>Manta alfredi</i> | 1-P | 1 | o | o | - | - | - | - | - |
| 28 | 16/02/2007 | Rasfari North | North Malé | 7 | <i>Manta alfredi</i> | 1-P | 1 | o | - | - | - | - | - | - |
| 29 | 08/03/2007 | Dhonkalo Thila | Ari | 11 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 30 | 01/08/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 7 | - | o | o | - | - | - | - |
| 31 | 03/09/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 2 | o | - | - | - | - | - | - |
| 32 | 20/09/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 2 | o | - | - | - | - | - | - |
| 33 | 20/09/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 34 | 15/10/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 35 | 17/10/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 4 | - | o | - | - | - | - | - |
| 36 | 29/10/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 2-P | 3 | - | o | o | - | - | - | - |
| 37 | 01/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 5 | - | o | - | - | - | - | - |
| 38 | 03/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 39 | 03/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 40 | 07/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 26 | - | o | o | - | - | - | - |
| 41 | 08/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 1 | o | - | - | - | - | - | - |
| 42 | 15/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 43 | 22/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 44 | 24/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 45 | 24/11/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 46 | 13/12/2007 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 2 | o | o | - | - | - | - | - |
| 47 | 31/12/2007 | Boduhithi Thila | North Malé | 6 | <i>Manta alfredi</i> | 1 | 6 | - | o | - | - | - | - | - |
| 48 | 01/03/2008 | Dhonkalo Thila | Ari | 11 | <i>Manta alfredi</i> | 1 | 8 | - | o | o | - | - | - | - |
| 49 | 25/05/2008 | Dhigu Thila | Baa | 3 | <i>Manta alfredi</i> | 1 | 1 | o | o | - | - | - | - | - |
| 50 | 12/08/2008 | Dhigu Thila | Baa | 3 | <i>Manta alfredi</i> | 1 | 7 | - | o | o | - | - | - | - |
| 51 | 20/09/2008 | Hanifaru Bay | Baa | 4 | <i>Manta alfredi</i> | 1-P | 5 | - | o | o | - | - | - | - |
| 52 | 27/10/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 53 | 29/10/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 54 | 04/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 2 | - | o | - | - | - | - | - |
| 55 | 04/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 56 | 05/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 57 | 05/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 58 | 08/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 59 | 08/11/2008 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | o | o | - | - | - | - |
| 60 | 12/11/2008 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 4 | - | o | - | - | - | - | - |
| 61 | 23/09/2009 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 62 | 04/10/2009 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 2 | 2 | - | o | o | - | - | - | - |
| 63 | 14/11/2009 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1-P | 7 | - | o | o | - | - | - | - |
| 64 | 01/12/2009 | Dhonkalo Thila | Ari | 11 | <i>Manta alfredi</i> | 1-P | 1 | - | o | - | - | - | - | - |
| 65 | 30/08/2010 | Hanifaru Bay | Baa | 4 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 66 | 01/09/2010 | Hanifaru Bay | Baa | 4 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 67 | 05/11/2010 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 68 | 13/01/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 69 | 17/01/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 70 | 17/01/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 71 | 09/03/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 72 | 15/03/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 73 | 15/03/2011 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 74 | 15/03/2011 | Maamigili Beyru | Ari | 15 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 75 | 08/04/2012 | North Point | Fuvahmulah | 17 | <i>Manta birostris</i> | 1 | 8 | - | o | o | - | - | - | - |
| 76 | 03/11/2012 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 77 | 04/11/2012 | Huravalhi Falhu | Ari | 13 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 78 | 08/11/2012 | Huravalhi Falhu | Ari | 13 | <i>Manta alfredi</i> | 1 | 2 | o | o | - | - | - | - | - |
| 79 | 15/11/2012 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 80 | 15/11/2012 | Hurai Faru | Baa | 2 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 81 | 01/01/2013 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 4 | - | o | - | - | - | - | - |
| 82 | 15/01/2013 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 5 | - | o | o | - | - | - | - |
| 83 | 04/03/2013 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 84 | 14/03/2013 | Rangali Madivaru | Ari | 14 | <i>Manta alfredi</i> | 1 | 3 | o | o | - | - | - | - | - |
| 85 | 05/06/2013 | Hanifaru Bay | Baa | 4 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 86 | 06/06/2013 | Hurai Faru | Baa | 2 | <i>Manta alfredi</i> | 1 | 3 | - | o | o | - | - | - | - |
| 87 | 06/07/2013 | Hanifaru Bay | Baa | 4 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 88 | 10/07/2013 | Maaneigaa Faru | Baa | 5 | <i>Manta alfredi</i> | 1 | 7 | - | o | - | - | - | - | - |
| 89 | 27/07/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 90 | 01/09/2013 | Gastinolu Beyru | North Malé | 8 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 91 | 24/09/2013 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 2 | - | o | o | - | - | - | - |
| 92 | 01/10/2013 | Lankan Beyru | North Malé | 10 | <i>Manta alfredi</i> | 1 | 1 | o | - | - | - | - | - | - |
| 93 | 29/10/2013 | Hiithadhoo Corner | Laamu | 16 | <i>Manta alfredi</i> | 1 | 2 | - | o | - | - | - | - | - |
| 94 | 30/10/2013 | Hiithadhoo Corner | Laamu | 16 | <i>Manta alfredi</i> | 1 | 4 | - | o | - | - | - | - | - |
| 95 | 30/10/2013 | Hiithadhoo Corner | Laamu | 16 | <i>Manta alfredi</i> | 1-P | 1 | o | - | - | - | - | - | - |
| 96 | 01/11/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 2 | o | - | - | - | - | - | - |
| 97 | 05/11/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 3 | - | o | - | - | - | - | - |
| 98 | 09/11/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 2 | 1 | o | o | - | - | - | - | - |
| 99 | 21/11/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 2 | - | o | - | - | - | - | - |
| 100 | 08/12/2013 | Sunlight Faru | North Malé | 9 | <i>Manta alfredi</i> | 1 | 1 | - | o | - | - | - | - | - |
| 101 | 28/12/2013 | Boduhithi Thila | North Malé | 6 | <i>Manta alfredi</i> | 2 | 5 | - | o | - | - | - | - | - |

The courtship and mating events recorded in this study have allowed a clear picture of manta behaviour to emerge, specifically as a result of several noteworthy events amongst the observations. These events are described below in the sequence in which manta courtship and mating occurs.

3.4.1 Stage 1 (initiation)

See table 3.2, event 58: 08/11/2008 – Site 10, Lankan Beyru, North Malé Atoll

Of the 101 courtship events recorded throughout the Maldives, 55 (55%) occurred at Lankan Beyru, indicating this cleaning station is a focal site for courtship of *M. alfredi*. At 9:24am as three adult female *M. alfredi* were observed via SCUBA to circle the cleaning station, an approaching male swam straight towards them, before manoeuvring himself directly above one (Fig. 3.2), unfurling his cephalic fins and placing them onto the female's head. The female reacted by rapidly raising her body forcefully into the male's ventral surface, physically pushing him backwards. This upward thrust was followed by a flick of one pectoral fin in what appeared an attempt to dislodge the male. This behaviour was seen more clearly during another courtship event (Fig. 3.3), which resulted in the displacement of the male from the female's back and cessation of further courtship. After these events the females resumed their previous cleaning behaviour.



Figure 3.2 A male *Manta alfredi* (top centre) initiates courtship behaviour with the larger female (foreground) by attempting to position himself directly on top of her dorsal surface at Lankan Beyru, North Malé Atoll, Maldives.

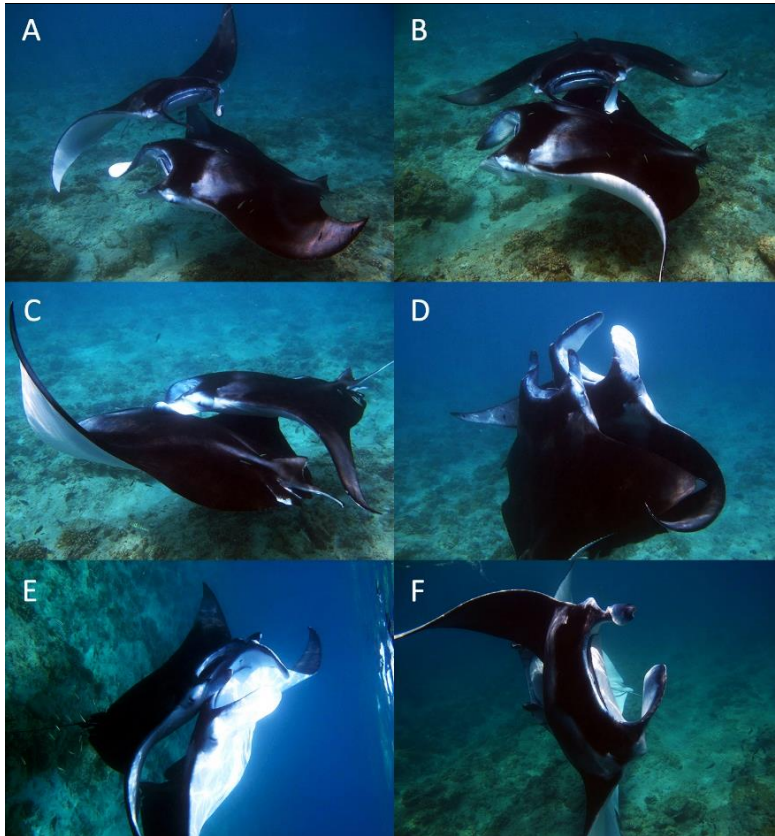


Figure 3.3 A male *Manta alfredi* approaches a female being cleaned and positions himself on the dorsal surface of a female (A – C), as the female 'bucks' herself upwards into the head of the male (D – F), dislodging him. Image taken at Rasfari North, North Malé Atoll, Maldives. (Image © Chiara Fumagalli).

3.4.2 Stages 2 (endurance) and 3 (evasion)

See table 3.2, event 59: 08/11/2008 – Site 10, Lankan Beyru, North Malé Atoll

Event 59 began immediately after event 58 finished. The male who had been displaced by the first of the females described above attempted to position himself onto the back on another mature female which had just arrived at the cleaning station. This time the female reacted to the male's presence by rapidly swimming forwards along the reef. The male followed, attempting to maintain his position on top of the female's back (Fig. 3.4).



Figure 3.4 A male *Manta alfredi* (right foreground) attempts to position himself on top of the female's dorsal surface, while the female (centre foreground) accelerates forward away from the male. Image taken at Lankan Beyru, North Malé Atoll, Maldives.

The ensuing courtship train was observed for several minutes while the pair remained within sight along the reef crest between 5 – 20 metres. Periodically the female undertook several forward flips and backward somersaults, while the male, shadowing her movements, remained within one or two metres at all times (Fig. 3.5). Between flips and somersaults the female swam faster than is usual, and made several quick changes in direction while the male stayed close. During this encounter fresh mating wounds were visible on the tip of the female's left pectoral fin, indicating she had recently mated (Fig. 3.5 circled).

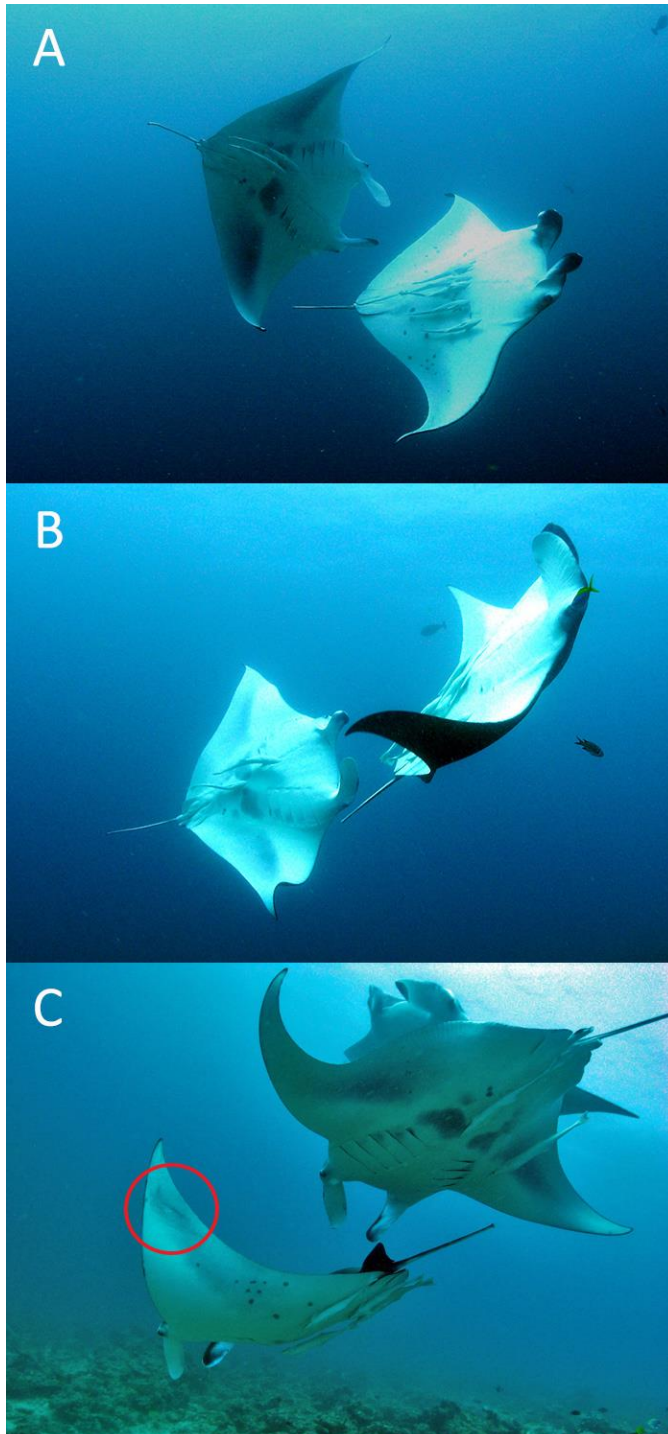


Figure 3.5 A female *Manta alfredi* (right) takes evasive action from the courting male (left) by flipping her body into a series of tight forward flips (A), while the male attempts to maintain his position behind and on top of the female's back (B). The female swims at higher than average speed along the reef crest while a pursuing male (now at right) attempts to maintain a position directly behind, or on top of, the female (C). Note the fresh mating wound on the female's left pectoral fin (circled) indicating recent mating activity. Images taken at Lankan Beyru, North Malé Atoll, Maldives.

See table 3.2, event 40: 07/11/2007 – Site 10, Lankan Beyru, North Malé Atoll

This event spanned two dives which were both approximately 60 minutes long. 16 minutes into the first dive at 07:16 am, while observing three adult female *M. alfredi* at the main cleaning station on the reef at 20 metres depth, another female swam rapidly overhead followed by 26 males in a courtship train. Due to the large number of males their trailing line was less delineated than if the average number of just 3 males were involved (Fig. 3.6).



Figure 3.6 A mass courtship train of 26 male *Manta alfredi* (of which 13 are captured in this image) pursue a single female (not visible) along the reef crest at Lankan Beyru, North Malé Atoll, Maldives.

Over the next fourteen minutes the courtship train remained within sight in the vicinity of the cleaning station. During this time, as described in event 59, the female undertook multiple tight turns, forward flips and backward somersaults (Fig. 3.7 female circled). Often she would loop back on herself to slot behind the following males, causing apparent confusion, resulting in an unstructured group around the cleaning station.

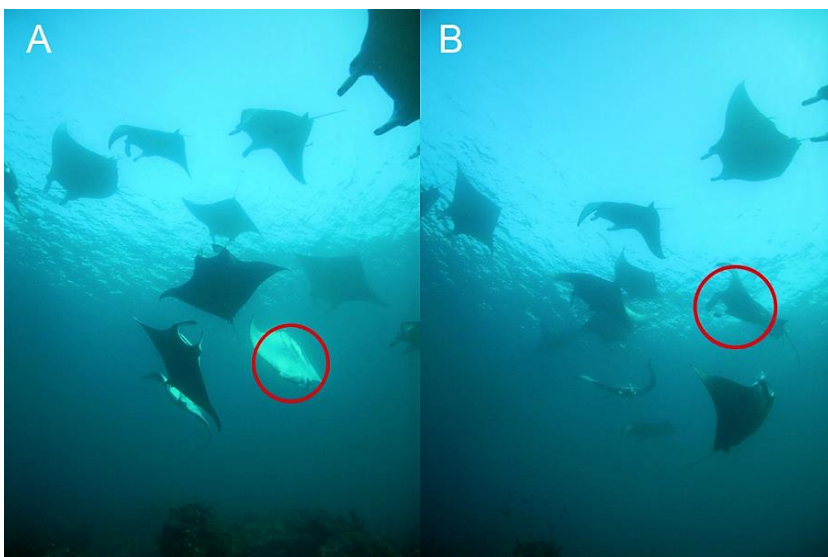


Figure 3.7 Mass courtship of one female by 26 male *Manta alfredi* above Lankan Beyru cleaning station in North Malé Atoll, Maldives. The female (circled) performs a forward flip (A) which confuses the pursuing males and causes her to become positioned at the tail of the courtship train (B).

The female also appeared to chase individual males at times, closely following one of the leading males in the courtship train while multiple other pursuing males attempted to position themselves onto her dorsal surface. Each time one of the pursuing males succeeded in getting within touching distance of her back she would perform another series of forward flips, or backward somersaults. After 14 minutes the female rapidly swam off followed by the train of males at which point observations ceased until the next dive (Fig. 3.8).



Figure 3.8 The tail end of a courtship train showing the last 8 of 26 male *Manta alfredi* chasing a single female at Lankan Beyru, North Malé Atoll, Maldives.

At 11:44 on the second SCUBA dive, the same female from the previous courtship train, again swam along the reef crest at 15 metres, this time pursued by eight males. Six were later identified as present at the earlier courtship event. The courtship train passed quickly and remained in sight for only three minutes. The total time between first and last sighting of this courtship train was 4 hrs. 31 min.

See table 3.2, event 51: 20/09/2008 – Site 4, Hanifaru Bay, Baa Atoll

This event took place at 15:26 while the observers were free-diving inside Hanifaru Bay and involved five males and a single near-term pregnant female. The observation lasted 2 minutes. The pregnant female swam rapidly into the vicinity of the observers, where visibility was only 12 metres, followed by the five males in a courtship train. In ten metres of water the female undertook four tight backwards somersaults while the following males attempted to maintain position close behind, or directly on top of the female's dorsal surface (Fig. 3.9A – L). After the last somersault the female rapidly swam out of sight followed by all of the males.



Figure 3.9 A near-term pregnant female *Manta alfredi* (A – left at front) is chased by five males in a courtship train at Hanifaru Bay in Baa Atoll, Maldives. Each of the males attempts to position himself closest to the female and directly on top of her dorsal surface (B – L), while the female performs a series of four consecutive backward somersaults (Images © Tim Davies).

See table 3.2, event 86: 06/06/2013 – Site 2, Hurai Faru, Baa Atoll

A courtship event similar to event 51 at Hanifaru Bay occurred several years later at the nearby *M. alfredi* feeding site of Hurai Faru, in which a female was engaged in a courtship train with three males. The female employed similar evasive flipping and somersault behaviour, although in this instance on two occasions she intentionally swam at increased speed within a metre of the freediving observers. On the second of these approaches the female, pursued by the three males, swam directly towards one observer, leaping clear of the water before landing partially on top of the observer, who was filming. The footage can be viewed at: <https://youtu.be/9tpkVjcxqK8>.

See table 3.2, events 31, 32, 62, 82 and 101

During event 86 described above the female also actively switched from being pursued, to following one of the males for approximately 30 seconds after a series of flipping manoeuvres which resulted in her looping back. Similar female pursuit behaviour was also observed in courtship event 82. In events 62 and 101 female pursuit behaviour was also observed, however during these events a lead female was chased by a second female, which was followed by 2 and 5 males respectively. In event 62 both females were near-term pregnant.

Two other events (31 and 32), separated by 17 days (03/09/2007 and 20/09/2007), involved a female engaged in courtship behaviour with two unidentified males. At both events this female was observed to be in the late stages of gestation. In the following seven weeks she was sighted five more times, still heavily pregnant, although not then involved in courtship.

See table 3.2, event 75: 08/04/2012 – Site 17, North Point, Fuvahmulah Atoll

During the study only one courtship event involved *M. birostris*. This occurred at the remote atoll of Fuvahmulah in the far south of the Maldives, a known aggregation site for the species. During this event one large female (disc width ~5 m) was chased by eight smaller males (average disc width ~4 m) along the reef crest of the northern tip of the atoll. The observation lasted only a minute, but the behaviour was similar to courtship trains recorded for *M. alfredi*.

3.4.3 Stage 4 (pre-copulation positioning)

See table 3.2, event 2: 06/01/2004 – Site 14, Rangali Madivaru, Ari Atoll

This event occurred on the shallow reef crest in water three metres deep close to another well-known *M. alfredi* cleaning station frequented by tourists. At 15:20 the sight of a leaping manta ray and the resulting splash alerted observers to the presence of several *M. alfredi*. They were followed by snorkel for the next three minutes. The courtship event involved a lead female and two males. When first spotted the two males were both attempting to position themselves onto the dorsal surface of the female, which remained almost motionless one metre above the reef (Fig. 3.10A). Both the male's mouths were open as each physically competed to engulf the left pectoral fin of the female (Fig. 3.10C – D). After 60 seconds one of the males succeeded, whereupon he rotated and flipped his body underneath the female's to align his abdomen against hers (Fig. 3.10E – H). Throughout this process the

female remained motionless, while the other male carried on trying to gain purchase on the female's left pectoral fin, using his head and body to ram the successful male which remained firm (Fig. 3.10G).

The lack of forward swimming motion by the three negatively buoyant manta rays, and the very shallow water, caused all three to sink onto the reef (Fig. 3.10I). Upon collision the male holding the female released his grasp, allowing her to rapidly swim off with both males in pursuit. Copulation was not seen.

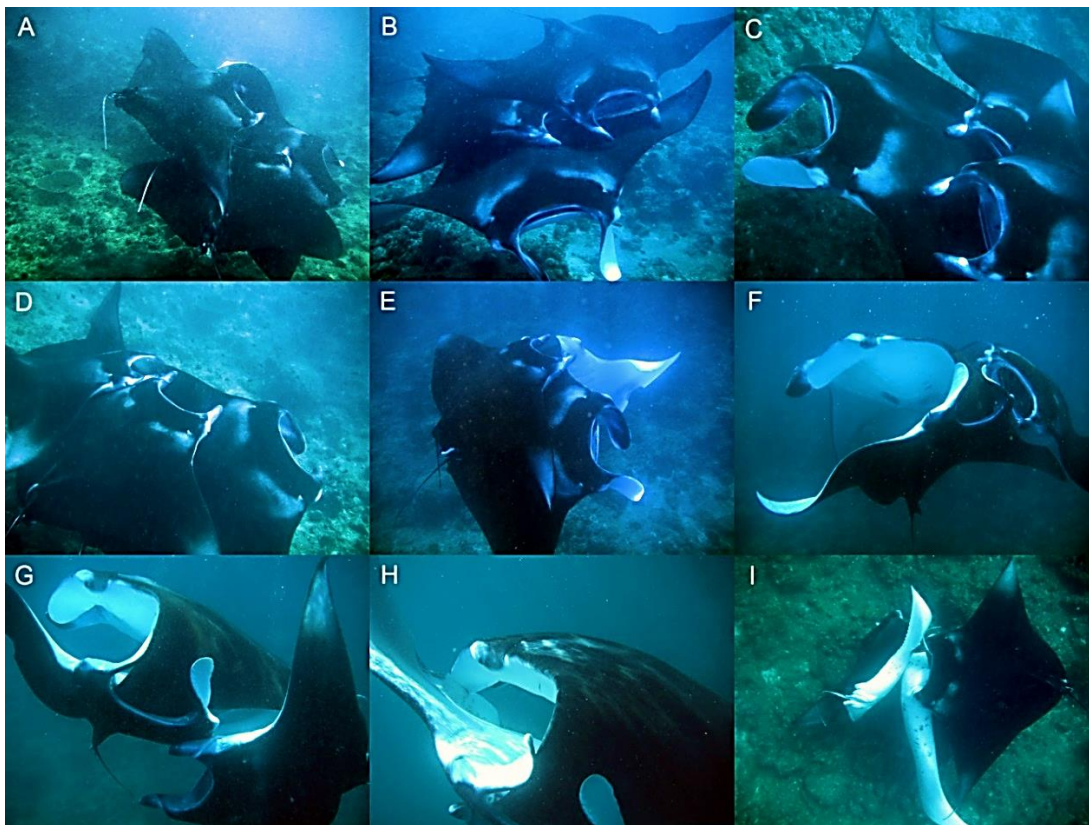


Figure 3.10 During a courtship event two male *Manta alfredi* compete to position themselves on top of a female's dorsal surface at Rangali Madivaru in Ari Atoll, Maldives. The female remains almost motionless while both males attempt to grasp her left pectoral fin with their open mouths (A – D). One of the males manages to engulf the female's pectoral fin; grasping hold with his mouth he flips and rotates his body underneath her (E – H). Copulation is unsuccessful as the second male uses his head and cephalic fins to try and dislodge the male (G). All three sink onto the reef (I) whereupon the grasping male releases his hold and the female swims off pursued by both males.

3.4.4 Stages 5 (copulation) and 7 (separation)

See table 3.2, event 1: 19/11/2003 – Site 10, Lankan Beyru, North Malé Atoll

Observers encountered a single male *M. alfredi* following a female at 10:52am at 20 metres depth on SCUBA. They were swimming rapidly along the reef crest at approximately 15 metres depth close to the nearby *M. alfredi* cleaning station where several other mantas

were being cleaned. The female's swimming behaviour was erratic; making tight twists and turns she swam directly at the observers, passing within less than a metre, while the male manta maintained a position less than two metres behind the female at all times. The pair quickly disappeared from view, swimming too fast to be followed. Five minutes later they re-appeared from the direction in which they had departed with the female in the lead. Their swimming speed was now reduced to normal cruising and erratic movements had ceased. When the mantas were parallel to the observers the male drew closer to the female, positioning himself directly on to her dorsal surface (Fig. 3.11A). The female reacted by slowly swimming up, at which point the male began to slide his open mouth down along the leading edge of her left pectoral fin, using his cephalic fins to guide the tip into his mouth; engulfing 1 metre, the male then grasped hold of the fin (Fig. 3.11B – D). The female ceased swimming while the male flipped his body underneath her, abdomen to abdomen in the water column within 10 metres of the surface. The male then inserted a clasper into the female's cloacal opening, while continuing to slowly beat his pectoral fins (Fig. 3.11E). He made rapid pelvic thrusts which lasted for 10 seconds as the copulating pair slowly spiralled clockwise while sinking. Copulation lasted for approximately 30 seconds before they separated and swam off in different directions. Removal of the male's cephalic fin resulted in a small milky cloud of fluid, presumably sperm, being released into the water column from the female's cloaca. This action occurred simultaneously with the male releasing his grip on the female's pectoral fin. No post-copulation holding occurred.

3.4.5 Supplementary on-line observational footage

An internet search on the video platform *YouTube* revealed ten manta ray courtship and mating event videos which show behaviour rarely observed (Table 3.3). Footage came from a wide variety of locations and for both species provided rarely seen copulation (Table 3.3, events 1, 2 and 10). In all three, as in the copulation observed in this study, there was no post-copulatory holding by males, with copulation ceasing simultaneously when the male released his grip on the female's pectoral fin. Event 2 of the on-line observations records behaviour very similar to the mass courtship event observed during this study (Table 3.2, event 40). On-line events 3 and 5 (Table 3.3) recorded near-term pregnant females engaged in courtship behaviour that is consistent with the 15 courtship events in the Maldives where near-term pregnant females were engaged in initiation, endurance and evasion behaviours. The on-line event number 9 (Table 3.3) also documented initiation behaviour for *M. birostris*, which means the complete sequence of courtship and mating behaviour for both species has

now been observed and documented. All other on-line courtship and mating behaviour recorded in Table 3.3 is consistent with observations recorded in the field in the Maldives in this study.

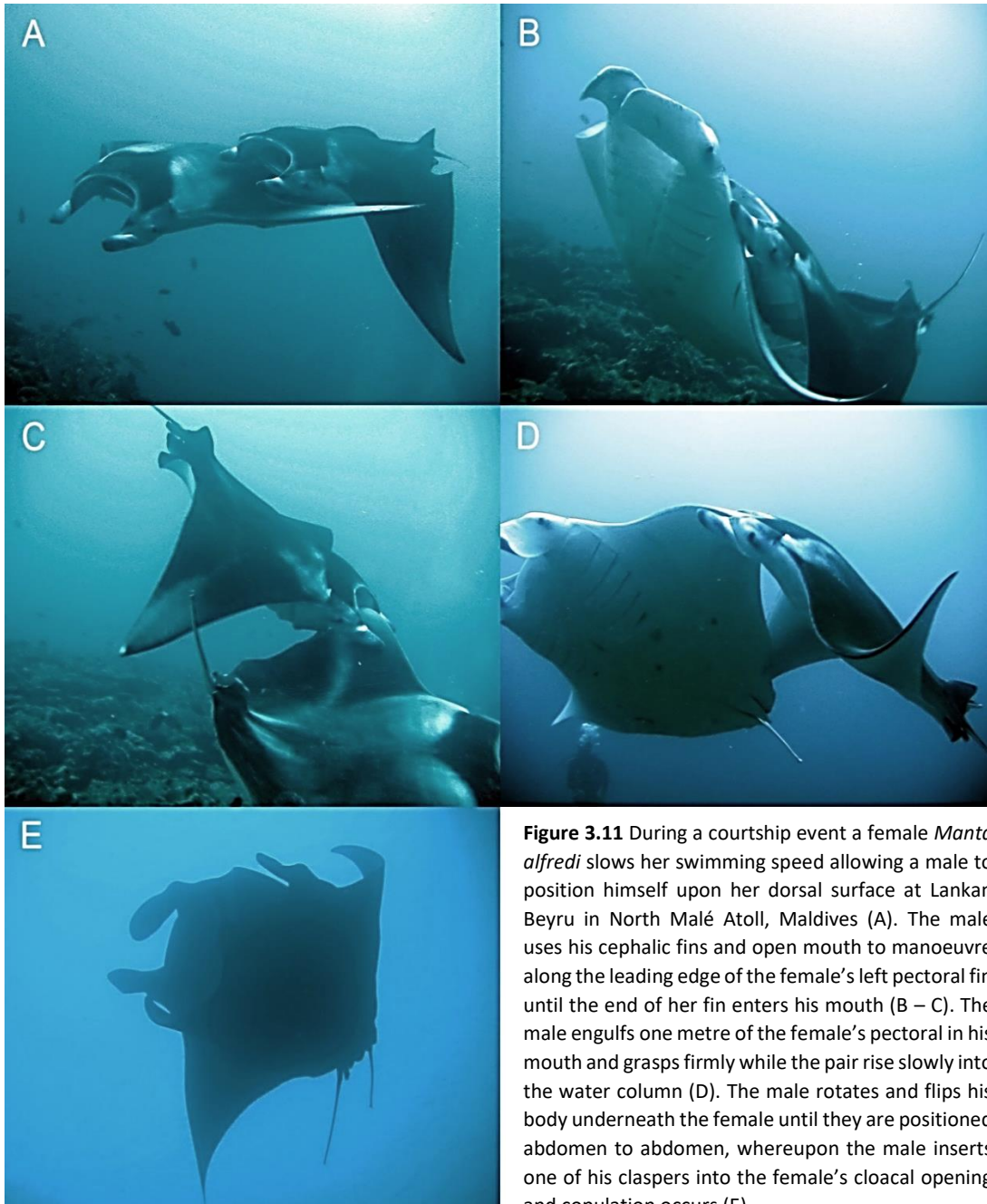


Table 3.3 Courtship and mating events of manta rays (*Manta alfredi* and *Manta birostris*) records obtained from the on-line search engine *YouTube*. (-) signifies courtship and/or mating stage was not observed, (o) signifies courtship / mating was observed, (x) signifies courtship / mating did not occur, (P) signifies visible pregnancy.

| Event | Date | Videographer | Web-link | Location | Species | No. females | No. males | Stage 1 (initiation) | Stage 2 (endurance) | Stage 3 (evasion) | Stage 4 (pre-copulation positioning) | Stage 5 (copulation) | Stage 6 (post-copulation holding) | Stage 7 (separation) |
|-------|---------|-------------------|---|-----------------------------------|------------------------|-------------|-----------|----------------------|---------------------|-------------------|--------------------------------------|----------------------|-----------------------------------|----------------------|
| 1 | 2006 | Peter Schneider | www.youtube.com/watch?v=leXmFbRyO6Y | Rangiroa, French Polynesia | <i>Manta alfredi</i> | 1 | 1 | - | - | - | o | o | x | o |
| 2 | 2011 | Jim Friend | www.youtube.com/watch?v=K2GtZu47Z4p | Olowalo, Maui, Hawaii | <i>Manta alfredi</i> | 2 | 20+ | - | o | o | o | o | x | o |
| 3 | 2011 | Jim Friend | https://www.youtube.com/watch?v=2XmFMvZ87Gc | Olowalo, Maui, Hawaii | <i>Manta alfredi</i> | 1-P | 14 | - | o | - | - | - | - | - |
| 4 | Aug-11 | Julia Sumerling | www.youtube.com/watch?v=CmRkqjlnk | Osprey Reef, Coral Sea, Australia | <i>Manta alfredi</i> | 1 | 3 | - | o | o | - | - | - | - |
| 5 | Sep-12 | Lembongan Zone | www.youtube.com/watch?v=CK0QySmN0CY | Nusa Penida, Bali, Indonesia | <i>Manta alfredi</i> | 1-P | 6 | - | - | o | - | - | - | - |
| 6 | 2013 | Robert Frixenhoff | www.youtube.com/watch?v=8UWvGZF6GsA | Bora Bora, French Polynesia | <i>Manta alfredi</i> | 1 | 2 | - | o | o | - | - | - | - |
| 7 | Mar-14 | SunLight On Water | www.youtube.com/watch?v=YgrTmG3D5mQ | Kona, Big Island, Hawaii | <i>Manta alfredi</i> | 1 | 1 | - | o | o | - | - | - | - |
| 8 | Unknown | Craig Rudnick | www.youtube.com/watch?v=zVVTzplzN0Q | Dhonkalo Thila, Maldives | <i>Manta alfredi</i> | 1 | 1 | - | - | o | - | - | - | - |
| 9 | Unknown | Larry Chow | www.youtube.com/watch?v=oMB4vi2hwU | Isla de Le Plata, Ecuador | <i>Manta birostris</i> | 1 | 1 | o | o | - | - | - | - | - |
| 10 | Unknown | Fabricio | www.youtube.com/watch?v=2SW4v41ajzE | Galápagos Archipelago | <i>Manta birostris</i> | 1 | 1 | - | - | - | o | o | x | o |

3.5. Discussion

For the first time a detailed photographic record of manta courtship and reproductive behaviour is presented, with the observations collated here significantly adding to what has been reported on this subject in the scientific literature. Seven distinct stages to the courtship and mating process are identified, which encompass both manta species: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulation positioning, (5) copulation, (6) post-copulation holding and, (7) separation (Fig. 3.12).

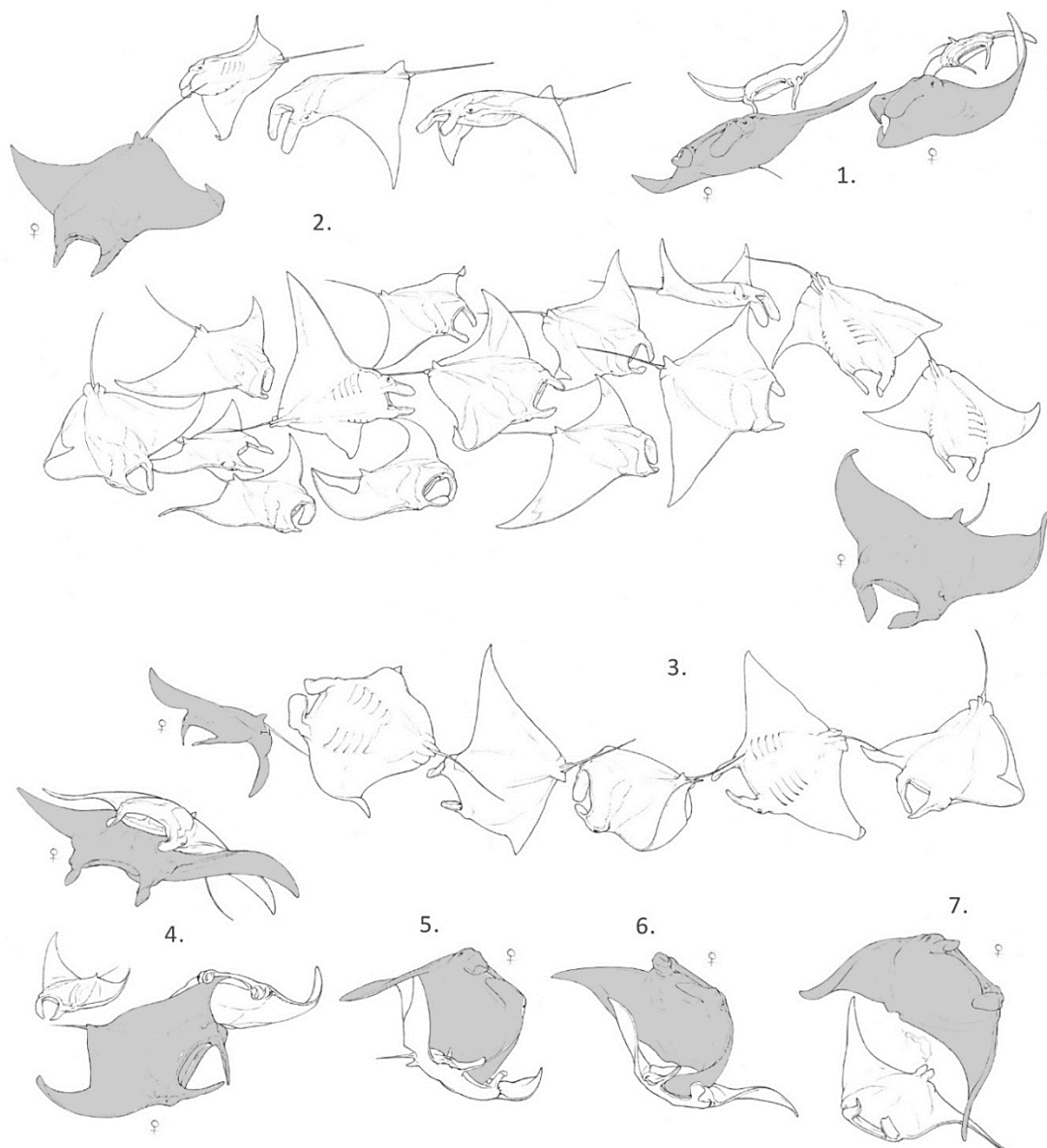


Figure 3.12 Illustration of the seven courtship and mating stages: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulation positioning, (5) copulation, (6) post-copulation holding and, (7) separation] of manta rays (*Manta birostris* and *Manta alfredi*). Female is shaded grey to aid identification (Illustrations © Marc Dando).

Yano et al. (1999) proposed five key stages of the courtship and copulatory behaviour of *M. birostris* [(1) chasing, (2) biting, (3) copulating, (4) post-copulation holding and, (5) separation]. Marshall and Bennett (2010) concurred based on a study of *M. alfredi* in East Africa, however they also proposed a sub-division of the first stage (chasing) into three categories; (a) following and chasing, (b) turning and flipping and, (c) evasion or avoidance. My study largely agrees with both Yano et al. and Marshall & Bennett, but advances their work by more accurately defining manta courtship and mating and by describing a new stage (initiation). I also consider that Marshall and Bennett's sub-division of turning and flipping (b above) and evasion or avoidance (c above) should be combined into a single category because turning and flipping (b above) is the action taken by females trying to evade or avoid males (c above).

Although the male manta rays appear to physically initiate courtship activity, it is possible that females could trigger this behaviour through releasing olfaction-mediated pheromones of readiness to mate. This could help explain the close following and courtship train behaviour observed in mantas and other elasmobranchs (Gordon 1993; Klimley 1980; Luer & Gilbert 1985; Tricas 1980), although no experimental evidence is available (for review also see Demski 1991). By contrast, the use of 'sex' pheromones to attract mates in the wider animal kingdom is well documented (Hurst 2005; Wyatt 2003).

Several particularly compelling accounts of proposed olfaction-mediated sex attraction in black-tip reef sharks (*Carcharhinus melanopterus*) were reported from French Polynesia (Johnson & Nelson 1978). Here one shark was observed to track down another (which was initially out of view) and then follow it closely with the snout directed towards the leader's vent. Very similar behaviour in manta rays was also observed in French Polynesia during a courtship event involving a near-term pregnant female *M. alfredi* and three males (M. de Rosemont 2010, pers. comm.). The four mantas were initially engaged in a courtship train above a cleaning station interspersed with evasive behaviour by the female while the three males attempted to closely follow her at all times. After several minutes of observations the mantas moved out of view due to the high swimming speed of the courting animals. However, a few minutes later the female returned, now alone, swimming at above average speed along the reef in a straight line. The female continued on the same course until reaching the cleaning station, at which point she changed course and headed into deeper water away from the reef, disappearing from view again within 15 seconds. During the next 60 seconds all three of the males which had been following the female returned one by one.

Travelling in the same direction as the female, they swam along the reef in a zig-zag motion with cephalic fins unfurled. Upon reaching the point where the female changed course each of the males appeared to sense the direction in which she had departed as all altered course to head seaward at precisely the same point she did. Similar 'searching' behaviour by male *M. alfredi* was observed during this study in the Maldives, although the event described above provides the strongest circumstantial evidence to support the hypothesis that olfaction-mediated cues are important in the courtship and mating processes of manta rays.

Further supporting the use of olfaction-mediated cues, a study on captive sandtiger sharks (*Carcharias taurus*) by Gordon (1993) suggested the observed action of flaring and cupping of the pelvic fins by females during courtship may serve as a pumping action, excreting a chemical stimulant (pheromone) into the water, attracting nearby males. During courtship event 40 (Table 3.2) in this study, the pursued female excreted an almost clear liquid into the water from her cloacal opening during the courtship train, repeatedly everting her intestine in the process to pump the discharge into the water (Clark et al. 2007) (Fig. 3.13A). This was quite unlike the cloudy mass of reddish-brown material which mantas produce when defecating (Fig. 3.13B) (pers. obs.). After the female released the clear liquid, her pursuing males' sped towards her and attempted to position themselves closely behind her. In response she then exhibited evasive behaviour.

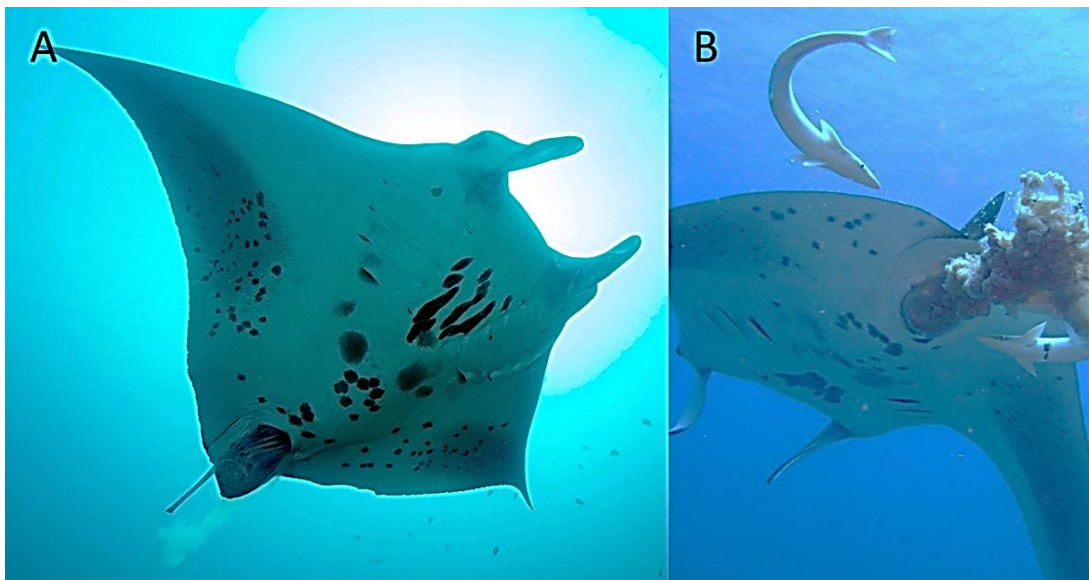


Figure 3.13 A female *Manta alfredi* everts her intestine, excreting a milky liquid (A), unlike the more normal brown cloud of digested zooplankton excreted during defecation (B).

During courtship trains the female's cephalic fins usually remain tightly rolled up, while the males' are usually unfurled. These modified 'head-fins' are primarily used by manta rays

during feeding where they act as a funnel to channel planktonic food into their mouths (Paig-Tran et al. 2013; pers. obs.). When mantas are 'cruising' these fins are curled up, presumably to improve hydrodynamic efficiency. Mantas have two small nostrils, the outer openings of which are situated on the upper jaw either side of their mouth (Fig. 3.14). The nostrils are aligned so that while the manta swims forward water flows into the them, passing over sensory folds, allowing the mantas, like other elasmobranchs, to detect tiny concentrations of dissolved chemicals (Theisen et al. 1986; Zeiske et al. 1987). It thus seems possible that male mantas unfurl their cephalic fins to increase the flow of water to the nostrils, helping them smell any sexual pheromones released by females.



Figure 3.14 The two nostrils of this bottom feeding *Manta alfredi* can be clearly seen on either side of its mouth (circled).

The endurance stage of manta courtship during this study consisted on average of a 3:1 (SE \pm 0.3) ratio of males to females, rising to 5:1 (SE \pm 1.2) if the courtship train escalated to the evasion stage. Female manta rays and elasmobranchs in general invest heavily in their offspring, while males invest little (Conrath & Musick 2012; Chapter 4). Therefore, the females are more likely to be selective in mate choice (Bleu et al. 2012; Trivers 1972), driving contest competition among males (Cox & Le Boeuf 1977), and the female evasive behaviour observed in manta rays and other shark and ray species (Deakos 2011; Pratt & Carrier 2005; Whitney et al. 2004).

Female manta rays determine the speed, duration and direction of the courtship trains, which can last for hours. Each sexually receptive female may also engage in multiple courtships trains (Chapter 4), which may not all result in copulation, although there is evidence to suggest multiple matings as a result of multiple courtship events do occur and may be commonplace (Chapter 4). A courtship train may on occasion also involve two

females. During these events the lead female is usually followed closely by the second which appears to be deliberately following her, while males trail behind. It is unclear why this behaviour occurs. However, if multiple sexually receptive females are present in an area, a passing courtship train may attract additional females to become engaged in the behaviour, as the train of males provides a ready source of potential suitors for the joining female. Furthermore, the presence of two females engaged in a single courtship train doubles the chances of each male successfully copulating, which potentially should attract more males. 83% of the courtship events recorded during this study occurred at cleaning stations, which may also function as leks (Chapter 4). These sites may therefore create a focal point for courting mantas, where individuals can join passing courtship trains to assess or compete for prospective mates (Beehler & Foster 1988).

The dorsal surface of manta rays is covered by a layer of mucus which contains dark pigmentation, creating darker shading where the mucus layer is thickest. The layer is easily rubbed off through light contact. During courtship the males' unfurled cephalic fins rub the back of the female's head, revealing a lighter skin tone underneath (Fig. 3.15). These marks quickly darken and the natural skin pigmentation returns within a few weeks, unlike the permanent scars on the dorsal surface of the females' pectoral fin tips which can arise from mating.



Figure 3.15 The marks left behind by repeated rubbing from a male's cephalic fins during courtship can clearly be seen on the head of the female *Manta alfredi* in this image (circled).

Manta rays are sensitive to touch (pers. obs.) and it is possible this tactile stimulation may serve as a way for males to pacify a female and trigger the onset of copulation, during which the females enter a passive, almost hypnotic state. Anecdotal experiments have shown that if a free diver places their hands on the head of a female *M. alfredi* at a cleaning station where a male manta touches her during courtship, then a tonic-like state can be induced in the female while this contact is maintained (pers. obs.). Such tonic immobility is also known from many ray and shark species and may help induce females to copulate and reduce risk of injury during copulation (Henningsen 1994; Whitman et al. 1986). This behaviour could also be linked to the fascinating interactions that have been reported between humans and mantas, especially *M. birostris*, whereby larger female mantas allow human SCUBA or free-divers to 'ride' them in a similar fashion to courting males. Indeed, it can appear that female mantas sometimes solicit physical interactions with people (pers. obs.) (Fig. 3.16).

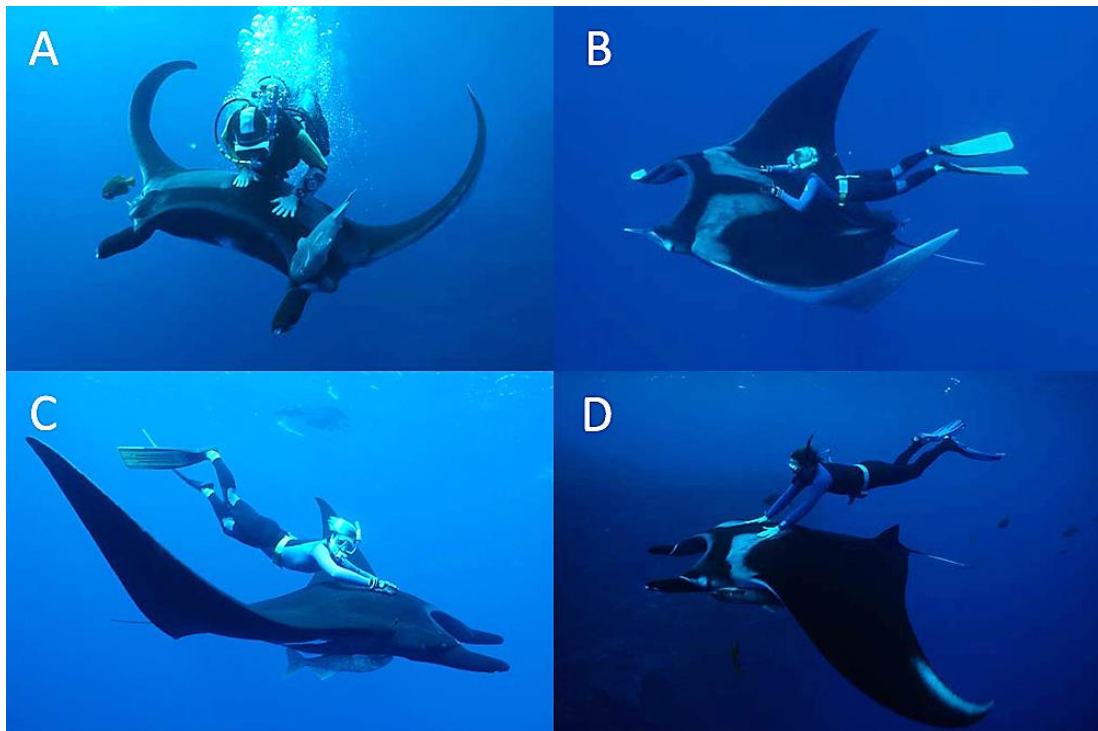


Figure 3.16 Image composite of a diver (A) and free-divers (B–D) 'riding' several different large *Manta birostris* at San Benedicto Island (Revillagigedo Islands), Baja California, Mexico (Images © Phillip Colla 1990).

At the onset of pre-copulation positioning, whether tactile stimulation plays a role or not, something causes the female to reduce her swimming speed and cease evasive behaviour, allowing the male to grasp her pectoral fin. As the majority of copulation events observed consisted of just a single male and female, it is likely that the lengthy duration of courtship trains constitutes a form of control by females to selectively reduce competing males until only the most persistent remains, similar to the 'heat runs' exhibited by humpback whales

(*Megaptera novaeangliae*), where the Principal Escort (male) attempts to hold his position next to the female throughout her late pregnancy (Baker & Herman 1984; Herman et al. 2007). In this way females may ensure only the fittest males are selected as a mate. During the study near-term pregnant females were regularly involved in courtship trains, suggesting that females are likely to mate soon after giving birth. Indeed, fresh mating wounds were recorded on females soon after parturition, although any subsequent gestation often appears to be delayed for many months or even years in the wild (Chapter 4). These field observations are supported by the mating behaviour of a female *M. alfredi* held in captivity in Okinawa Churaumi Aquarium, Japan (Okinawa Churaumi Aquarium 2010). From four consecutive pregnancies this individual gave birth to a single pup then copulated within hours of parturition. These observations are to date the only record of a manta ray of either species giving birth.

Protracted courtship periods may also increase reproductive success in other ways. Females that allow copulation while multiple males are still engaged in courtship activity with her also run the risk of injury through collision with the reef. The presence of multiple males during the later stages of courtship is also likely to reduce the chance of a successful copulation as competing males prevent each other from successfully positioning themselves. Previous mating experience of a female may also play a role in the timing of the pre-copulation positioning trigger.

Post-copulation holding behaviour was observed in six of the seven previously described manta mating events (Marshall & Bennett 2010a; Yano et al. 1999). However, of the four documented copulation events in this study, no post-copulation was observed, with separation commencing simultaneously upon cessation of copulation. It is unclear what benefit, or significance, may be derived from this post-copulatory behaviour, therefore future studies will need to determine if it warrants the current stage categorisation.

This study confirms that both *M. alfredi* and *M. birostris* appear to engage in the same elaborate courtship rituals, exhibiting similar behaviours during all stages of the courtship and mating process. These courtship rituals are most prevalent at cleaning stations in *M. alfredi*, which may also function as lekking sites. The study suggest female manta rays invest heavily in mate choice, shaping the reproductive strategies of these species. These strategies, and how they may influence the fitness and reproductive periodicity of individual *M. alfredi*, are investigated further in Chapter 4.

Chapter 4

Reproductive strategies and fecundity
of the reef manta ray (*Manta alfredi*)
in the Maldives

4.1 Abstract

Low rate of reproduction, long maturation time, small highly fragmented populations, and aggregating behaviour, all characterize the reef manta ray (*Manta alfredi*). These life history and species characteristics mean even small increased mortality rates or reduced fecundity are likely to have severe consequences for a population's survival. During a decade long study from January 2005 through 2014, 5,321 surveys were undertaken in the Republic of Maldives using photo-ID techniques and behavioural observations to characterise fecundity and reproductive strategies of *M. alfredi*. A total of 30,345 photo-ID sightings of 3,570 individuals were recorded in 20 of the Maldives' 26 atolls at 193 different sites. Three hundred and seven pregnancies were recorded among 745 adult females. Generalized additive models significantly linked the highly variable fecundity observed inter-annually to variability in Chl-a and SST. Variations in monsoon driven productivity, which in turn may be linked to larger-scale climatic phenomena in the Indian Ocean, such as the Indian Ocean Dipole and El Niño-Southern Oscillation, may be affecting the availability of the manta's planktonic food, which in turn affects their fecundity. Significant intra-annual variations in instances of courtship behaviour and pregnancies suggest *M. alfredi* in the Maldives exhibit a partially defined annual reproductive cycle, with one or two peaks in reproductive activity each year occurring prior to transitions between the two monsoons. Significant seasonal shifts in the sex-ratio of adult *M. alfredi* at cleaning stations, intra-annual differences in fecundity among female sub-populations, and behavioural observations throughout the study suggest reproductive behaviour in *M. alfredi* may be characterised by female mate choice and undertaken primarily at cleaning stations which are utilised as lekking sites. Intervals between presumed copulation and parturition were also highly variable among individuals, suggesting females may utilise sperm storage or embryonic diapause to maximise fecundity. Based on consistent sightings of a core group of adult female *M. alfredi* (N = 150) throughout the decadal study period, on average each females had one pregnancy every 7.3 years, enabling the production of only 3 – 4 pups on average during a females' estimated lifetime, making *M. alfredi* as one of the least fecund vertebrate animals in the world and highly vulnerable to over-exploitation.

Keywords: leks, courtship, mate choice, mating behaviour, cleaning stations, monsoons

4.2 Introduction

Manta rays are amongst the most conspicuous and charismatic of marine creatures. Born into a life of perpetual motion these zooplanktivorous elasmobranchs belong to the Mobulidae family which currently comprises nine species in the genus *Mobula* (Notarbartolodi-Sciara 1987; Rafinesque 1810) and two in the genus *Manta* (Bancroft 1829). Reef manta rays – *Manta alfredi* (Krefft 1868) are widely distributed throughout the tropical and subtropical waters of the Pacific and Indian Oceans, although populations are highly fragmented (Couturier et al. 2012; Kashiwagi et al. 2011), by resource and habitat needs (Anderson et al. 2011b; Braun et al. 2015; McCauley et al. 2014). *M. alfredi* occur in shallow waters along the coastal reefs of continents and around remote oceanic islands and archipelagos (Kashiwagi et al. 2011; Marshall et al. 2011b), venturing offshore and into the mesopelagic zone (Braun et al. 2014; Braun et al. 2015; Jaine et al. 2014).

The Maldives contains the world's largest known population of *M. alfredi* (Chapter 2). This island nation rarely experiences severe weather events (Gischler et al. 2014), but is strongly influenced by the South Asian monsoon, especially the northern and central atolls which are closer to India (Anderson et al. 2011b; Gischler et al. 2014). It is within these most productive atolls that the greatest concentrations of *M. alfredi* feed all year round, migrating across the archipelago with the changing monsoons to exploit the richest zooplankton feeding grounds (Anderson et al. 2011b).

Variations in current flow through channels separating atolls in the Maldives have been shown to develop island wake eddies as a result of the island mass effect (Doty & Oguri 1956; Sasamal 2006). These current eddies, driven by the South Asian monsoon, enhance primary productivity on the leeward side of the atolls through deep-water upwellings, bringing nutrient rich water into the euphotic zone (Sasamal 2007). Large-scale weather anomalies, such as the El Niño-Southern Oscillation (ENSO) and Indian Ocean Dipole, are thought to regulate annual variations in the strength of the South Asian monsoon (Annamalai et al. 2007; Ashok et al. 2001; Wilkinson et al. 1999), where stronger monsoon winds lead to increased upwelling, which in turn promotes biological productivity (Gupta et al. 2004; Singh & Gupta 2004). While seasonality affects the migratory behaviour of the *M. alfredi* population in the Maldives intra-annually (Anderson et al. 2011b), inter-annual variations in the fecundity of the *M. alfredi* population may be linked to inter-annual variations in the strength of the monsoons and the resulting ocean productivity.

Long-term sighting records of *M. alfredi* at established aggregation sites suggest this species is highly philopatric, often undertaking short seasonal migrations between favoured sites (Braun et al. 2015; Couturier et al. 2011; Couturier et al. 2014; Deakos et al. 2011; Dewar et al. 2008; McCauley et al. 2014). Site fidelity has been linked to areas of high primary productivity (Jaine et al. 2014), and may also vary by sex and age-class (Couturier et al. 2011; Chapter 2).

Photo identification studies have shown that *M. alfredi* can live for at least 30 years (Clark 2010; Couturier et al. 2014; Homma et al. 1999; Kashiwagi et al. 2010; Chapter 2) and they are estimated to live for about 40 years, although the maximum longevity for this species remains unknown (Couturier et al. 2012). Natural mortality in *M. alfredi* is thought to be low, and current estimates of life history parameters have generation span (female age at maturity, plus gestation time) at 11 years and generation time (average age of adult female, approximated as halfway between age at first maturity and maximum age) at 25 years (Dulvy et al. 2014b; Marshall et al. 2011b).

Manta rays are ovoviviparous matrotrophs (Dulvy & Reynolds 1997; Wourms 1977) whereby the developing embryo is initially nourished by the egg yolk, then by uterine milk (Conrath & Musick 2012; Hamlett et al. 2005; Musick 2010; Tomita et al. 2012; Wourms 1981). The gestation time of *M. alfredi* is reported to be one year in both the wild and captivity (Deakos 2011; Marshall & Bennett 2010a; Okinawa Churaumi Aquarium 2010). In wild *M. alfredi*, reproductive cycles often include resting periods, with biennial cycles reported as the norm for some individuals in Hawaii (Deakos 2011) and Mozambique (Marshall & Bennett 2010a) and an average reproductive periodicity of ~3.6 – 3.9 years in Japan (Kashiwagi 2014). *M. alfredi* normally give birth to a single large pup (Beebe & Tee-Van 1941b; Bigelow & Schroeder 1953; Coles 1916; Compagno & Last 1999) and occasionally twins (Marshall 2009). During copulation the male bites down hard on the end of one of the female's wing-tips, working as much as one metre of her pectoral fin deep into his mouth before inserting one of his paired claspers into her cloaca (Marshall & Bennett 2010a; Yano et al. 1999). These bites often result in permanent mating scars to the female's left pectoral fin tip (Marshall & Bennett 2010a).

As female *M. alfredi* perform all post coital parental investment, mate selection theory predicts that to maximize personal fitness, females should be more choosy than males in selecting partners (Fisher 1958, Trivers 1972). Manta rays and their close relatives the bat ray (*Myliobatis californica*), spotted eagle ray (*Aetobatus narinari*), spinetail devil ray

(*Mobula japonica*) and sicklefin devil ray (*Mobula tarapacana*) all exhibit elaborate courtship in which females lead males on a chase called a 'mating or courtship train' which can last for hours (Deakos 2011; Marshall & Bennett 2010a; Pratt & Carrier 2001; Tricas 1980; Uchida et al. 1990; Yano et al. 1999; Chapter 3), perhaps to assess male fitness (Deakos 2011). As female mantas can remain sexually receptive for several weeks (Chapter 3), they may perform multiple matings with a variety of partners (Yano et al. 1999; Chapter 3).

Sexually receptive females spurn and/or solicit males in many species (Cox & Le Boeuf 1977; Poole 1989; Wolff 1998) and there are also parallels between manta courtship trains and, for example, the tending behaviour in ungulates, where a male will associate with an oestrous female until they either copulate or he is displaced by another male (Mysterud et al. 2004; Pelletier et al. 2006; Wolff 1998). In humpback whales (*Megaptera novaeangliae*), competitive courtship groups, known as 'heat runs', were first described by Tyack and Whitehead (1982), who noted that these typically contain a central, relatively passive 'Nuclear Animal', usually a female, and a 'Principal Escort', usually the largest male (Spitz et al. 2002), who maintains position close to the Nuclear Animal and frequently fends off challenges to his position. To ensure he can mate with the female after she gives birth, the Principal Escort tries to hold his position next to the female throughout her late pregnancy (Baker & Herman 1984; Herman et al. 2007). Similar escorting behaviour by male *M. alfredi* of females which were close to parturition has also been observed in the Maldives (Chapter 3). To better understand the ultimate and proximate causations of mating behaviour in *M. alfredi*, inferences from findings on better studied species are useful (Clapham et al. 1992; Clapham 1996; Wasser & Starling 1988).

M. alfredi have among the lowest fecundity of all elasmobranchs (Dulvy et al. 2014b) and their small, highly fragmented populations make their global population hard to estimate. In known subpopulations, numbers are typically estimated in the 100's, although they can reach up to several thousand (Couturier et al. 2014; Deakos et al. 2011; Kitchen-Wheeler et al. 2011; Marshall et al. 2011a). The low rate of reproduction, late maturity, small size of subpopulations, and aggregating behaviour make them particularly vulnerable to fishery over-exploitation from which they are extremely slow to recover (Dulvy et al. 2014b). The greatest threat to *Manta* spp. are targeted fisheries, increasingly driven by the international trade in their gill plates which are used in Asian medicine for a health tonic purported to treat a wide variety of conditions (Lewis et al. 2015; O'Malley et al. 2016). Mantas and mobulas

also get caught as bycatch in other fisheries, which occurs to worrying levels (Croll et al. 2015).

M. alfredi's life history characteristics mean that even small increases in mortality rates or decreases in fecundity will affect population survival. Consequently, and in response to increased demand for gill plates, both *Manta* species (Walbaum 1792) were listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) in March 2013. Since November 2014, they have also been listed on Appendix I and II of the Convention on Migratory Species (CMS).

The aim of this ten year study was to characterise fecundity and reproductive strategies of *M. alfredi* in the Maldives using photo-ID techniques and behavioural observations. Temporal changes in reproductive output were then examined in relation to variability in ocean productivity. In the Maldives *M. alfredi* has never supported a fishery and is currently protected from fishing (Anderson et al. 2011b; EPA 2014), offering the opportunity to examine the species' reproductive output under near natural conditions.

4.3 Methods

4.3.1 Study area

The 26 coral atolls of the Maldives archipelago extend 870 km from 7° north to half a degree south of the equator in the Indian Ocean (Fig. 4.1). The maximum natural height of the country is 2.4 m above sea level and the atolls have steep reef slopes that drop to 2 – 3,000 m. The Maldives exclusive economic zone contains 3.1% of the world's coral reefs and encompasses 923,000 km² of ocean. By contrast the land area is only 300 km² (Sea Around Us Project 2014).

4.3.2 Study sites and duration

During a decade long study from January 2005 through to the end of 2014, 2,064 surveys for manta rays were undertaken throughout the year in the Maldives at the primary study sites of Hanifaru Bay in Baa Atoll and at Lankan Beyru in North Malé Atoll. These atolls are situated 108 km apart and separated by 50 km of open sea, 500 m at its deepest (Fig. 4.2). A further 301 surveys were performed during the same period at secondary study sites called Rasfari North, Dhonkalo Thila and Rangali Madivaru in North Malé and Ari Atolls (Fig. 4.3). A further 2,956 surveys were undertaken sporadically at an additional 188 known manta ray aggregation sites in 20 different atolls throughout the country.

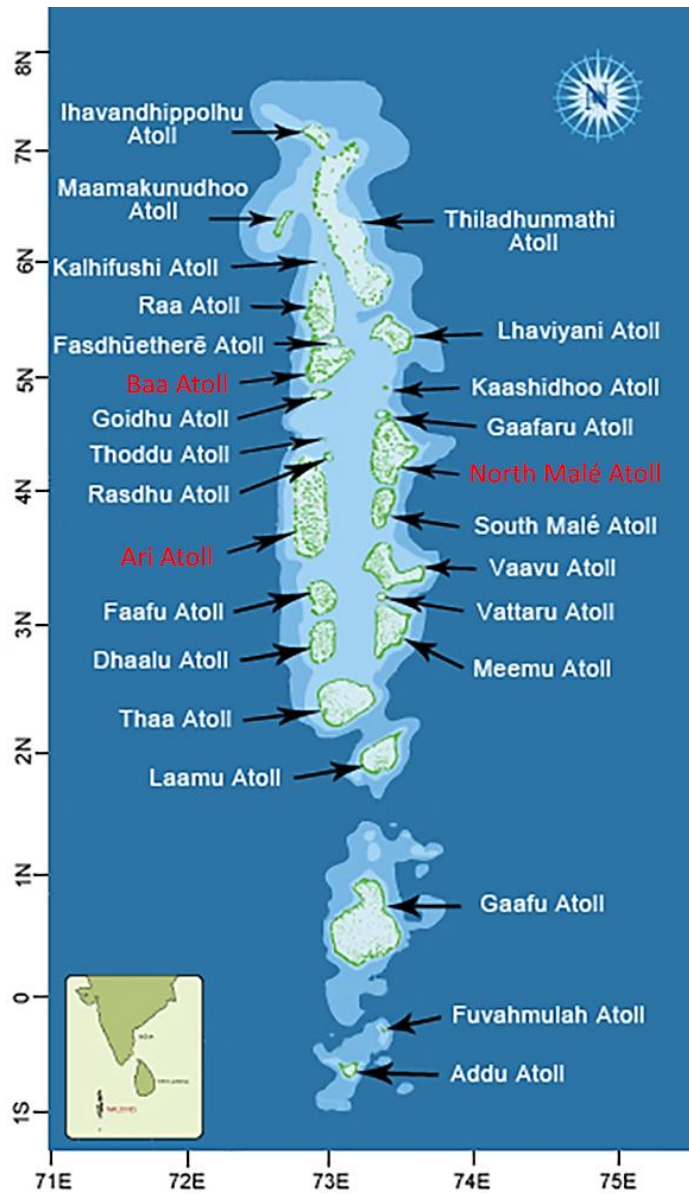


Figure 4.1 Map of the Maldives Archipelago showing the 26 geographical atolls, illustrated in green. The primary and secondary study atolls are labelled in red. Dark blue indicates where the sea floor exceeds 1,000 m; middle toned blue where it is between 500 – 1,000 m and the lightest blue where 500 m or shallower. The depth of the sea floor inside the lagoon of all the atolls is < 100 m. 1° latitude = 111 km.

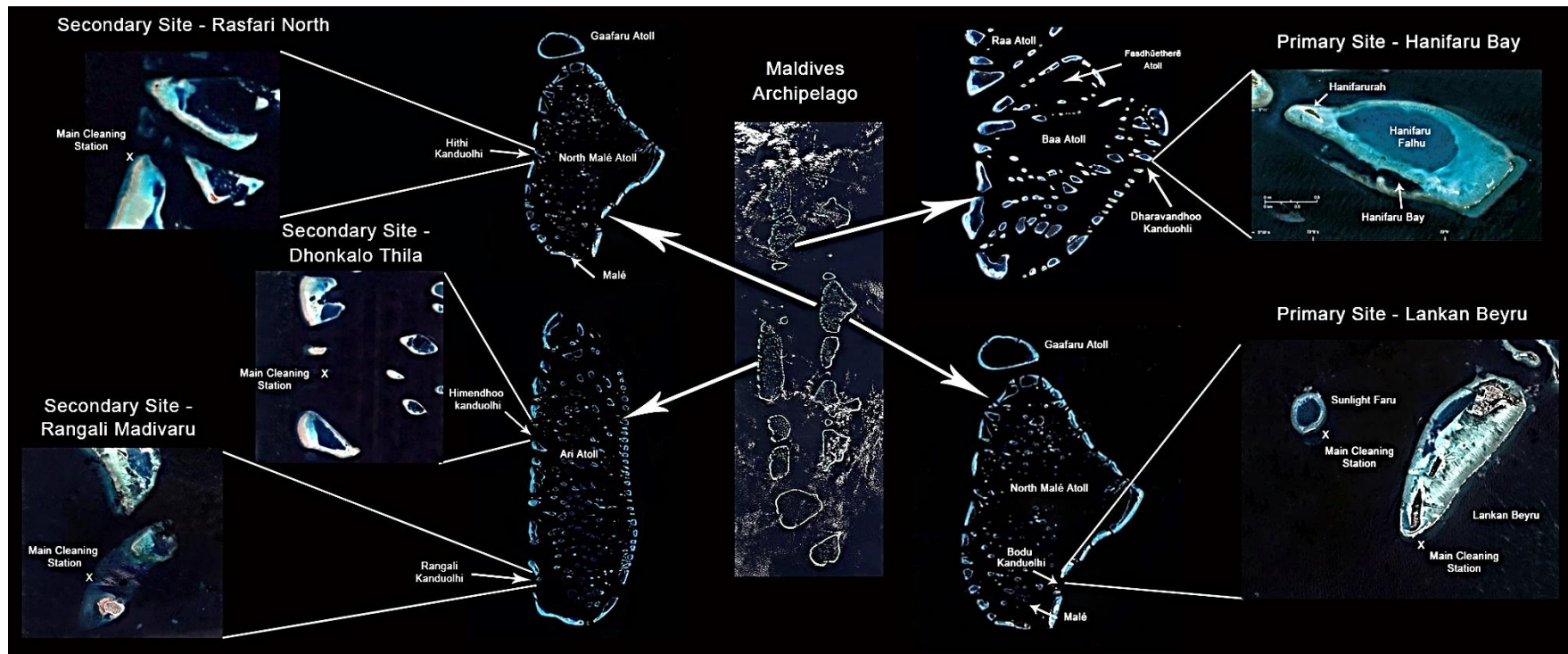


Figure 4.2 Maldives Archipelago showing the five study sites within North Malé, Baa and Ari Atolls.

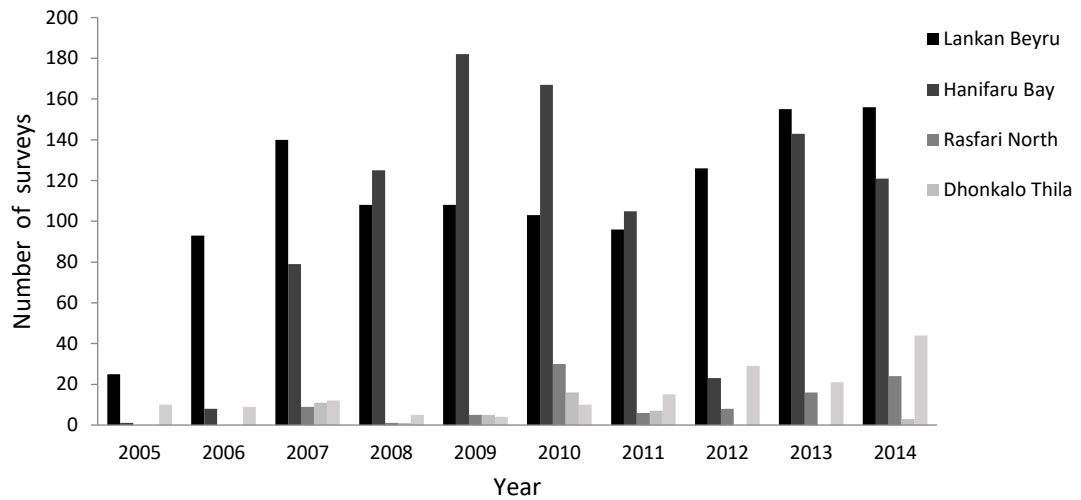


Figure 4.3 Temporal distribution of surveys conducted at the two primary study sites of Lankan Beyru and Hanifaru Bay, and the three secondary study sites of Rasfari North, Dhonkalo Thila and Rangali Madivaru.

The two primary sites were known to be key aggregation areas for mature *M. alfredi*. At Hanifaru Bay, few surveys were undertaken during the first two years of the study due to logistical constraints, while in 2012 survey numbers were down due to restrictions on research permits. Surveys at the three secondary sites (Fig. 4.3), were undertaken more sporadically throughout the study due to logistical constraints.

Surveys at all five sites were timed to coincide with peak manta occurrence (Fig. 4.4). Manta rays frequent the two primary sites in the greatest numbers during the Southwest monsoon (May – November), while greater manta visitations occur at the three secondary sites most frequently during the Northeast monsoon (December – April) (Fig. 4.5).

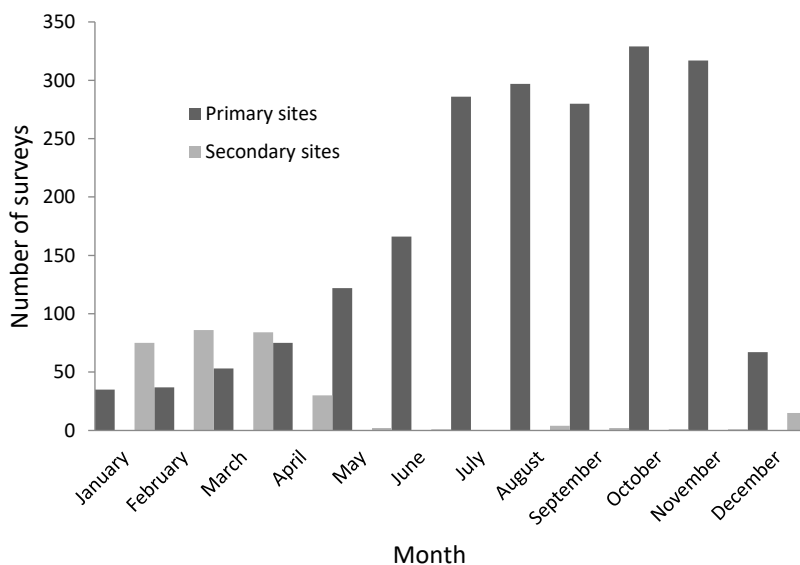


Figure 4.4 Distribution of surveys undertaken at primary and secondary study sites throughout the study period (2005 – 2014).

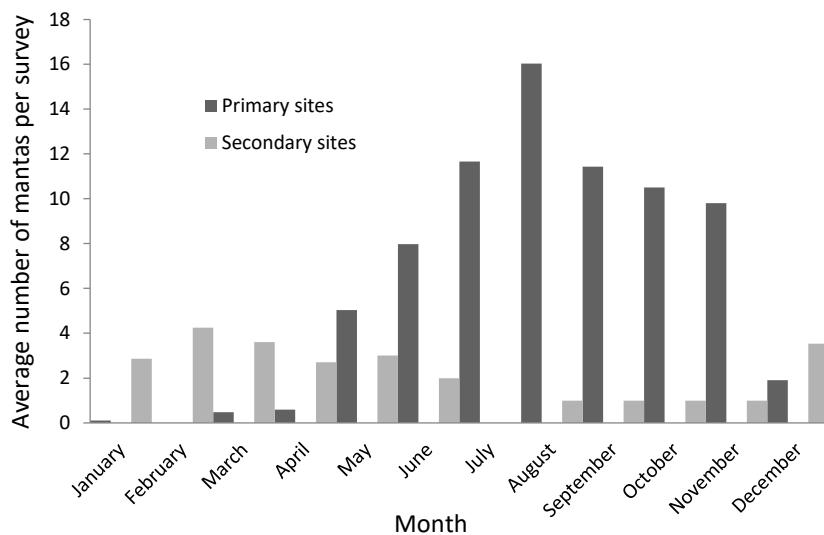


Figure 4.5 *Manta alfredi* sightings throughout the study period (2005 – 2014) at primary and secondary study sites.

It is known that courtship and mating behaviour of *M. alfredi* occurs at all study locations (Chapter 3), that Lankan Beyru and all three secondary study sites attract manta rays to cleaning stations (Coté 2000; Feder 1966; Losey 1972) (Chapter 2), and that Hanifaru Bay acts predominantly as a manta ray feeding aggregation site (Chapter 5).

Primary site: Lankan Beyru, North Malé Atoll

Lankan Beyru is a stretch of reef along the outside eastern edge of North Malé Atoll and is one of the best known tourist locations in the world to dive with *M. alfredi*. Although several cleaning stations occur along the outer reef drop-off the majority of cleaning activity is focused on one main coral patch. This is situated 500 m from a channel, through which strong monsoonal and lunar currents sweep plankton rich water into the atoll's lagoon. The same manta rays which frequent Lankan Beyru also visit a cleaning site inside the atoll called Sunlight Faru, which is just 3.2 km from Lankan Beyru. In this study all sightings collected from both locations are amalgamated together and referred to hereafter as Lankan Beyru.

Primary site: Hanifaru Bay, Baa Atoll

Hanifaru Bay is situated on the eastern edge of Baa Atoll and is a small (700 x 200 m), shallow (22 m max depth) sandy, reef inlet (essentially a cul-de-sac), surrounded by shallow reef (<1 m) along all but a 75 m section at the western end. Hanifaru in the local Dhivehi language translates to narrow 'Hani' reef 'faru'. Due to the unique structure of the bay's outer reef, large aggregations of feeding *M. alfredi* frequently occur inside the bay during the Southwest monsoon. Here strong lunar tides overcome the force of the prevailing monsoonal current, sucking plankton rich water from ocean depths outside the atoll back into the shallow atoll

channel (P. Hosegood, unpublished data). In an atoll pass adjacent to Hanifaru Bay called Dharavandhoo Kanduolhi, these currents form a back eddy which traps and concentrates plankton into the shallow bay of the study site.

Secondary sites: Rasfari North (North Malé Atoll), Dhonkalo Thila and Rangali Madivaru (Ari Atoll)

Secondary sites are situated on the western edges of their respective atolls and serve as important seasonal aggregation sites for *M. alfredi* during the Northeast monsoon. The cleaning stations at Rasfari North and Rangali Madivaru are located along the outer reef crest adjacent to one of the atoll's channels. At both these sites the cleaning stations are only loosely defined as cleaning occurs over a broad area of approximately 100 m, mostly <5 m at Rasfari North, and a little deeper between 10 – 20 m at Rangali Madivaru. The cleaning station at Dhonkalo Thila is inside the one of the atoll's channels at 20 – 30 m, and is also loosely defined.

4.3.3 Sampling protocol

In the Maldives, manta rays are accustomed to interacting with tourist divers and snorkellers at aggregation sites where the rays predictably gather at certain times of the year to feed, clean and socialise (see Chapter 2 methodology for site type descriptions). A typical survey during this study entailed diving or freediving at one of these aggregation sites, where close encounters with the unperturbed rays easily allowed photo-ID images to be taken and observations recorded of the individuals present. Manta surveys were performed on SCUBA or while freediving from either a dedicated research vessel or commercial diving vessels. Surveys were performed at different times of day in all months of the year throughout the month. SCUBA surveys lasted about 60 minutes and ranged to a maximum depth of 30 metres. Freediving surveys generally took 120 minutes, except at Hanifaru Bay where they were on average 4.5 hours as feeding events there lasted longer. Myself, or trained staff or volunteers from the Manta Trust conducted the surveys (www.mantatrust.org/in-the-field/maldives/).

When manta rays were encountered, where possible the following information was recorded for each individual: (1) species, (2) photo-ID, (3) sex, (4) female pregnancy status, (5) maturity status), and (6) behavioural activity. Behavioural activity was further broken down into four major groups: (1) feeding, (2) cleaning, (3) cruising, and (4) courtship. If during the course of an encounter an individual performed several different behavioural activities, the one which

dominated was recorded as the primary behaviour. For a detailed description of each of the criteria listed above and the complete methodology, see Chapter 2.

Individual manta rays are recognisable by their unique pattern of ventral markings (Marshall & Pierce 2012). Using a combination of manual and automated matching systems, all photo-IDs collected during study surveys, or submitted to the Manta Trust by the general public, were compared against a database of identified individuals by one of the Maldives Manta Trust staff (www.mantatrust.org/about-us/the-manta-team/). To confirm a match, or record a new individual, every photo-ID was double checked by the Maldives Manta Trust Project Leader, then triple checked by myself. Each manta ray was assigned a unique ID-code, and every sighting logged and the corresponding photo-ID image archived. Repeat sightings of the same individual on the same day were logged as a single sighting event. For a detailed description of photo-ID and matching methodology, see Chapter 2.

In this study, 75% of the identification pictures used were taken with either a Nikon D700 SLR with a 16mm wide angle lens, or a Sea & Sea DX1G compact underwater camera with wide angle converter lens. The remaining 25% were taken by a wide variety of other compact underwater cameras. Observations on reproductive behaviour were also recorded photographically and scientific notes about these made on underwater slates.

4.3.4 Core group study

Capitalising on the philopatric lifestyle of *M. alfredi* (especially the females, see Chapter 2), which enables reliable encounters with the same individuals at the same locations over time, the fifty most sighted adult females from each of the two primary study sites were selected for a detailed study of their reproductive strategies and fecundity. This sample of one hundred females allowed me to examine if reproductive frequency and periodicity differed within and between the two primary study sites over time. To achieve these aims it was crucial to obtain regular sightings of the individuals within these core groups to determine when, and how often, each individual became pregnant and/ or showed evidence of reproductive activity through at least one of the following: fresh mating wounds, mating scars or courtship behaviour.

To provide a comparative data reference to the two primary study sites where *M. alfredi* aggregate during the Southwest monsoon, data for a further fifty mature females (selected on the same basis as those at the primary sites) were pooled from the three secondary study sites where *M. alfredi* aggregate during the Northeast monsoon. Each of the one hundred

and fifty females were exclusively assigned to one of the three study groups, but all sightings of an individual were included in the data analysed.

4.3.5 Ocean productivity

Chlorophyll-a (chl-a) pigment concentration at the ocean's surface is a convenient index of phytoplankton biomass which often correlates to sea surface temperature (SST), an important influencing factor on the growth of phytoplankton. To investigate the possible influence of primary productivity on *M. alfredi* fecundity, mean monthly SST (°C) and chl-a concentrations were obtained from January 2004 to December 2014 from measurements produced daily by the MODIS sensors on NASA's Aqua satellite. Data were made available by the Distributed Active Archive Centre of the Goddard Space Flight Centre/NASA at <http://disc.sci.gsfc.nasa.gov> (downloaded February 2015). Chl-a and SST data were available continuously throughout the sampling period at a spatial resolution of 9 km, and mean monthly data were converted to yearly averages. Chl-a data were log transformed to improve the spread of skewed values.

4.3.6 Data analysis

Photographic records were analysed to obtain presence/absence records and because expected values were greater than five for all instances (Dytham 2010), Pearson's Chi-Square Tests were performed to examine if: 1) courtship behaviour varied inter-annually or seasonally; 2) pregnancy rates varied seasonally; and 3) visitation rates between sexes at Lankan Beyru showed inter-annual or seasonal variation. To determine if courtship behaviour was a useful predictor of pregnancies, a Cross-Correlation analysis using a lag range (years) of between -7 to 7 was performed on the percentage of adult females observed pregnant each year against the percentage of adults observed to engage in courtship behaviour. As visitations by adult *M. alfredi* to Lankan Beyru were not normally distributed, Kruskal–Wallis one-way analysis of variance tests were used to examine if the mean number of visitations varied inter-annually or seasonally at Lankan Beyru.

Four Generalized Additive Models (GAM) based on Poisson family errors were created to determine whether environmental fluctuations influence the number of manta pregnancies and episodes of courtship behaviour. As SST and chl-a expressed strong co-linearity (i.e. $r > 0.7$), they went into separate models. To avoid model overfitting, and to produce ecologically interpretable results, the models were constrained by three knots (GAMs take

each predictor variable in the model and separate it into sections, delimited by 'knots') and by the number of surveys to account for variations in sampling effort.

All statistical analysis were performed using SPSS version 19.0 (SPSS, Inc. 2011) or the statistical package R (www.r-project.org). Where applicable, data were plotted using t-distributions with 95% confidence intervals (CI) around the mean.

4.4 Results

4.4.1 Total population

From January 2005 through December 2014 30,345 photo-ID sightings of 3,570 individual *M. alfredi* were recorded in 20 of the Maldives' 26 atolls at 193 different sites. Of the animals seen, 1,786 (50%) were female, 1,742 (49%) male, and the sex of 42 (1%) could not be determined (Chapter 2). 1,959 (55%) animals were adult (Chapter 2), of which 745 (38%) were female and 1,214 (62%) male. This equates to an operational sex ratio, defined as the average ratio of fertilisable females to sexually active males at any given time (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996) of 1.63 adult males per adult female, assuming every adult *M. alfredi* in the sampled population was present and able to reproduce each year. This is similar to the operational sex ratio that Deakos (2011) reported for Hawaii of 1.34 males per female.

550 (74%) of the adult females were confirmed to have visible reproductive scars, and/or were recorded pregnant. 192 (76%) of the observed pregnant females (N = 254) had visible reproductive scars, 31 (12%) had no visible reproductive scars, and in a further 31 (12%), presence or absence of reproductive scarring could not be determined. In 195 females where visible reproductive scars or a pregnancy bulge could not be used to determine maturity (Chapter 2), maturity was assumed on the basis of a disc width ≥ 3.2 m.

For the 745 adult females recorded from January 2005 through December 2014, a total of 307 pregnancies were recorded among 254 (34%) individuals (Table 4.1). The average reproductive periodicity for each adult female was calculated by averaging the percentage of the annually sighted adult female population which were pregnant each year, across all years, then dividing 100 by this figure to express the average reproductive periodicity in years. On average, 12.7% of females sighted were pregnant each year, resulting in an average reproductive periodicity rate of one pregnancy per adult female every 7.9 years. When grouped by total years sighted, the average number of pregnancies per adult female

produces similar fecundity periodicity results, with one pregnancy per individual reached between 8 – 9 years (Fig. 4.6). 66% (N = 491) of the adult females in this study were never recorded pregnant, despite being sighted on average 8 times per individual across multiple years. 28% (N = 207) were observed pregnant once and 6% (N = 47) two, three or four times (Fig. 4.7), with none more than this. 2% (N = 12) were pregnant in two consecutive years.

Table 4.1 Annual sightings of adult *Manta alfredi* in the Maldives, distinguished on the basis of sex and pregnancy status.

| Year | Adult males | | Adult females | | Pregnancies | |
|--------------|---------------------|--|---------------------|--|----------------------|---|
| | Individuals sighted | Percent of total adult male population sighted | Individuals sighted | Percent of total adult female population sighted | Individuals pregnant | Percent of adult females sighted pregnant |
| 2005 | 64 | 5% | 92 | 12% | 26 | 28% |
| 2006 | 126 | 10% | 137 | 19% | 7 | 5% |
| 2007 | 285 | 23% | 288 | 39% | 88 | 31% |
| 2008 | 428 | 35% | 361 | 49% | 71 | 20% |
| 2009 | 300 | 25% | 311 | 42% | 37 | 12% |
| 2010 | 383 | 32% | 366 | 50% | 0 | 0% |
| 2011 | 342 | 28% | 268 | 36% | 0 | 0% |
| 2012 | 414 | 34% | 236 | 32% | 2 | 1% |
| 2013 | 495 | 41% | 262 | 35% | 12 | 5% |
| 2014 | 596 | 49% | 249 | 34% | 64 | 26% |
| Total | 3433 | Av.28.3% | 2570 | Av.34.8% | 307 | Av.12.7% |

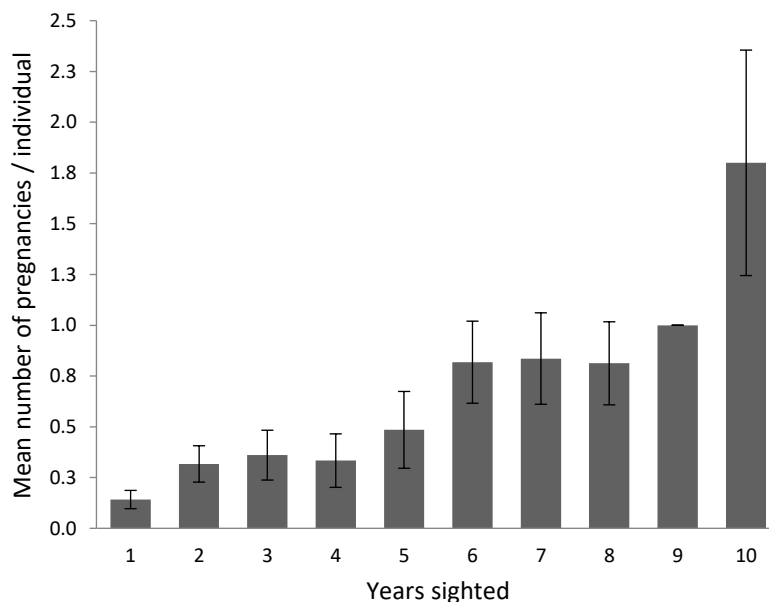


Figure 4.6 Number of pregnancies per adult female *Manta alfredi* (N = 745) Maldives wide, grouped by years sighted during the study period (2005 – 2014). Error bars represent 95% confidence intervals.

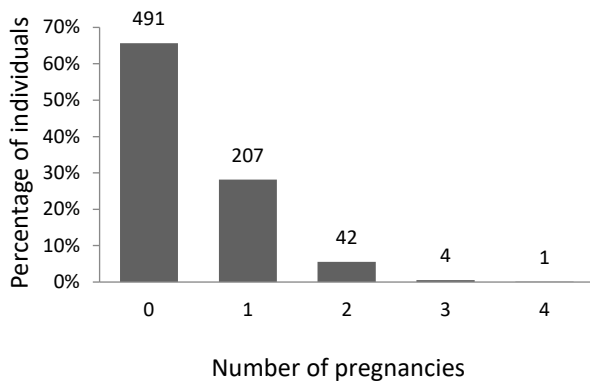


Figure 4.7 Maldives wide figures for pregnancies observed in adult female *Manta alfredi* sighted (N = 745) during the study period (2005 – 2014). Actual numbers presented at the top of bars.

Pregnancy rates varied greatly among years (Fig. 4.8), with 73% (N = 222) occurring in three of the ten years studied (i.e. 2007, 2008 and 2014). For a three year span from 2010 – 2012 reproduction virtually stopped, with just two pregnancies recorded from the entire archipelago, despite continued high sighting rates of adult females (N = 484) (Fig. 4.8). During five of the ten years studied (2006 and 2010 – 2013) $\leq 5\%$ of adult females were visibly pregnant, while in the most fecund years the figure was about a quarter (25 – 31%) of the population.

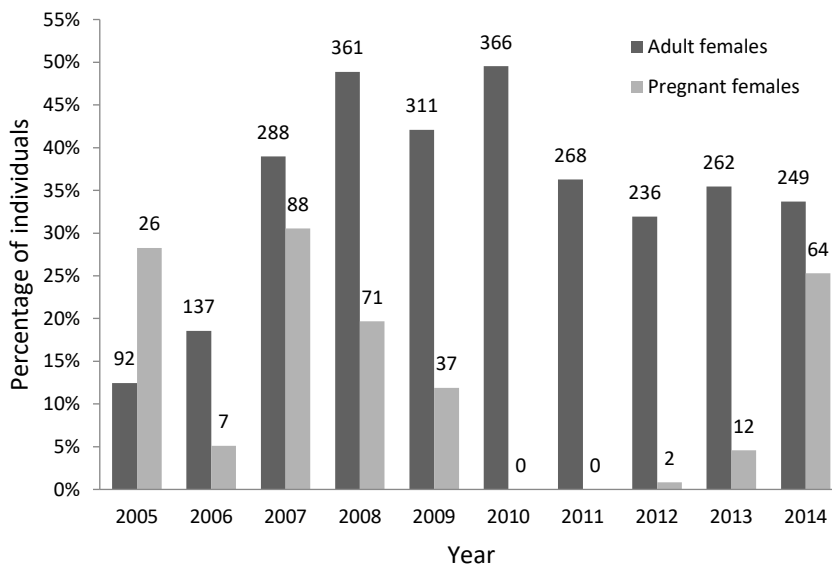


Figure 4.8 Maldives wide annual sightings of adult female *Manta alfredi* (N = 745), and the figures of those recorded pregnant in the same year. Actual numbers presented at the top of bars.

4.4.2 Core group study

4,522 photo-ID sightings were obtained of the 100 adult females from the two primary sites (N = 50 per site), with each individual sighted 45 times on average (Table 4.2). Of these, 81 were visibly pregnant once, 26 twice, and 3 three times, equating to 113 pregnancies with 19 individuals never visibly pregnant. 94 of the 100 females showed visible reproductive scars, and the six that didn't were all observed pregnant at least once. On average, 71% of these

frequently-sited females from Lankan Beyru and 90% from Hanifaru Bay were observed each year. These sightings predominantly occurred during the Southwest monsoon, the annual period when the greatest proportion of late-term pregnancies are observed, reducing the chances of missing any pregnancies.

At Lankan Beyru 15.6% of adult females were observed pregnant each year on average, which equates to an average reproductive periodicity per female of 6.4 years. For Hanifaru Bay the figures were 13.6% and 7.4 years respectively, excluding data from 2005 and 2006 due to the low sampling effort then. Results from the secondary study sites produced similar values at 12.5% and 8.0 years (Table 4.3).

Table 4.2 Reproductive and sighting periodicity of each adult female *Manta alfredi* surveyed within the core group at both primary study sites (N = 50 per site). (-) no sighting, (S) sighted and (P) visibly pregnant. All females possessed visible reproductive scars, except for those marked *.

| Primary site: Lankan Beyru | | | | | | | | | | | | | Primary site: Hanifaru Bay | | | | | | | | | | | | |
|----------------------------|-----------------|------|------|------|------|------|------|------|------|------|------|-----------------|----------------------------|------|------|------|------|------|------|------|------|------|------|----|--|
| Manta ID number | Total sightings | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | Manta ID number | Total sightings | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | | |
| MV-MA-0001 | 46 | S | S | S | S | S | S | S | S | - | - | MV-MA-0055 | 48 | S | S | S | P | S | S | S | S | P | S | | |
| MV-MA-0002 | 29 | - | S | P | S | S | S | - | - | - | - | MV-MA-0261 | 64 | - | P | - | S | S | S | S | S | S | S | | |
| MV-MA-0003 | 36 | S | S | P | S | S | S | S | S | - | - | MV-MA-0298 | 92 | - | - | - | P | S | S | S | S | S | P | | |
| MV-MA-0008 | 23 | S | S | P | S | S | S | S | S | - | - | MV-MA-0358 | 84 | - | - | P | S | S | S | S | S | S | S | | |
| MV-MA-0011 | 20 | S | S | S | S | S | - | - | - | - | - | MV-MA-0412* | 56 | S | S | S | P | S | S | - | S | S | S | | |
| MV-MA-0012 | 35 | S | S | P | S | S | S | S | - | - | - | MV-MA-0416 | 87 | - | - | S | P | S | S | S | S | P | S | | |
| MV-MA-0014 | 37 | P | S | P | S | S | - | - | S | - | S | MV-MA-0421 | 61 | - | - | S | S | S | S | S | S | S | P | | |
| MV-MA-0025 | 84 | P | S | P | S | S | S | S | - | - | - | MV-MA-0441 | 55 | - | S | S | S | S | S | S | S | S | P | | |
| MV-MA-0027 | 28 | S | S | P | S | S | S | S | S | S | - | MV-MA-0444 | 42 | - | - | P | S | S | S | S | S | S | S | | |
| MV-MA-0028 | 39 | S | S | P | S | S | S | S | S | - | - | MV-MA-0447 | 48 | - | - | S | P | P | S | - | S | S | - | | |
| MV-MA-0031 | 43 | S | S | P | S | S | S | S | S | S | P | MV-MA-0451 | 59 | - | - | S | S | S | S | S | S | S | - | | |
| MV-MA-0032 | 19 | S | S | S | S | S | S | S | - | - | - | MV-MA-0460 | 48 | - | - | S | S | P | S | - | S | S | S | | |
| MV-MA-0058* | 29 | S | S | P | S | S | S | S | - | - | - | MV-MA-0462 | 55 | - | - | S | S | P | S | S | S | S | S | | |
| MV-MA-0059 | 43 | P | S | P | S | S | S | S | - | - | - | MV-MA-0464 | 77 | - | - | S | S | P | S | S | S | S | S | | |
| MV-MA-0060 | 39 | P | S | P | S | S | S | S | S | - | - | MV-MA-0502 | 66 | - | - | S | P | S | S | S | S | S | P | | |
| MV-MA-0062 | 68 | S | S | P | S | S | S | S | S | P | - | MV-MA-0539 | 40 | - | - | S | S | S | S | S | S | S | S | | |
| MV-MA-0063 | 21 | S | S | P | S | S | S | - | - | S | S | MV-MA-0541 | 43 | - | - | S | P | S | S | S | - | S | P | | |
| MV-MA-0066 | 45 | S | P | S | S | S | S | - | - | S | S | MV-MA-0546 | 30 | - | - | S | S | S | S | - | S | S | P | | |
| MV-MA-0067 | 18 | S | S | S | S | S | S | S | - | - | - | MV-MA-0585 | 41 | - | - | S | S | - | S | S | S | S | - | | |
| MV-MA-0068 | 30 | S | S | P | S | S | S | S | - | - | - | MV-MA-0606 | 60 | - | - | S | S | S | S | S | S | S | P | | |
| MV-MA-0069 | 62 | P | S | S | S | P | S | S | S | S | S | MV-MA-0623 | 101 | S | - | - | P | S | S | S | S | P | S | | |
| MV-MA-0071 | 75 | P | S | P | S | S | S | S | S | - | - | MV-MA-0665 | 32 | - | S | - | P | P | S | S | S | S | S | | |
| MV-MA-0073 | 30 | P | S | P | S | S | S | - | - | - | - | MV-MA-0747 | 54 | - | - | S | S | S | S | S | S | S | P | | |
| MV-MA-0075 | 24 | P | S | S | S | S | S | - | - | - | - | MV-MA-0771 | 89 | - | - | S | S | S | S | - | S | S | S | | |
| MV-MA-0078 | 18 | P | - | P | S | S | S | S | - | - | - | MV-MA-0778 | 73 | - | - | P | S | S | S | S | S | S | S | | |
| MV-MA-0090 | 53 | S | S | P | S | S | S | S | S | S | S | MV-MA-0784 | 39 | - | - | S | S | - | S | S | S | S | S | | |
| MV-MA-0100 | 28 | P | S | P | S | P | S | - | - | - | - | MV-MA-0798 | 53 | - | - | S | S | S | S | S | S | S | S | | |
| MV-MA-0102 | 38 | P | S | P | S | S | S | S | - | - | - | MV-MA-0850 | 41 | - | - | S | P | S | S | S | - | S | S | | |
| MV-MA-0104 | 57 | S | S | P | S | S | S | - | - | - | - | MV-MA-0890 | 35 | - | - | S | S | - | S | S | S | S | P | | |
| MV-MA-0105 | 54 | S | S | P | S | S | S | - | - | - | - | MV-MA-0891 | 40 | - | - | S | S | S | S | S | S | S | S | | |
| MV-MA-0118 | 23 | S | S | S | S | P | S | - | - | - | - | MV-MA-0895 | 83 | - | - | S | P | S | S | S | S | S | S | | |
| MV-MA-0127 | 79 | - | S | P | S | S | S | S | S | S | S | MV-MA-0909 | 36 | - | - | S | S | S | S | S | - | S | S | | |
| MV-MA-0128 | 50 | S | S | P | S | P | S | - | - | - | - | MV-MA-0946* | 32 | - | - | - | P | P | S | S | - | S | S | | |
| MV-MA-0129 | 33 | - | S | P | S | S | S | - | - | - | - | MV-MA-1019* | 40 | - | - | - | S | S | S | S | S | S | P | | |
| MV-MA-0132 | 24 | - | S | P | S | S | S | - | - | - | - | MV-MA-1054* | 48 | - | - | - | P | S | S | - | S | P | S | | |
| MV-MA-0150 | 27 | - | S | P | S | S | S | S | S | S | S | MV-MA-1081 | 43 | - | - | - | P | S | S | S | S | S | - | | |
| MV-MA-0151 | 28 | S | S | P | S | S | S | S | - | - | - | MV-MA-1100 | 45 | - | - | - | P | S | S | S | S | S | P | | |
| MV-MA-0156 | 26 | - | S | P | S | S | S | S | - | - | - | MV-MA-1111* | 82 | - | S | - | P | S | S | - | S | S | S | | |
| MV-MA-0167 | 21 | - | S | P | S | - | S | S | - | S | S | MV-MA-1124 | 52 | - | - | - | S | S | S | S | S | S | S | | |
| MV-MA-0173 | 24 | S | S | S | S | S | S | - | - | - | - | MV-MA-1146 | 30 | - | - | - | P | S | S | - | S | S | P | | |
| MV-MA-0174 | 21 | S | S | S | S | S | S | - | - | - | - | MV-MA-1152 | 36 | - | - | - | S | S | S | S | S | S | S | | |
| MV-MA-0181 | 27 | - | S | P | S | S | S | S | - | - | - | MV-MA-1162 | 33 | - | - | - | S | P | S | - | S | P | S | | |
| MV-MA-0198 | 22 | - | S | P | S | S | S | S | - | - | - | MV-MA-1176 | 79 | - | - | - | P | S | S | S | S | S | P | | |
| MV-MA-0211 | 25 | S | S | S | S | S | S | - | S | S | P | MV-MA-1184 | 34 | - | - | - | S | S | S | S | S | S | S | | |
| MV-MA-0232 | 33 | S | P | - | S | S | S | S | - | - | - | MV-MA-1260 | 49 | - | - | - | S | S | S | S | S | S | P | | |
| MV-MA-0255 | 21 | S | S | P | S | - | S | S | - | - | - | MV-MA-1365 | 63 | - | - | - | S | S | S | S | S | S | S | | |
| MV-MA-0272 | 25 | P | S | P | S | P | S | S | S | - | - | MV-MA-1369 | 61 | - | - | - | P | S | S | S | S | S | S | | |
| MV-MA-0275 | 15 | - | S | P | - | S | S | - | - | - | S | MV-MA-1389 | 50 | - | S | S | S | S | S | S | S | P | S | | |
| MV-MA-0329 | 25 | P | S | P | S | P | S | S | - | - | - | MV-MA-1734 | 64 | - | - | - | S | S | S | S | S | S | P | | |
| MV-MA-0534 | 28 | - | - | S | S | S | S | S | - | S | P | MV-MA-1932 | 42 | - | - | - | - | S | S | S | S | S | S | | |
| Sightings | 1748 | 39 | 48 | 49 | 49 | 48 | 49 | 32 | 17 | 12 | 13 | Sightings | 2715 | 3 | 7 | 29 | 49 | 47 | 50 | 41 | 46 | 50 | 46 | | |
| Pregnancies | 62 | 13 | 2 | 37 | 0 | 6 | 0 | 0 | 0 | 0 | 4 | Pregnancies | 51 | 0 | 1 | 3 | 19 | 7 | 0 | 0 | 0 | 0 | 6 | 15 | |

Table 4.3 Pregnancy rates of the core population of adult female *Manta alfredi* observed at each of the two primary sites (N = 50 at both), and at the three secondary sites combined (N = 50).

| Primary site: Lankan Beyru | | | | | Primary site: Hanifaru Bay | | | | | Secondary sites combined | | | | |
|----------------------------|-------------------|---------------------|----------------------|---|----------------------------|-------------------|---------------------|----------------------|---|--------------------------|-------------------|---------------------|----------------------|---|
| Year | Number of surveys | Individuals sighted | Individuals pregnant | Percent of sighted individuals pregnant | Year | Number of surveys | Individuals sighted | Individuals pregnant | Percent of sighted individuals pregnant | Year | Number of surveys | Individuals sighted | Individuals pregnant | Percent of sighted individuals pregnant |
| 2005 | 25 | 39 | 13 | 33% | 2005 | 1 | 3 | 0 | Exc. | 2005 | 10 | 11 | 3 | 27% |
| 2006 | 93 | 48 | 2 | 4% | 2006 | 8 | 7 | 1 | Exc. | 2006 | 9 | 11 | 1 | 9% |
| 2007 | 140 | 49 | 37 | 76% | 2007 | 79 | 29 | 3 | 10% | 2007 | 32 | 23 | 8 | 35% |
| 2008 | 108 | 49 | 0 | 0% | 2008 | 125 | 49 | 19 | 39% | 2008 | 7 | 27 | 6 | 22% |
| 2009 | 108 | 48 | 6 | 13% | 2009 | 182 | 47 | 7 | 15% | 2009 | 14 | 21 | 2 | 10% |
| 2010 | 103 | 49 | 0 | 0% | 2010 | 167 | 50 | 0 | 0% | 2010 | 56 | 33 | 0 | 0% |
| 2011 | 96 | 32 | 0 | 0% | 2011 | 105 | 41 | 0 | 0% | 2011 | 28 | 30 | 0 | 0% |
| 2012 | 126 | 17 | 0 | 0% | 2012 | 23 | 46 | 0 | 0% | 2012 | 37 | 26 | 0 | 0% |
| 2013 | 155 | 12 | 0 | 0% | 2013 | 143 | 50 | 6 | 12% | 2013 | 37 | 30 | 0 | 0% |
| 2014 | 156 | 13 | 4 | 31% | 2014 | 121 | 46 | 15 | 33% | 2014 | 71 | 31 | 7 | 23% |
| Total | 1110 | 356 | 62 | Av.15.6% | Total | 954 | 368 | 51 | Av.13.6% | Total | 301 | 243 | 27 | Av.12.5% |

4.4.3 Variations within and between study sites

Pregnancy rates fluctuated greatly among study sites and years. For example, in 2007 at Lankan Beyru, 76% (N = 37) of sighted females (N = 49) were visibly pregnant, while in the following seven years at this site just 10 pregnancies were recorded in total, all in 2009 (N = 6) or 2014 (N = 4) (Fig. 4.9). Towards the end of the study, despite high sampling effort, sightings of the core 50 adult females at Lankan Beyru began to decline, reaching a low of only 24% (N = 12) in 2013. The absent females were not recorded elsewhere in the archipelago.

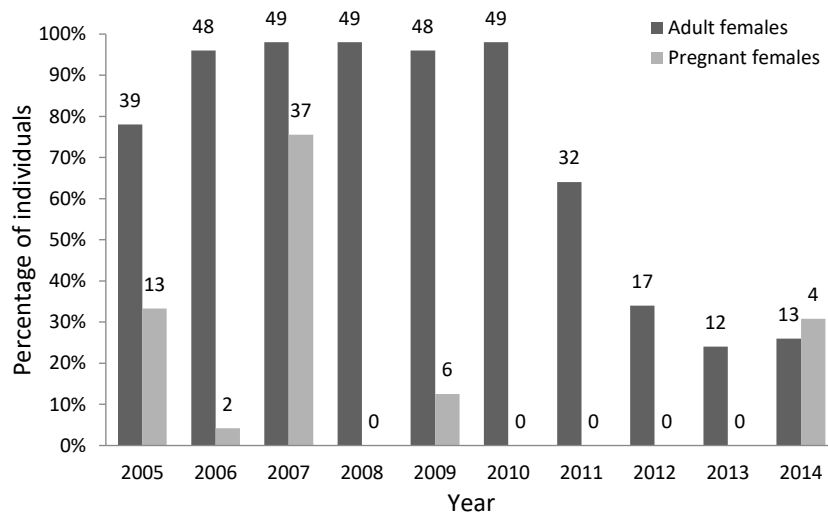


Figure 4.9 Annual sightings of Lankan Beyru's core population of adult female *Manta alfredi* (N = 50), and the figures of those recorded pregnant in the same year. Actual numbers presented at the top of bars.

At Hanifaru Bay during 2007, just 10% (N = 3) of core females were observed pregnant compared to the 76% at Lankan Beyru the same year. However, in the following year when no pregnancies were recorded at Lankan Beyru, 39% (N = 19) of the sighted core females that year (N = 49) were pregnant (Fig. 4.10). As at Lankan Beyru, no core animal at Hanifaru Bay appeared pregnant for three years between 2010 and 2012, but at Hanifaru Bay pregnancies re-appeared in 2013 and 2014, not just in 2014, as at Lankan Beyru.

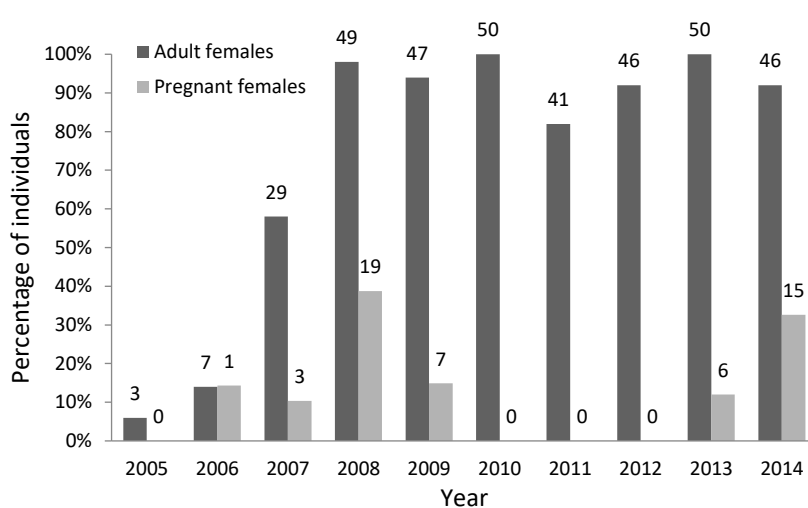


Figure 4.10 Annual sightings of Hanifaru Bay’s core population of adult female *Manta alfredi* (N = 50), and the figures of those recorded pregnant in the same year. Actual numbers presented at the top of bars.

At the three combined secondary study sites frequented by mantas during the Northeast monsoon, the years with the greatest rates of pregnancy were 2005, 2007, 2008 and 2014 (Fig. 4.11), which corresponded with the four highest years of recorded pregnancies at the two primary sites. Individuals at these sites appeared to completely cease reproduction for a 4 year period 2010–2013.

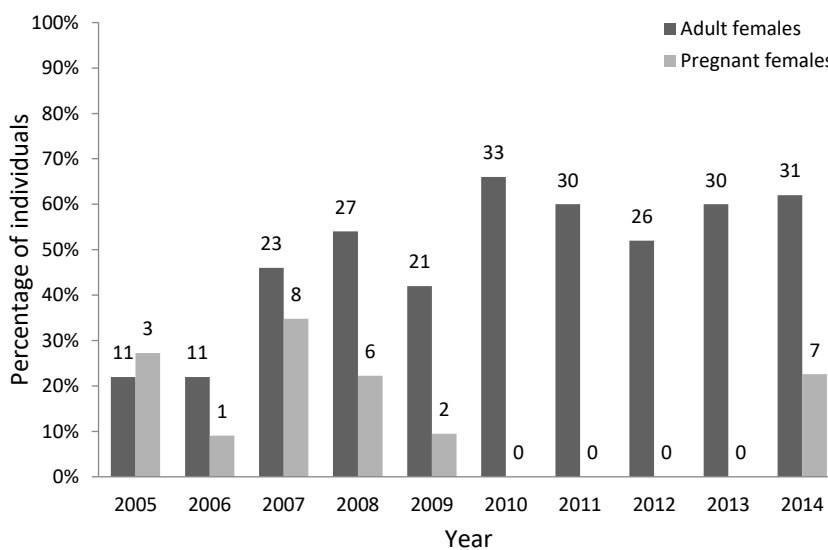


Figure 4.11 Annual sightings of core adult female *Manta alfredi* at secondary sites combined (N = 50), and the figures of those recorded pregnant in the same year. Actual numbers presented at the top of bars.

4.4.4 Courtship behaviour and seasonality

Observed frequencies of courtship behaviour showed significant inter-annual variation ($\chi^2 = 174.55$, $df = 9$, $N = 321$, $p < 0.001$), with the greatest amount observed in 2006, 2007, 2013 and 2014, exhibited by 19% (N = 51), 13% (N = 72), 8% (N = 60), and 6% (N = 53) respectively of the observed adult *M. alfredi* (Fig. 4.12). These figures were followed by corresponding peaks in pregnancies. Likewise, low pregnancy numbers followed for years with little prior courtship behaviour (Fig. 4.12).

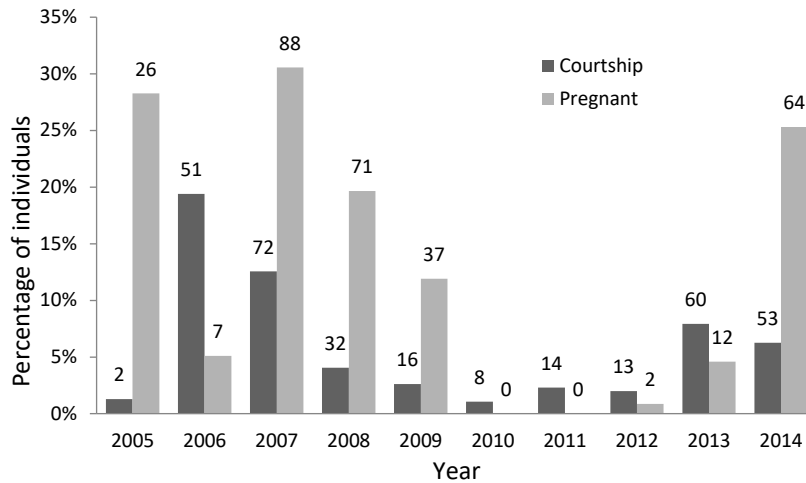


Figure 4.12 Annual figures for Maldives wide adult female *Manta alfredi* sighted visibly pregnant, and all adults sighted which engaged in courtship behaviour. Actual numbers presented at the top of bars.

To help determine if courtship behaviour was a useful predictor of pregnancy, a Cross-Correlation analysis was performed on the percentage of adult females observed pregnant each year against the percentage of adult *M. alfredi* seen to engage in courtship behaviour, using a lag range of -7 to 7. A lag of 1 year between courtship behaviour and pregnancies produced the highest Cross-Correlation Function of 0.804 (SE = +/- 0.333).

Courtship behaviour varied significantly with season ($\chi^2 = 419.99$, $df = 11$, $N = 302$, $p < 0.001$), with 181 (60%) of the 302 individuals observed engaged in this behaviour doing so in October and November at the end of the Southwest monsoon. By contrast, courtship behaviour during the first five months of the Southwest monsoon (May – Sept) was lower than throughout the rest of the year (Fig. 4.13).

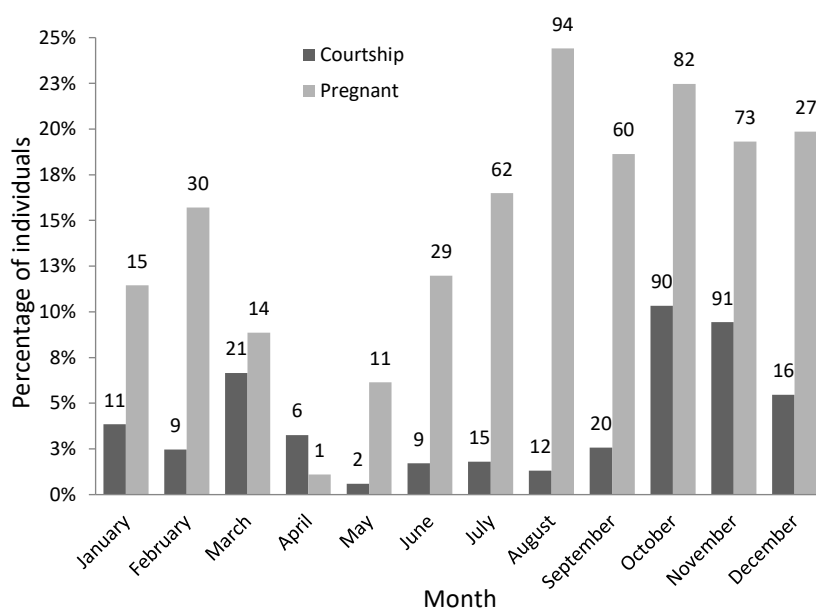


Figure 4.13 Monthly figures for Maldives wide adult female *Manta alfredi* sighted visibly pregnant, and all adults sighted which engaged in courtship behaviour (2005 – 2014). Actual numbers presented at the top of bars.

Pregnancy rates also showed significant seasonal variation throughout the study ($\chi^2 = 257.33$, $df = 11$, $N = 498$, $p < 0.001$). Numbers of visibly pregnant females peaked in August at 94 individuals, remained high throughout the rest of the year, dropping to a low in April, when only one pregnant individual was seen (Fig. 4.13). Lows in records of visibly pregnant females (April) and courtship behaviour (May), coincide with the period of change between the Northeast and Southwest monsoons.

When courtship behaviour is investigated by primary site function, a much higher percentage of courtship activity was observed at cleaning sites (Lankan Beyru) (Fig. 4.14), than at feeding sites (Hanifaru Bay) (Fig. 4.15). All previous studies on *M. alfredi* courtship and reproductive activity have been undertaken at cleaning stations.

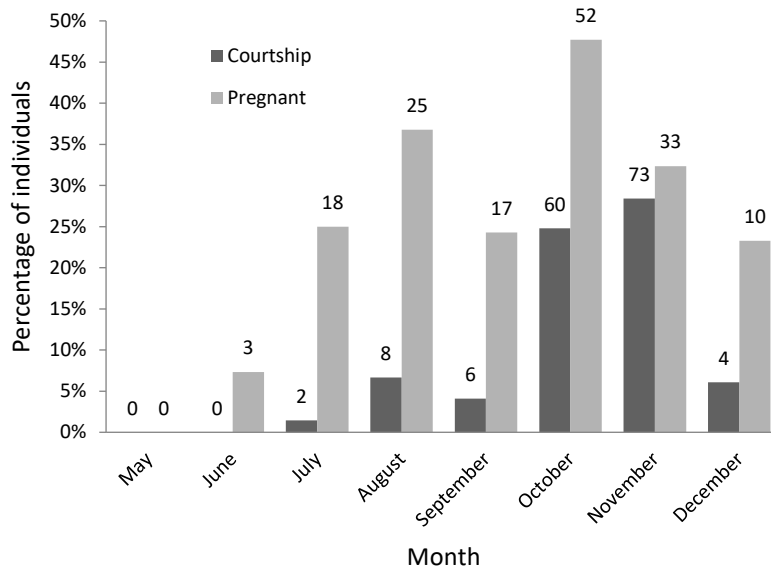


Figure 4.14 Monthly figures for adult female *Manta alfredi* sighted at Lankan Beyru visibly pregnant, and all adults sighted which engaged in courtship behaviour (2005 – 2014). Actual numbers presented at the top of bars.

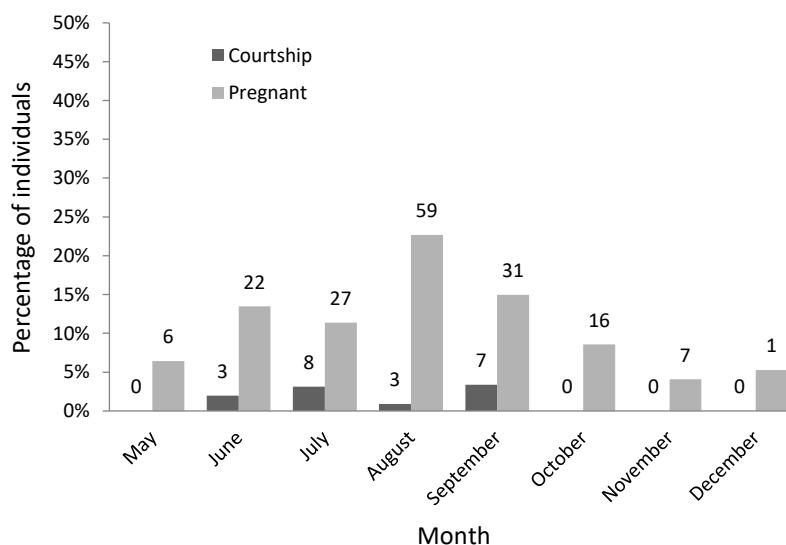


Figure 4.15 Monthly figures for adult female *Manta alfredi* sighted at Hanifaru Bay visibly pregnant, and all adults sighted which engaged in courtship behaviour (2005 – 2014). Actual numbers presented at the top of bars.

Courtship behaviour peaked at Lankan Beyru during November, the month directly after the month when observations of pregnant females peaked (N = 52) (Fig. 4.14). At Hanifaru Bay, the highest number of visibly pregnant females observed was two months earlier in August (N = 59) (Fig. 4.15).

87 different adult females were observed to engage in courtship trains during the study, 25% (N = 22) of which were visibly pregnant. 23% (N = 5) of them were observed again within a month having giving birth. A small number of individuals were sighted with such regularity during the period of high reproductive activity in 2006 – 7 that their reproductive periodicity could be tracked in detail (Table 4.4). During the monsoon transitional months of October and November in 2006, all five of the Lankan Beyru’s adult females listed in Table 4.4 engaged in reproductive behaviour, or bore fresh mating wounds. During the next six months these same individuals were all sighted at Lankan Beyru and/or Rasfari North, although none were visibly pregnant. However, by July 2007 they all were, and by November all had given birth and were seen to engage in courtship behaviour (Sept – Nov), with three of the five doing so while heavily pregnant (MV-MA-0003, MV-MA-0014, and MV-MA-0090). MV-MA-0025 was seen on the 28th October 2007 visibly close to parturition, then again four days later (1st November 2008) having given birth and with fresh mating wounds on her pectoral fin. Two days later she was seen again in a courtship train. MV-MA-0071 was also seen to engage in courtship behaviour within weeks of parturition and to have fresh mating wounds. All five individuals were sighted frequently during the next 12 months, but despite all engaging in courtship behaviour and/or copulating in late 2007, none were visibly pregnant the following year. During this time they showed no signs of injury or poor health which could have increased the likelihood of miscarriage if a female had become pregnant.

Table 4.4 Summary of sightings of reproductive periodicity and behaviour of five female *Manta alfredi* at Lankan Beyru from Oct-06 to Nov-07. (-) = no sightings, NVP = no visible pregnancies, VP = visible pregnancy, GB = birth occurred since last encounter, FMW = fresh mating wounds, and CT = courtship train. * indicates the sighting was recorded at the secondary study site of Rasfari North.

| Manta ID number | Oct-06 | Nov-06 | Dec-06 | Jan-07 | Feb-07 | Mar-07 | Apr-07 | May-07 | Jun-07 | Jul-07 | Aug-07 | Sep-07 | Oct-07 | Nov-07 | |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-----------|----------|-----------|
| MV-MA-0003 | FMW | - | - | - | *NVP | - | - | - | - | VP | - | VP | CT-VP | VP | GB |
| MV-MA-0014 | CT | - | - | *NVP | - | - | NVP | - | - | VP | VP | VP | CT-VP | VP | GB |
| MV-MA-0025 | FMW | CT | - | - | *NVP | - | - | NVP | - | - | VP | VP | - | VP | GB-FMW-CT |
| MV-MA-0071 | CT-FMW | NVP | - | *NVP | *NVP | - | NVP | NVP | VP | VP | - | VP | GB-FMW-CT | NVP | VP |
| MV-MA-0090 | FMW | NVP | NVP | *NVP | *NVP | - | NVP | NVP | - | VP | - | - | VP | CT-VP-GB | VP |

During the study’s second period of high reproductive activity in 2013 – 14, 28% (N = 14) of Hanifaru Bay’s 50 core females were observed to engage in sufficient reproductive behaviour to track their reproductive periodicity (Table 4.5). All did this between June and October

2013, or showed fresh mating wounds then. However, over the following 12 months, and unlike the females from Lankan Beyru (Table 4.4), the reproductive patterns of the Hanifaru females varied greatly. 71% (N = 10) became visibly pregnant in the following 12 months. One individual (MV-MA-1162) became visibly pregnant just four months after being sighted with fresh mating wounds, but was no longer visibly pregnant when sighted again 8 months later. Two other females (MV-MA-1019 & MV-MA-1146) also appeared to commence gestation directly after copulation (presumed by the presence of fresh mating wounds). When sighted 10 – 12 months after their copulation was presumed to have occurred, both these females were visibly close to parturition, then again within a month having given birth. MV-MA-1146 was also observed engaging in a courtship train in July 2014 while close to parturition. Of the remaining seven visibly pregnant females, one (MV-MA-0606) gave birth 15 months after copulation was presumed to have occurred, while the remaining six were not visibly observed pregnant until 15 – 17 months after copulation was presumed to have taken place and were still visibly pregnant when last sighted during this study in November 2014. The remaining four core individuals listed in Table 4.5 had fresh mating wounds in both 2013 and 2014, yet no visible pregnancies were recorded in the months between or thereafter. It is important to note that the observation of engagement in courtship behaviour and the presence of fresh mating wounds does not necessarily mean a successful copulation or insemination occurred at this time.

Table 4.5 Summary of sightings of reproductive periodicity and behaviour of fourteen female *Manta alfredi* at Hanifaru Bay from June-13 to Nov-14. (-) = no sightings, NVP = no visible pregnancies, VP = visible pregnancy, GB = birth occurred since last encounter, FMW = fresh mating wounds, and CT = courtship train. * indicates the sighting was recorded at a different study site.

| Manta ID number | Jun-13 | Jul-13 | Aug-13 | Sep-13 | Oct-13 | Nov-13 | Dec-13 | Jan-14 | Feb-14 | Mar-14 | Apr-14 | May-14 | Jun-14 | Jul-14 | Aug-14 | Sep-14 | Oct-14 | Nov-14 |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| MV-MA-0298 | FMW | NVP | NVP | NVP | NVP | NVP | - | - | *NVP | - | - | NVP | NVP | NVP | NVP | NVP | VP | VP |
| MV-MA-0358 | NVP | FMW | FMW | NVP | NVP | NVP | - | - | - | - | - | NVP | NVP | - | FMW | NVP | NVP | NVP |
| MV-MA-0462 | FMW | - | NVP | NVP | NVP | NVP | - | - | - | - | - | - | FMW | NVP | NVP | NVP | NVP | NVP |
| MV-MA-0464 | - | NVP | NVP | NVP | FMW | NVP | - | - | - | *NVP | - | - | NVP | FMW | NVP | - | NVP | NVP |
| MV-MA-0502 | - | FMW | - | NVP | NVP | - | NVP | - | - | - | *NVP | - | - | NVP | NVP | NVP | NVP | VP |
| MV-MA-0541 | - | CT-FMW | - | - | NVP | NVP | - | - | - | - | - | - | - | NVP | NVP | NVP | NVP | VP |
| MV-MA-0606 | - | FMW | NVP | NVP | NVP | - | - | - | - | - | - | - | VP | VP | VP | VP | GB | NVP |
| MV-MA-0895 | - | - | - | NVP | FMW | - | - | - | - | - | - | - | - | FMW | NVP | - | NVP | NVP |
| MV-MA-1019 | - | CT | NVP | NVP | NVP | NVP | - | - | - | - | - | - | VP | VP | GB | NVP | NVP | - |
| MV-MA-1146 | - | - | FMW | NVP | NVP | - | - | - | - | - | - | - | - | VP-CT | GB | NVP | NVP | NVP |
| MV-MA-1162 | FMW | NVP | - | NVP | VP | VP | - | - | - | - | - | - | - | NVP | - | - | - | - |
| MV-MA-1176 | FMW | NVP | NVP | - | NVP | NVP | - | - | - | - | - | - | NVP | NVP | - | - | - | VP |
| MV-MA-1260 | FMW | NVP | - | - | NVP | - | - | - | - | *NVP | - | - | - | NVP | NVP | - | - | NVP |
| MV-MA-1734 | - | NVP | FMW | NVP | NVP | NVP | - | - | - | - | - | - | NVP | NVP | - | NVP | NVP | VP |

Throughout the study period at Lankan Beyru the mean number of adult *M. alfredi* sighted per survey varied significantly, seasonally (averaged across all years) ($K = 319.71$, $df = 9$, $N = 1110$, $p < 0.001$) (Table 4.6), and among years ($K = 251.62$, $df = 11$, $N = 1110$, $p < 0.001$) (Table 4.7). At Lankan Beyru sightings increased steadily throughout the year (Fig. 4.16), peaking during the months of October and November when reproductive behaviour was also at its highest (Fig. 4.14), before dropping off steeply when the monsoon changed in December.

Inter-annually, sightings at Lankan Beyru showed a significant decrease from 2010 onwards (Fig. 4.17).

Table 4.6 Monthly sightings of adult *Manta alfredi* at Lankan Beyru (2005 – 2014).

| Month | Adult females | Adult males | Total individuals | Total sightings | Number of surveys | Mean number of mantas / survey |
|--------------|---------------|-------------|-------------------|-----------------|-------------------|--------------------------------|
| January | 4 | 0 | 4 | 4 | 25 | 0.16 |
| February | 0 | 0 | 0 | 0 | 26 | 0.00 |
| March | 12 | 6 | 18 | 19 | 44 | 0.57 |
| April | 22 | 2 | 24 | 36 | 67 | 0.66 |
| May | 44 | 23 | 67 | 108 | 71 | 1.85 |
| June | 41 | 31 | 72 | 95 | 70 | 1.86 |
| July | 72 | 67 | 139 | 312 | 114 | 3.60 |
| August | 68 | 52 | 120 | 275 | 120 | 3.10 |
| September | 70 | 76 | 146 | 339 | 130 | 3.44 |
| October | 109 | 133 | 242 | 888 | 176 | 5.63 |
| November | 102 | 155 | 257 | 1058 | 216 | 5.88 |
| December | 43 | 23 | 66 | 110 | 51 | 2.43 |
| Total | 587 | 568 | 1155 | 3244 | 1110 | 2.43 |

Table 4.7 Yearly sightings of adult *Manta alfredi* at Lankan Beyru.

| Year | Adult females | Adult males | Total individuals | Total sightings | Number of surveys | Mean number of mantas / survey |
|--------------|---------------|-------------|-------------------|-----------------|-------------------|--------------------------------|
| 2005 | 60 | 36 | 96 | 141 | 25 | 6.08 |
| 2006 | 85 | 80 | 165 | 461 | 93 | 5.90 |
| 2007 | 101 | 134 | 235 | 890 | 140 | 7.59 |
| 2008 | 68 | 96 | 164 | 458 | 108 | 5.21 |
| 2009 | 66 | 57 | 123 | 368 | 108 | 4.37 |
| 2010 | 69 | 58 | 127 | 444 | 103 | 4.61 |
| 2011 | 39 | 32 | 71 | 157 | 96 | 1.75 |
| 2012 | 18 | 21 | 39 | 71 | 126 | 0.94 |
| 2013 | 16 | 39 | 55 | 117 | 155 | 1.08 |
| 2014 | 16 | 57 | 73 | 137 | 156 | 1.40 |
| Total | 538 | 610 | 1148 | 3244 | 1110 | 3.89 |

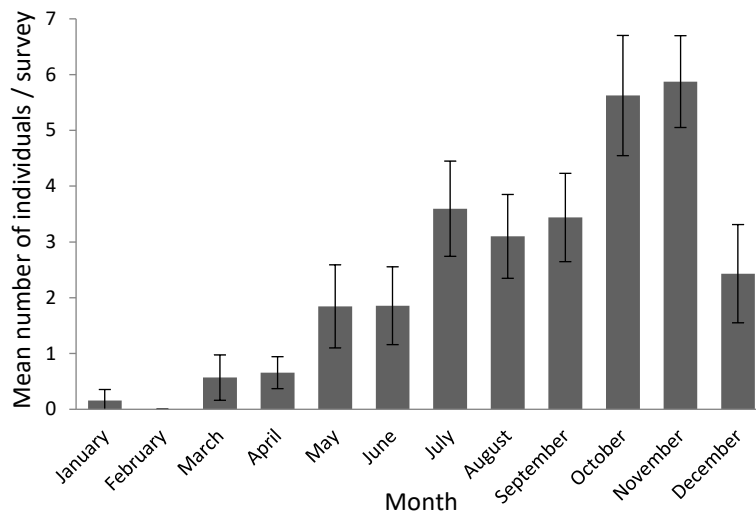


Figure 4.16 Monthly sightings of adult *Manta alfredi* per survey at Lankan Beyru, averaged across all study years (2005 – 2014). Error bars represent 95% confidence intervals.

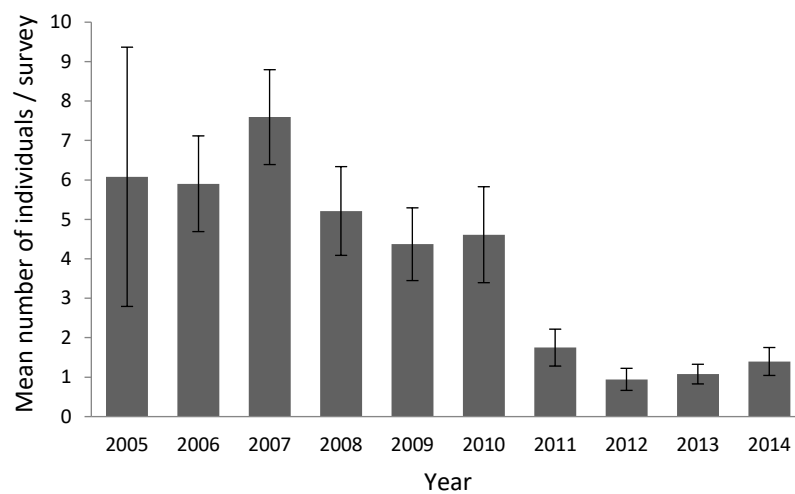


Figure 4.17 Yearly sightings of adult *Manta alfredi* per survey at Lankan Beyru. Error bars represent 95% confidence intervals.

The mean increase in the number of adult *M. alfredi* sighted per survey each month at Lankan Beyru corresponded to a shift in population demographics at this site. As the Southwest monsoon advanced (May – November), the sex-ratio of the adult population shifted significantly from 34% male in May to a peak of 60% male in November, then back down to 35% male in December ($\chi^2 = 29.80$, $df = 7$, $N = 1109$, $p < 0.001$) (Fig. 4.18). Courtship activity peaked in November when the ratio of males to females was highest (Fig 4.14).

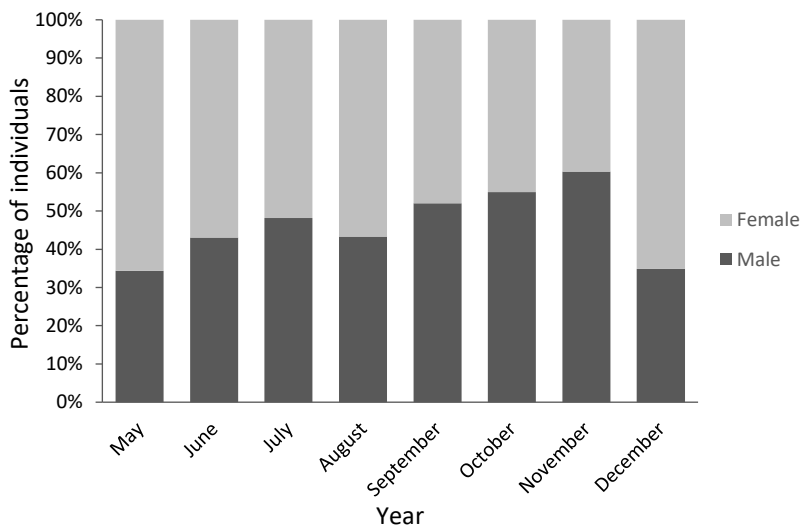


Figure 4.18 Average composition of adult *Manta alfredi* at Lankan Beyru during the Southwest monsoon (2005 – 2014).

There were also significant shifts in sex-ratio of the adult *M. alfredi* population inter-annually at Lankan Beyru ($\chi^2 = 46.11$, $df = 9$, $N = 1148$, $p < 0.001$). Between 2006 and 2008, and again between 2012 and 2014, the demographics shifted towards a majority of adult males (Fig.4.19), when courtship activity was also at its highest (2006 – 2007 and 2013) (Fig 4.20).

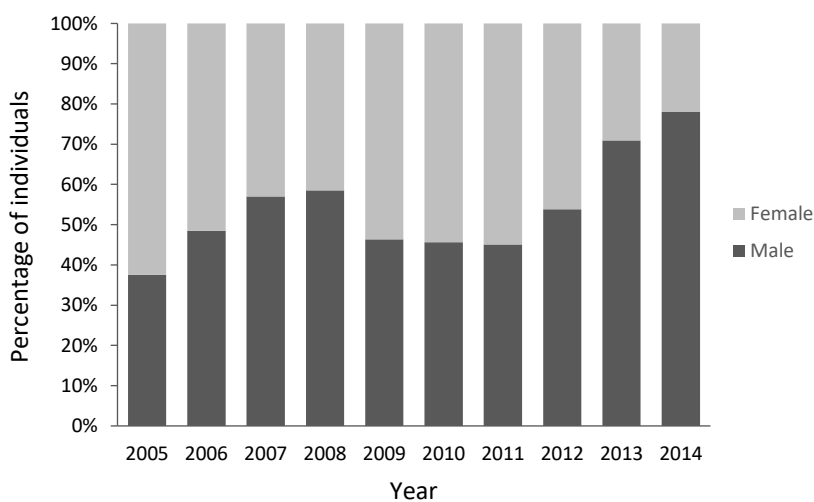


Figure 4.19 Demographic composition of the adult *Manta alfredi* from annual sightings at Lankan Beyru.

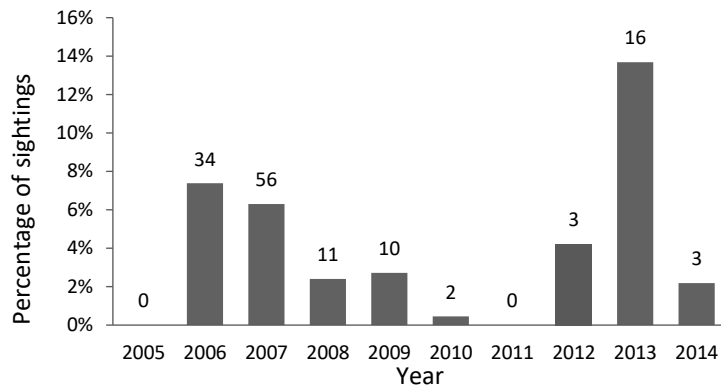


Figure 4.20 Annual sightings of adult *Manta alfredi* at Lankan Beyru in which courtship was the primary behaviour observed. Actual numbers presented at the top of bars.

4.4.5 Ocean productivity

Figure 4.21A shows interannual changes in marine Chl-a concentrations around the Maldives, while Figure 4.22B shows annual deviations in values from the long-term mean across the entire region. Productivity was highest in 2005, and thereafter dropped to a low in 2012 before recovering towards the mean. Over this timeframe, SST's showed a generally contrary pattern, with lowest temperatures in 2005 and highest in 2012 (Fig. 4.21B and 4.22A).

Models suggest that deviation from the mean concentration of chl-a, deviation from the mean SST, and sampling effort all significantly influenced both number of manta pregnancies and instances of courtship behaviour observed (Table 4.8). Chl-a accounted for 39.4% of the variance in number of manta pregnancies and 30.4% of the variance in courtships observed, while SST accounted for 41.1% of the former and 27.1% of the latter. When chl-a concentrations were moderately elevated it increased the chance of observing a pregnant manta by 14 fold and made the likelihood of observing courtship behaviour 16 times higher. During periods of average SST the chance of observing a pregnant manta is 56 times greater than during periods of extreme low and high temperatures, and courtship behaviour is 10 times more likely to be observed.

Between 2010 and 2012, when virtually no pregnancies were recorded and courtship behaviour was at its lowest, three of the four warmest SST years and three of the four least productive years for Chl-a occurred. In 2013, when Chl-a concentrations rose above the mean and SSTs dropped below it, courtship behaviour and pregnancies once again increased (Fig. 4.22).

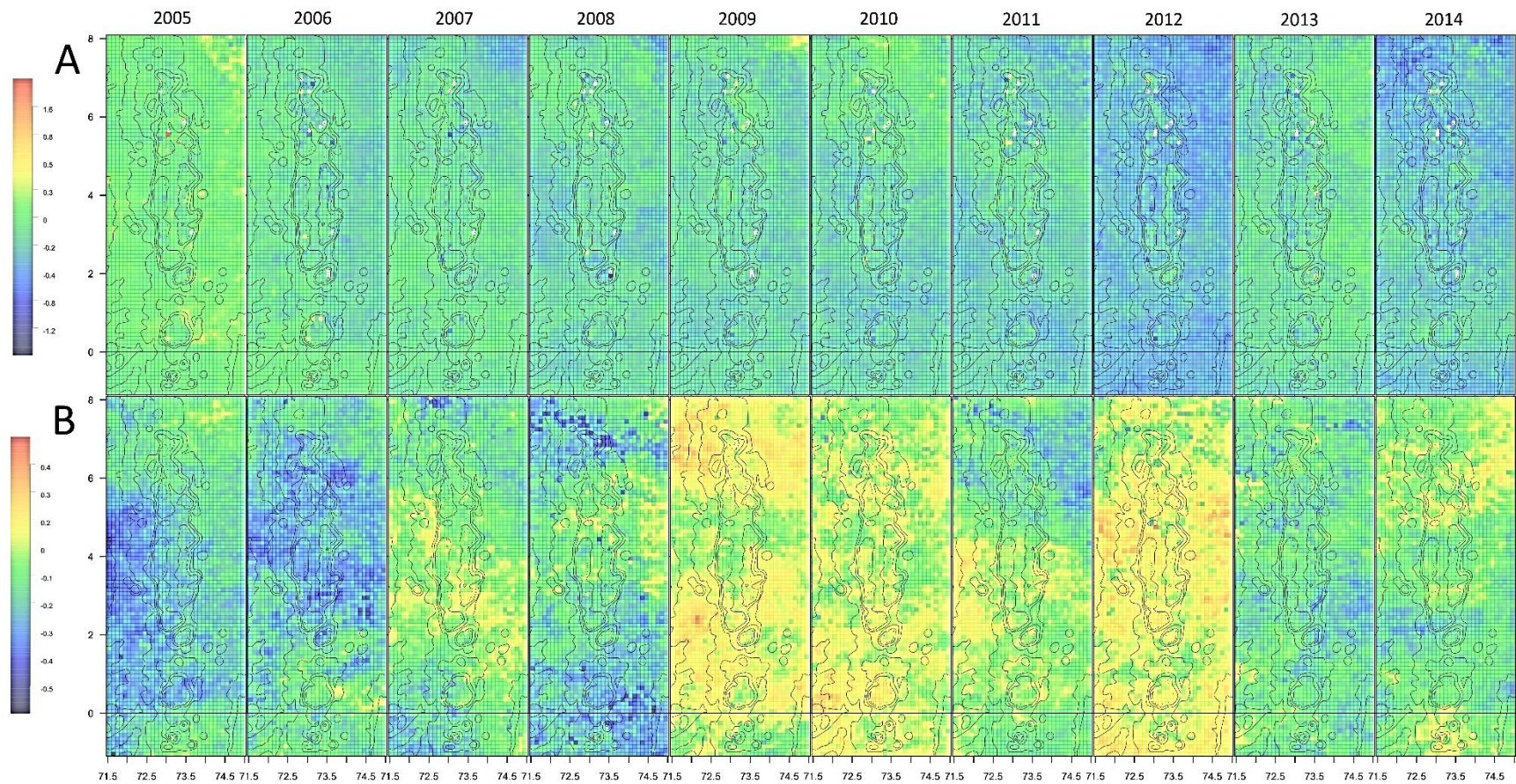


Figure 4.21 Deviation from the mean concentrations of Chlorophyll-a (-1.96 mg/m^3) (A), and mean Sea Surface Temperature (29.6°C) (B), in Maldives waters within the study period, as revealed by composite MODIS-Aqua satellite images.

Table 4.8 The four GAM models which tested whether environmental fluctuations influenced the number of *Manta alfredi* pregnancies and the number of *M. alfredi* exhibiting courtship behaviour. Chl-a = deviation from the mean concentration of chlorophyll a; SST = deviation from the mean sea surface temperature; N = number of surveys (2005 – 2014).

| Response | Predictors | χ^2 | p | Deviance (%) |
|-------------|------------|----------|-----------|--------------|
| Pregnancies | Chl-a | 54.4 | * < 0.001 | 39.4 |
| | N | 318.4 | * < 0.001 | |
| Pregnancies | SST | 58.2 | * < 0.001 | 41.1 |
| | N | 259.7 | * < 0.001 | |
| Courtships | Chl-a | 42.8 | * < 0.001 | 30.4 |
| | N | 163.9 | * < 0.001 | |
| Courtships | SST | 26.1 | * < 0.001 | 27.1 |
| | N | 124.9 | * < 0.001 | |

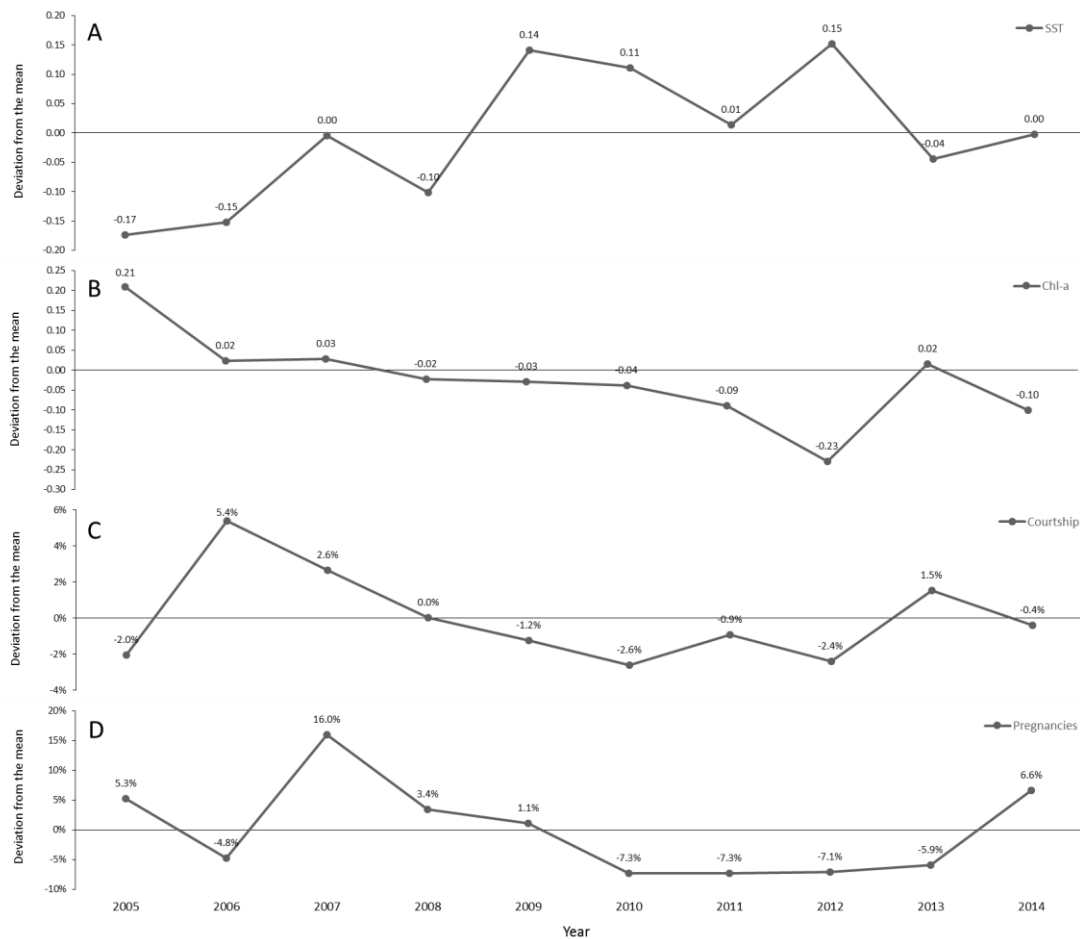


Figure 4.22 Maldives wide figures for: deviation from mean Sea Surface Temperature (SST = 29.6°C) (A), the mean concentrations of Chlorophyll-a (Chl-a = -1.96 mg/m³) as revealed by composite MODIS-Aqua satellite images (B), the mean percentage of the total sightings of adult *Manta alfredi* in which courtship was the primary behaviour engaged (2.9%) (C), and the mean percentage of the total adult female *M. alfredi* recorded that were pregnant (7.3%) (D).

4.5 Discussion

4.5.1 Fecundity and reproductive periodicity

The findings of this decade long study suggest that *M. alfredi* in the Maldives is one of the least fecund vertebrates in the world, given the average pregnancy rate per female of once every 7.3 years (mean of all 150 core females). Instances of courtship behaviour and pregnancies were highly variable inter-annually and linked to variations in primary productivity. Fecundity was also variable among sites intra-annually, possibly driven by the function of cleaning stations as lekking sites and female mate choice. Intervals between presumed copulation and birth were highly variable among individuals, suggesting female may utilise sperm storage or embryonic diapause to maximise fecundity.

Figures for other large vertebrates with conservative life history strategies are one offspring every 3 – 5 years in elephants, dolphins and whales (Lee & Moss 1986; Mann et al. 2000; Whitehead & Mann 2000), 4 – 6 years in gorillas (Robbins et al. 2004; Watts 1991), 5 – 7 years in chimpanzees (reviewed in Boesch & Boesch-Achermann 2000) and 8 – 9 years in orang-utans (Galdikas & Wood 1990; van Noordwijk & van Schaik 2005; Wich et al. 2004). All these mammals invest heavily in the post-parturition nurturing of their offspring, necessitating the lengthy interbirth intervals (Lee & Moss 1986; Ross & Jones 1999; Watts 1991). However, in *M. alfredi* there is no parental care of the pup, suggesting a long recovery period between pregnancies.

As pregnancy is only discernible in *M. alfredi* after 4 – 6 months of their year-long gestation, my finding of a reproductive periodicity of 7.9 years for the entire adult female population may be an overestimate given some pregnant individuals might not have been recognised as such. To reduce the possibility of this source of error, the study focused on multiple study sites where repeat sightings of the same individuals were high, and on sub-sets of the most-frequently sighted individuals. At all three sites the average reproductive periodicities were similar –i.e. once per 6.4, 7.4 and 8.0 years (average 7.3 years) at Lankan Beyru, Hanifaru Bay and the three other sites combined respectively, suggesting these findings are representative of the wider population. Furthermore, few neonates were observed each year, despite consistent sampling effort at juvenile *M. alfredi* aggregation sites during the study (Chapter 2), further supporting the low fecundity estimates.

The two previous peer-reviewed investigations into *M. alfredi*'s fecundity have suggested a biennial reproductive strategy is likely to be the norm for females (Deakos 2011; Marshall & Bennett 2010a). These studies were in Mozambique (Marshall & Bennett 2010a) and Hawaii (Deakos 2011), and both failed to account for mature females which didn't reproduce during their study periods, which were just five and six years respectively, compared to my ten. Their sample sizes were also much lower than mine, with just 62 females considered in Mozambique and 20 in Hawaii, of which on average, only 29% and 41% respectively were sighted each year. A long-term (23 years) unpublished study upon a small population of *M. alfredi* (N = 144 females) in Japan by Kashiwagi (2014) estimated the average reproduction frequency among females to be 1 pup in 3.61 ~3.93 years. However, this study also did not account for mature females which didn't reproduce during their study periods when calculating the average reproduction frequency, despite consistent sightings of these individuals.

Assuming that a female *M. alfredi* can live for at least 40 years (Couturier et al. 2014; Marshall et al. 2011b; Chapter 2) and takes 13 – 17 years to reach sexual maturity (Chapter 2) and only gives birth to a single large pup (Beebe & Tee-Van 1941; Bigelow & Schroeder 1953; Coles 1916; Compagno & Last 1999, Marshall 2009), after a gestation of one year (Okinawa Churaumi Aquarium 2010) then a reasonable lifetime fecundity for a female *M. alfredi* is 3 – 4 pups. This is based on the average fecundity estimates from this study (7.3 years), and assumes that females remain reproductively active from maturity until death. Indeed, in this study where 307 pregnancies were recorded from 254 individuals, only 6% (N = 47) of adult females were recorded pregnant twice or more, while 66% (N = 491) were not recorded pregnant once. These findings of such low fecundity in *M. alfredi* have significant implications for the conservation management of this species, both within the Maldives and internationally, especially given the recent development of manta fisheries for gill plates for Asian medicine (O'Malley et al. 2016). In such a slow reproducing species, even small negative pressures which increase mortality rate or reduce fecundity, are likely to have severe consequences for population persistence.

4.5.2 Annual reproductive variability and seasonality

The results of this study, and the findings of Marshall & Bennett (2010) and Deakos (2011), all suggest that *M. alfredi* falls into Wourms (1977) second type of elasmobranch reproductive cycle, namely one which has a partially defined annual reproductive cycle with

one or two peaks in reproductive activity each year. In Marshall & Bennett's Mozambique study the peak in reproductive activity occurred in summer, while in Deakos' study in Maui, Hawaii it was in winter. In the Maldives peaks in courtship activity occurred in October and November, prior to the transition between the southwest and the northeast monsoon, and in March when the monsoon reversed.

The findings of this study conform to Dodd's (1983) analysis of elasmobranch reproduction, finding that synchronous matings, gestation and parturition are often followed by immediate mating. Given *M. alfredi*'s one year gestation time (Table 4.4) (Deakos 2011; Marshall & Bennett 2010a; Okinawa Churaumi Aquarium 2010), if mating occurs immediately after parturition and gestation automatically ensues, then all mature females within the Maldives population should be continuously gestating. However, my study only recorded twelve back-to-back gestations, with 95% of females taking at least a year between them and an average time of 7.3 years across the primary and secondary sites. Such variability may reflect how the large size of manta neonates (~1.6m DW) in comparison to adults (~3.5m DW) can impose long post pregnancy recovery periods. As in this study, Marshall and Bennett (2010a) also noted that observed mating in *M. alfredi*, or presence of fresh mating wounds, did not always result in visible pregnancy the following year. While it is possible that a small number of pregnant mantas may abort in response to attempted predation (Marshall & Bennett 2010a), or fail to conceive after mating, our study suggests that a rest phase in females could be triggered by a physiological response to low fat reserves resulting from a combination of high energy expenditure during pregnancy and/or low food availability.

Although ovulation and fertilization in elasmobranchs often occurs immediately after mating (Callard et al. 2005), suspended animation, or embryonic diapause, has been confirmed in Australian sharpnose sharks (*Rhizoprionodon taylori*) and bluntnose stingrays (*Dasyatis say*) and there is evidence to suggest diapause in another sixteen species (Waltrick et al. 2012). In ovulating viviparous elasmobranchs, eggs are first fertilized then enclosed in a membranous egg case as oocytes pass through the oviducal gland and into the uterus (Conrath & Musick 2012; Wourms 1977). Where it has been examined in diapausing elasmobranchs, embryogenesis is arrested at the blastodisc stage and preserved in the uterus for periods of 4 – 10 months (Waltrick et al. 2012). In vertebrates embryonic diapause is widespread, with many mammals able to hold embryos in embryonic diapause for long periods prior to implantation before this is triggered subject to appropriate cues (Mead 1993).

Another explanation which could account for a rest phase between gestations in *M. alfredi* is the possibility that females can store sperm from multiple matings. This mechanism is taxonomically widespread within elasmobranchs (Hamlett et al. 2002; Hamlett et al. 2005; Pratt 1993), with sperm able to be stored for up to, or just over, a year within the oviducal gland (Hamlett et al. 2002; Hamlett & Koob 1999; Pratt 1993; Storrie et al. 2008). In 2011 at Manly SEA LIFE Sanctuary in Australia, two female southern eagle rays (*Myliobatis australis*), which were isolated from males in an aquarium for over two years, gave birth to 5 and 7 pups within two days of each other, presumably through sperm storage or embryonic diapause (Townsend 2011).

Embryonic diapause and sperm storage both allow the synchronization of gestation and parturition with times when environmental conditions are optimal for the gestating mother, and her new-born offspring (Waltrick et al. 2012). The findings of this study suggest that mating directly after parturition regularly occurs in *M. alfredi* and this is backed up by anecdotal reports from the Federated States of Micronesia, Indonesia and French Polynesia, where pregnant females which visually appeared close to parturition engaged in pre-mating courtship activity (B. Acker 2011, pers. comm.; S. Lewis 2013, pers. comm.; M. de Rosemont 2010, pers. comm.). If food is abundant, females could maintain high enough energy reserves to support a fertilized oocyte directly after parturition. Alternatively, if females lack adequate energy reserves they could undergo an appropriate phase of recovery prior to initiating gestation, which during periods of average productivity is likely to occur within a year or to two. However, if below average productivity occurs for a sustained period of several years, further copulation is likely to be required.

4.5.3 Environmental influences on reproduction

My results suggest that reproductive activity and fecundity in *M. alfredi* were adversely affected by extreme temperatures, whereas moderately elevated levels of chl-a increase them. Most probably high levels of chl-a do not benefit *M. alfredi* because of the negative relationship SST and chl-a have with each other. Weather in the Maldives is heavily influenced by the South Asian monsoon, which drives the wind and currents that influence primary productivity. As *M. alfredi* feed on zooplankton, which feed on phytoplankton, it might be expected that in years when strong monsoonal winds generate high primary productivity, the abundance of the manta ray's planktonic food will be high. This in turn would lead to increased *M. alfredi* courtship activity, followed by higher pregnancy rates the

following year, like those found in this study. Similar variations in inter-annual reproductive behaviour and fecundity linked to variations in food availability as a result of fluctuating climate conditions have been recorded in Japanese macaques (*Macaca fuscata*) (Suzuki et al. 1998). Inter-annual variations in the reproductive success of a variety of other large marine species has been linked to large-scale climate anomalies, such as the ENSO (Jenouvrier et al. 2003; Lake et al. 2008; Laurie & Brown 1990; Leaper et al. 2006; Vargas et al. 2006).

Fluctuations in the strength of the South Asian monsoon, which drives the prevailing weather in the Maldives, have been linked to ENSO and positive and negative phases of the Indian Ocean Dipole (Ashok et al. 2001). Very little is understood about the influences of these large-scale climate modifying events on the Maldives' monsoons; catch sizes of skipjack tuna (*Katsuwonus pelamis*) in the Maldives are known to be affected by large-scale climate events linked to ENSO (Adam 2010). Anecdotal observations of Maldivian fishermen suggests a weakening and less predictable seasonality of Maldivian monsoons in recent decades (pers. comm.). These local observations are supported by ocean basin studies which attribute a weakening South Asian summer monsoon to human-influenced aerosol emissions which slowdown the tropical meridional overturning circulation (Bollasina et al. 2011; Lau & Kim 2006; Turner & Annamalai 2012). It is therefore possible that variations in *M. alfredi* fecundity revealed by this study may reflect how large-scale climate events have affected their planktonic food (Byrne 2011; Hays et al. 2005). While it is not possible to determine whether the inter-annual variations in courtship behaviour and fecundity constitute a naturally occurring long-term cyclical process, and whether such variation is intensified by anthropogenic climate change events, it is unlikely these processes account for the variability observed between study sites within years. To make sense of this observation more research is required on the likely proximate causes of reproductive timing in *M. alfredi*.

4.5.4 Mate choice and lekking

Trivers (1972) stated that generally the sex with the lower parental investment will be the sex towards which the operational sex ratio is biased. Furthermore, the degree of bias determines the intensity of sexual competition among individuals of the sex with the operational sex ratio bias. Conversely, the outnumbered sex may be selective in mate choice (Bleu et al. 2012) and mate with the fittest mates as an outcome of contest competition (Cox & Le Boeuf 1977).

Female *M. alfredi* invest heavily in their offspring, while the only parental investment from males is sperm. For the Maldives, the average reproductive periodicity of female *M. alfredi* is 7.3 years. This equates to an operational sex ratio [average ratio of fertilisable females (N = 745) to sexually active males (N = 1,214) at any given time] of 11.9 males for each reproductively active adult female per year, given most females most of the time are not sexually receptive. This finding would explain female *M. alfredi* mate selection by 'courtship trains', where in the Maldives the average ratio of mature males to females was 3:1, with a peak of 26:1 (Chapter 3). Under such ratios, competition among males over limited females should be intense. Furthermore, with the females apparently controlling courtship trains and the initiation of copulation, it would seem female *M. alfredi* are able to be highly selective in their choice of mates.

In dominance polygyny mating systems, courtship is often centred around contests between males from which females choose their sexual partner (Emlen & Oring 1977). These communal displays called leks or courtship 'hot spots' allow females to judge male fitness, and through this maximize the chances of their own reproductive fitness (Beehler & Foster 1988; Bradbury 1981; Bradbury 2010; Young et al. 2009). It has been suggested that leks occur when males are unable to economically defend either females, or the resources they require (Bradbury & Gibson 1983; Emlen & Oring 1977). Leks should emerge in areas where females are likely to congregate, and where a reduction in predation pressure for individuals via the group effect will also help drive their evolution (Bradbury & Gibson 1983). For humpback whales (*Megaptera novaeangliae*), Clapham (1996) termed the phrase of 'floating lek' to describe the features of this species' courtship system, which have all of the same characteristics of a traditional lek, except for the rigid spatial structure. *M. alfredi* exhibit many of the characteristics described above as males: they lack parental care, are unable to defend females or the resources they require, and aggregate in the mating season to compete for access to sexually receptive females.

This study suggests that manta ray cleaning stations can function as a lek, not only for the courtship behaviour which is focused at them, but because of the variation in the extent to which different ages and sexes of *M. alfredi* visit them beyond what could reasonably be explained by differences in cleaning requirements. For example, normally adult female *M. alfredi* spend more time at cleaning stations than adult males (Chapter 2), but male visits increase significantly during the peak mating season. Juvenile and subadult manta rays, which do not engage in courtship activity, spend significantly less time at cleaning stations

than adults (Chapter 2). With females aggregating at cleaning stations to be cleaned, and possibly thermoregulate (Chapter 2), these sites provide a good place for males to competitively display via courtship trains, and through these entice copulation. Inter-annual and seasonal variations in reproductive activity may also explain why cleaning stations go through periods of visitation peaks and troughs, as occurred at Lankan Beyru during peak mating times when scarcely any reproductive activity was recorded (Figs. 4.16 & 4.17).

4.5.5 Polyandrous females and synchronisation

Although the concept of male dominance polygyny fits the mating systems of *M. alfredi* observed in this study, the data of Yano et al. (1999) and mine of female manta rays' engaging in multiple matings during each ovulation suggest polygamy may also play a key role in their mating system. Studies in behavioural ecology suggest that sexual reproduction actually promotes polyandry, despite potentially high risks and costs associated with copulation (Chapman et al. 1995; Holland & Rice 1998; Watson et al. 1998). In a 2001 review of reproductive modes and the genetic benefits of polyandry, Zeh & Zeh state that 'in both oviparous and viviparous species, polyandry and the accumulation of sperm from several males shifts the arena for sexual selection from the external environment to the female reproductive tract'. Female choice of competing sperm acts as a selective mechanism, favouring fertilization by more competitive (Laurent & Reeve 1995), genetically superior (Birkhead et al. 1993), or more compatible sperm (Zeh & Zeh 1997; Zeh & Zeh 1996).

Reproductive synchrony, often characterised in animals by the temporal clustering of ovulation, mating and births, is a strategy which individuals adopt to maximize their reproductive success and is controlled by environmental, ecological, and physiological factors (Bronson 1989; McClintock 1983; Sadler 1969). Although the seasonal and inter-annual variations in the reproductive synchrony and periodicity observed in *M. alfredi* in this study are likely to have been caused by environmental variations in the manta's food source, underlying sociobiological factors linked to social cues may have also helped control breeding seasonality. For example, there is evidence that social cues emanating from conspecifics influence reproductive synchrony in birds (Helm et al. 2006; Hinde & Steel 1978) and mammals (Berger 1992; Chemineau et al. 2008; Schiml et al. 1996; Wayne et al. 1989). In many cases where this exists the cues are olfactory pheromonal signals that work at relatively small scales (Ims 1990), and the females involved are part of stable social groups. In *M. alfredi* adult females regularly visit the same feeding and cleaning sites over multiple decades,

creating a core group of associates. Although no experimental evidence is available for elasmobranchs, it may be possible that pheromones are released by some females at social aggregation sites, such as cleaning stations, which trigger other adult females to ovulate and thereby attract mature males into the area. The release of pheromones by females could also help explain the close following and courtship train behaviour observed in mantas and many other elasmobranchs (Gordon 1993; Klimley 1980; Luer & Gilbert 1985; Tricas 1980; Chapter 3). To maximize mate choice, once ready to mate, female *M. alfredi* should attract as many potential suitors as they can. Reproductive synchronicity within populations may facilitate this clustering, and thereby act to increase individual fitness. Reproductive synchrony may also have driven the utilisation of sperm storage and/or embryonic diapause, explaining why females often mate straight after giving birth instead of waiting until they have regained the energy reserves to support another gestation.

Social cues exchanged between resident females within different sub-populations may also explain differences in the clustering of pregnancies observed between the two primary study sites in the same years. For example, at Lankan Beyru in 2007, 76% of the 50 core females were recorded pregnant, while in the same year at Hanifaru Bay this figure was just 10% for the core 50 females there. Conversely, during the following year none of the Lankan Beyru core females were recorded pregnant, while 39% at Hanifaru Bay were. If all the females within a population were acting independently based on their own physical readiness to mate, then the number of pregnancies between the two sites should have been about equal. Being only 108 km apart, and thus subject to the same over-riding weather patterns, inter-annual variability of food between the two sites should also be similar. While individuals from these two sites exhibited strong site fidelity, they did also move between locations, some several times a season. It therefore seems unlikely that the large variations in pregnancy rates between the sites during the same years could be explained by environmental variations in food alone.

4.5.6 Conclusions

In the Maldives, *M. alfredi* exhibit a partially defined annual reproductive cycle with one or two peaks in reproductive activity each year occurring prior to transitions between the southwest and northeast monsoons. In this study, fecundity varied considerably among years and appeared related to variability in abundance of the manta's planktonic food, which in turn could be linked to large-scale weather patterns such as the Indian Ocean Dipole and

ENSO. Variations in the intra-annual fecundity between female sub-populations studied may be linked to social cues among females associated with mate choice, and thereby individual fitness. Complex courtship behaviour at cleaning stations, which appear to double up as leks, are part of *M. alfredi*'s reproductive strategies which, possibly coupled with the utilisation of sperm storage and embryonic diapause, are much more complex than previously thought. These findings also validate the importance of undertaking long-term studies on species with conservative life history strategies if important ecological and evolutionary questions are to be answered (Clutton-Brock & Sheldon 2010). In this case, my findings produce fecundity estimates of *M. alfredi* two to more than three times lower than previously documented (Deakos 2011; Kashiwagi 2014; Marshall & Bennett 2010a). With the average reproductive periodicity for a female estimated at one gestation every 7.3 years, and an average lifetime production of 3 – 4 pups, *M. alfredi* are one of the world's least fecund vertebrates. These findings have significant implications for the management and conservation of *M. alfredi*, marking them as highly vulnerable to human impacts in general and especially to over-fishing, which is a major threat given the lucrative market for manta gill plates in Asian medicine. With such low fecundity and low population densities, there is no possibility of *M. alfredi* supporting sustainable fisheries and the species needs complete protection from exploitation.

Chapter 5

Feeding behaviour of manta rays (*Manta alfredi* and *M. birostris*) in the Maldives

5.1. Abstract

Manta rays (*Manta* spp.) are the largest and among the most conspicuous of the elasmobranchs. However, the feeding strategies employed by these pelagic zooplanktivorous rays have yet to be defined. The aim of this ten year-long study was to describe and elucidate all of the different feeding strategies exhibited by both manta species (*M. alfredi* and *M. birostris*) using behavioural observations and photographic records. From January 2005 through December 2014 over 5,000 observational dive or freediving surveys were performed at known manta ray aggregation sites in the Maldives to record their feeding activity. Nine hundred and fifty four of the surveys were conducted at the primary study site of Hanifaru Bay where, for a seven month period between May-Nov 2014, a visual estimate of the zooplankton density and the feeding strategies employed by individually identified *M. alfredi* were also recorded. Between 2009 and 2014 at Hanifaru Bay, the time of any observed manta breaching event was recorded to seek an explanation for this frequently observed behaviour. Feeding activity was observed at 150 different sites, resulting in 22,263 photo-ID sightings of 2,733 feeding individuals. 12,852 of these sightings, comprised of 1,427 individuals, were recorded at Hanifaru Bay. Two hundred and eighty six breaches, during 86 surveys, were seen. An ethogram of the eight manta feeding strategies identified, based on movement type and number of individuals involved, is presented here: (1) straight, (2) surface, (3) chain, (4) piggy-back, (5) somersault, (6) cyclone, (7) sideways, and (8) bottom. All strategies were recorded for *M. alfredi*, but only strategies 1, 2, 5 & 7 for *M. birostris*, which may reflect the greater time spent observing *M. alfredi*. Female *M. alfredi* were seen almost twice as often in Hanifaru Bay as males, and adults 40% more often than juveniles. In *M. alfredi* there was no significant difference in the feeding strategy employed between sex or maturity status, but as prey density increased *M. alfredi* were significantly more likely to feed in groups. We propose that predator escape responses of the manta's prey, hydrodynamic efficiency, and collision avoidance techniques, may all influence the feeding strategy adopted by manta rays. Breaches were significantly more likely to occur one hour before peak feeding time. We propose that this behaviour, and the resultant pressure wave the breach creates, may be a mechanism for manta communication, attracting other rays to the feeding site to aid group feeding.

Keywords: feeding strategies, coordination, breaching, group behaviour, communication

5.2. Introduction

Largest of all rays, the pelagic zooplanktivorous mantas' behaviour, specialised anatomical feeding adaptations, and flattened body shape, place these elasmobranchs among the most conspicuous and charismatic of all marine species. Manta rays belong to the Mobulidae family which currently comprises nine species in the genus *Mobula* (Notarbartolo-di-Sciara 1987; Rafinesque 1810) and two species in the genus *Manta* (Bancroft 1829). The genus *Manta* was re-classified in 2009 (Marshall et al. 2009) when the monospecific genus was split into the two species currently recognized: the reef manta (*Manta alfredi*) (Kreffft 1868) and oceanic manta (*Manta birostris*) (Walbaum 1792). A third putative species, the Caribbean manta (*Manta c.f. birostris*), has also been proposed and its taxonomic status is currently under review (Marshall et al. 2009).

M. alfredi are widely distributed throughout tropical and sub-tropical waters of the Indo-Pacific within 32° of latitude north and south of the equator, although populations appear highly fragmented (Couturier et al. 2012; Kashiwagi et al. 2011), most likely due to availability of resource and habitat requirements (Anderson et al. 2011b). *M. alfredi* frequent coastal reefs of continents and remote oceanic islands (Kashiwagi et al. 2011; Marshall et al. 2011b). They also venture 100's km off the continental shelf, diving to depths >400 m into the mesopelagic zone (Braun et al. 2014; Braun et al. 2015; Jaine et al. 2014). *M. birostris* is distributed throughout tropical, sub-tropical and temperate waters of the Pacific, Atlantic, and Indian oceans within 41° of latitude north and south of the equator (Kashiwagi et al. 2011). It is thought that *M. birostris* spend the majority of their time offshore, but they are also known to frequent upwelling areas along productive coastlines (Marshall et al. 2011c). Throughout their range *M. birostris* also aggregate around oceanic island groups and offshore pinnacles, seamounts and submarine ridge systems where they are known to engage in courtship activity (Compagno & Last 1999; Marshall et al. 2011c; Rubin 2002; Chapter 3).

A highly specialised diet has greatly shaped the anatomy of both manta species. Huge mouths and cephalic fins funnel plankton rich water through five pairs of modified gills which are encircled by pre-branchial appendages, or gill plates (Paig-Tran et al. 2013). As a manta ray ram feeds through the water these feathery structures sieve zooplankton larger than a grain of rice, redirecting the tiny morsels of food back towards the manta's throat (Paig-Tran et al. 2013). Once the manta has trapped a mouthful of planktonic food it closes its mouth, squeezing out the remaining water in the buccal cavity through the gill slits, before swallowing its prey (pers. obs.).

By targeting low trophic level plankton, the manta rays specialised feeding mechanism has enabled them to exploit one of the oceans' most abundant food sources. However, zooplankton is a patchy resource varying greatly spatially and temporally (Folt & Burns 1999; Martin 2003). Like many species, manta rays may employ optimised 'random walk' search behaviour to discover prey concentrations (Bartumeus et al. 2005; Sims et al. 2008). Once a manta has located its prey, probably using smell, taste, or visual recognition (Ari & Correia 2008), it may then adopt a feeding strategy which maximises feeding efficiency. Using acoustic telemetry to actively track tagged *M. alfredi* in the lagoon of Palmyra Atoll, Papastamatiou et al. (2012) found that mantas used a 'correlated random walk' search pattern at small spatial scales to move between prey patches, but at larger scales performed home-ranging behaviour. Similar prey search patterns may also be utilised by manta rays in other habitats. For example, zooplankton are known to make large diurnal vertical migrations in the water column, actively rising from the mesopelagic zone into the epipelagic zone at night to feed upon their phytoplankton prey, before sinking back into the depths during the day to reduce the risk of predation (Gliwicz 1986; Lampert 1989). Satellite tagged *M. alfredi* in the Red Sea (Braun et al. 2014; Braun et al. 2015) and in eastern Australia (Jaine et al. 2014), and actively tracked *M. alfredi* in Hawaii (Clark 2010), regularly moved offshore, possibly to exploit these rising layers of zooplankton (Lampert 1989; Lo et al. 2004). Braun et al. (2014) found that *M. alfredi* dived to greater depths at night than in the day, regularly making dives >150 m, with one individual diving to 432 m. During the day in Papua New Guinea *M. alfredi* were repeatedly observed from a manned submersible over several days feeding on mysid shrimp at a depth of between 190 – 200 m (M. Erdmann, pers. comm.). Jaine et al. (2014) also recorded several satellite tagged individuals diving to depths of almost 300 m, and in Nusa Penida, Indonesia and Ouvéa, New Caledonia, satellite tagged *M. alfredi* have been recorded to dive to depths of 480 m and 670 m respectively (M. Erdmann, pers. comm.; S. Lewis, pers. comm.). Based on field observations in the Maldives, Anderson et al. (2011b) have speculated that *M. alfredi* regularly feed at night upon zooplankton in the plankton concentration known as the 'deep scattering layer', named from its appearance on echo sounder traces.

It is known that during the day *M. alfredi* feed in shallow waters (<30 m) of inshore reef habitat throughout their range (pers. obs.). In the Maldives they have been recorded preying upon concentrated patches of zooplankton (including Copepoda, Mysida, Chaetognatha, Decapoda, Ostracoda and Ichthyoplankton) (Richardson et al. in prep.). These daytime surface feeding events in the Maldives are linked to the lunar phase and tidal cycle, which

generate strong currents that bring the deep zooplankton layer to the surface (pers. obs.). *M. alfredi* are also known to prey upon demersal zooplankton which emerge from the seabed at night (pers. obs.; Osada 2010). These observations are supported by stable isotopic analysis of muscle tissue taken from *M. alfredi* individuals along Australia's Great Barrier Reef (Couturier et al. 2013) and at Palmyra Atoll in the Pacific (McCauley et al. 2014), which indicated a strong demersal, reef-derived diet source. It therefore appears that both mesopelagic and demersal zooplankton comprise an important component of *M. alfredi* diet throughout its range.

Little is known about the feeding ecology of *M. birostris* and only two papers are currently available on the movements of this more elusive species (Graham et al. 2012; Stewart et al. 2016a). However, given the oceanic nature of *M. birostris* it seems likely that this species exploits mesopelagic zooplankton, undertaking deep dives to feed (Stewart et al. 2016b). Observations from a deep sea submersible have recorded it feeding at a depth of 300 m (Stewart et al. 2016b), and data from satellite tagged animals in Mexico show that they regularly dived to depths in excess of 300 m, with one individual reaching 648 m (Stewart et al. 2016b). It has been proposed that differential exploitation of prey may have driven the speciation of the *Manta* genus (Marshall et al. 2009), allowing the two species to occur sympatrically throughout their range (Kashiwagi et al. 2011).

Zooplankton species are often high in unsaturated fatty acids, which are critical for maintaining high growth, survival and reproductive rates for a wide variety of marine and freshwater organisms (Brett & Müller-Navarra 1997). Indeed, life as a marine filter feeder has proved successful for a variety of large marine species across a broad taxonomic spectrum; from the filter feeding baleen whales (mysticetes) to the planktivorous elasmobranchs (i.e. basking, whale and megamouth sharks, as well as the other mobulid rays) (Friedman et al. 2010; Sanderson & Wassersug 1993; Shimada et al. 2015). Driven by the rewards which come from preying upon zooplankton, and shaped by the physiological boundaries and life history characteristics which constrain and maximise this niche exploitation (Pauly 1998; Sanderson & Wassersug 1993), convergent evolution has resulted in the largest of all marine species arising from these lineages (Friedman 2012).

It is not known what quantity of zooplankton manta rays must consume in order to survive, however we do know that manta rays in aquaria are fed roughly 12% of their body weight weekly (K. Timmins, pers. comm.). For an average sized adult *M. alfredi* weighing approximately 500 kilograms, that equates to approximately 8.5 kilograms a day. However,

considering the manta's zooplankton prey are often patchy and ephemeral (Anderson et al. 2011b), it is therefore unlikely that wild mantas can consistently find the quantity of food provided for captive animals on a daily basis. Indeed, in oligotrophic tropical oceans it is likely their large size enables them to cope with periods of food scarcity. Being large increases energy storage capacity, which in elasmobranchs is aided by large oily livers (Hoffmayer et al. 2006; Rossouw 1987). Being large also increases foraging range; the manta rays' flattened body shape is perfectly suited for gliding, improving swimming efficiency of such large fish, enabling them to travel farther in search of prey (Braun et al. 2014).

When prey is located, manta rays must feed quickly, which they do by employing a wide variety of feeding strategies (pers. obs.). These strategies can often be complex, requiring coordination among multiple individuals to enhance feeding efficiency (pers. obs.). Coordinated group feeding behaviour has been documented in a variety of marine fish (Bshary et al. 2006; Domenici et al. 2014) and mammal species (Fertl et al. 1997; Similä & Ugarte 1993), including the filter feeding humpback whales (*Megaptera novaeangliae*), which use a technique known as 'bubble net feeding' to maximise capture of their prey (Wiley et al. 2011).

To date just one peer reviewed publication exists on the feeding strategies employed by manta rays (Gadig & Neto 2014), based on just 50 minutes of observations captured from a video recording of feeding *M. alfredi* in the Red Sea, Sudan. With major gaps still remaining in our knowledge of these varied feeding strategies, the primary goal of this ten year-long study was to elucidate all of the different feeding strategies exhibited by both manta species by creating an ethogram based upon behavioural observations and photographic records. To seek correlations which might further explain the different feeding strategies employed, I also investigated variations in the feeding behaviours employed between sexes, between adults and juveniles, and with changing prey density. I also recorded manta breaches (i.e. leaps) during feeding events to seek explanations for this behaviour. The research was done within the small island nation of the Maldives which straddles the equator in the middle of the Indian Ocean. The Maldives is home to the world's largest known population of *M. alfredi* (Kitchen-Wheeler et al. 2011; Chapter 2) and *M. birostris* also occurs there at several locations where remote seamounts rise from extremely deep water (Kashiwagi et al. 2011; Chapter 3). With legal protection from fishing and sightings of feeding individuals of both manta species virtually guaranteed, the Maldives offered an ideal location in which to perform this study.

5.3 Methods

5.3.1 Study area

The 26 coral atolls of the Maldives archipelago extend 870 km from 7° north to half a degree south of the equator in the Indian Ocean (Fig. 5.1). The maximum natural height of the country is 2.4 m above sea level and the atolls have steep outer reef slopes that drop to 2 – 3,000 m. In the central Maldives’ the atolls form a double chain, and here the seafloor between the atolls reaches a maximum depth of 500 m. The Maldives exclusive economic zone contains 3.1% of the world’s coral reefs and encompasses 916,000 km² of ocean. By contrast the total area encompassed by the 26 atolls is 21,600 km², only 300 km² of which is land (Sea Around Us Project 2014).

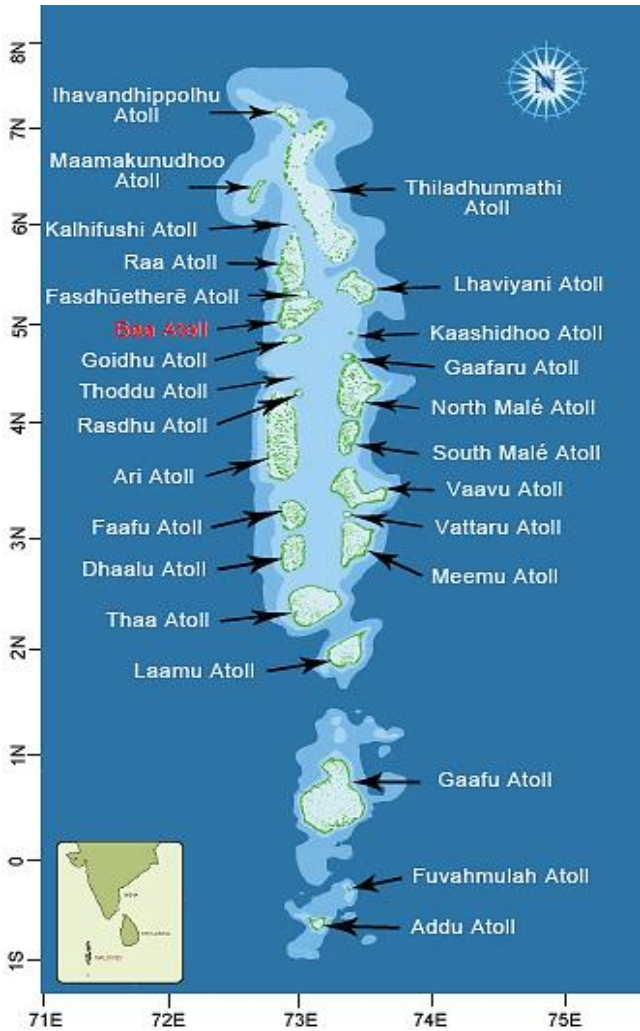


Figure 5.1 Map of the Maldives Archipelago showing the 26 geographical atolls, illustrated in green. The main study atoll is labelled in red. Dark blue indicates where the sea floor exceeds 1,000 m; middle toned blue where it is between 500 – 1,000 m and the lightest blue where 500 m or shallower. The depth of the sea floor inside the lagoon of all the atolls is <100 m. 1° latitude = 111 km.

5.3.2 Primary study site – Hanifaru Bay, Baa Atoll

Hanifaru Bay is situated on the eastern edge of Baa Atoll in the central atolls of the Maldives (Fig. 5.2). It is small (700 x 200 m) and shallow (22 m max depth) with a sandy seabed. It is a cul-de-sac reef inlet surrounded by shallow (<1 m) reef along all but a small 75 m section of the bay's circumference, situated at the western end. Hanifaru in the local Dhivehi language translates to narrow 'Hani' reef 'faru'. Hanifaru is a key aggregation site for *M. alfredi*, which frequent this site primarily to feed.

Due to the unique structure of the bay's outer reef, each year from May to December during the Southwest Monsoon (Hulhangu – local Dhivehi name), mass aggregations of feeding mantas frequently occur inside the bay. Strong lunar tides overcome the force of the prevailing monsoonal current, sucking plankton rich water from ocean depths outside the atoll back into the shallow atoll channels (P. Hosegood et al., unpublished data). In the atoll pass adjacent to Hanifaru Bay (Dharavandhoo Kanduoohi) these currents form a back eddy, trapping and concentrating plankton in the shallow bay. Although the feeding aggregations inside Hanifaru Bay are comprised of predominantly *M. alfredi*, *M. birostris* were also observed feeding at this location during the study.

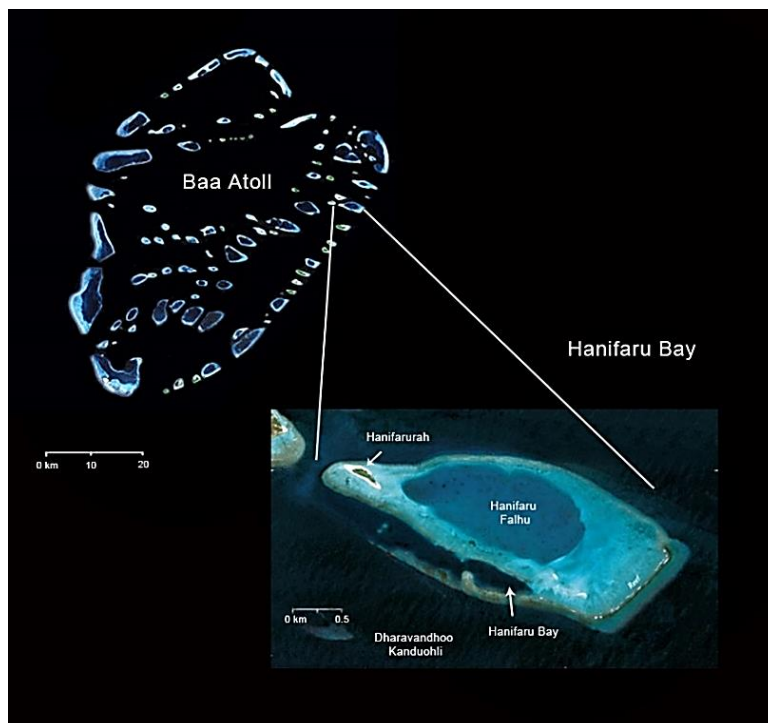


Figure 5.2 Primary study site, Hanifaru Bay, situated on the eastern edge of Baa Atoll.

5.3.3 Study duration and sampling protocol

In the Maldives, manta rays are accustomed to interacting with tourist divers and snorkellers at aggregation sites where the rays predictably gather at certain times of the year to feed, clean and socialise (see Chapter 2 methodology for site type descriptions). A typical survey during this study entailed diving or freediving at one of these aggregation sites, where close encounters with the unperturbed rays easily allowed photo-ID images to be taken and observations recorded of the individuals present. Manta surveys were performed on SCUBA or while freediving from either a dedicated research vessel or commercial diving vessels. Surveys were performed at different times of day in all months of the year throughout the month. SCUBA surveys lasted about 60 minutes and ranged to a maximum depth of 30 metres. Freediving surveys generally took 120 minutes, except at Hanifaru Bay where they were on average 4.5 hours as feeding events there lasted longer. Myself, or trained staff or volunteers from the Manta Trust conducted the surveys (www.mantatrust.org/in-the-field/maldives/).

During a ten year study from January 2005 through December 2014 over 5,000 surveys for the manta rays *M. alfredi* and *M. birostris* were undertaken at over 150 known aggregation sites for these species throughout the Maldives. Nine hundred and fifty four of the surveys were undertaken at the primary study site of Hanifaru Bay (Fig. 5.3). At each of the manta aggregation sites surveyed, the predominant behaviour undertaken by the observed manta rays enabled these sites to be defined as either predominantly feeding (90), or cleaning (61) sites (Coté 2000; Feder 1966; Losey 1972; Chapter 2). Manta rays were also recorded at a further 73 locations during an additional 158 surveys, however these sites were not considered to be manta aggregation areas because when sighted, the usually solitary individuals, were predominantly travelling through the area.

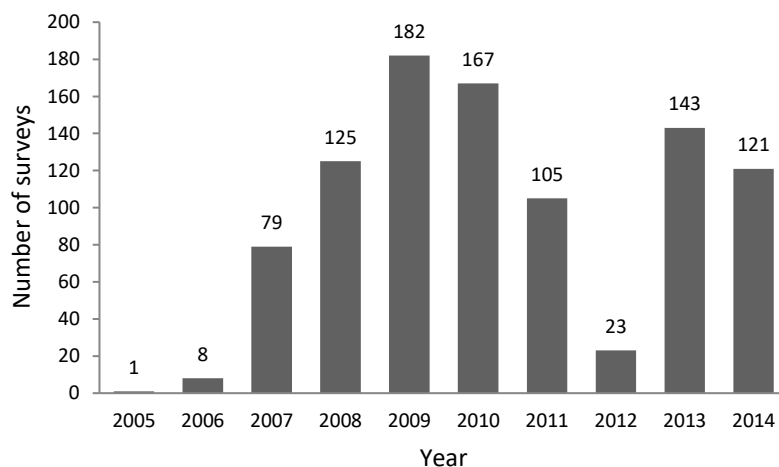


Figure 5.3 Number of surveys conducted at the primary study site of Hanifaru Bay each year.

When manta rays were encountered, where possible the following information was recorded for each individual: (1) species, (2) photo-ID, (3) sex, (4) maturity status, and (5) behavioural activity. Behavioural activity was further broken down into four major groups: (1) feeding, (2) cleaning, (3) cruising, and (4) courtship. If during the course of an encounter an individual performed several different behavioural activities, the one which dominated was recorded as the primary behaviour. For the purposes of this study, only the feeding behaviour is considered. For a detailed description of each of the criteria listed above, see Chapter 2.

Individual manta rays are recognisable by their unique pattern of ventral markings (Marshall & Pierce 2012). Using a combination of manual and automated matching systems, all photo-IDs collected during study surveys, or submitted to the Manta Trust by the general public, were compared against a database of identified individuals by one of the Maldives Manta Trust staff (www.mantatrust.org/about-us/the-manta-team/). To confirm a match, or record a new individual, every photo-ID was double checked by the Maldives Manta Trust Project Leader, then triple checked by myself. Each manta ray was assigned a unique ID-code, and every sighting logged and the corresponding photo-ID image archived. Repeat sightings of the same individual on the same day were logged as a single sighting event. For a detailed description of photo-ID and matching methodology, see Chapter 2.

At the primary study site, for a seven month period between May – Nov 2014, a randomly selected subset of the identified *M. alfredi* individuals were recorded using a GoPro Hero3 underwater video camera to identify feeding strategies employed. Throughout the rest of the study, all individuals were photographed or videoed for photo-ID purposes only. Of these, 75% were captured as still photographs using a Nikon D700 SLR with a 16 mm wide angle lens, or a Sea & Sea DX1G compact underwater camera with wide angle converter lens. The remaining 25% were taken by a wide variety of other compact underwater cameras and video cameras.

A simple visual underwater estimate of the zooplankton density, rated low, medium, or high, was recorded at the peak feeding time of each aggregation event during the study at Hanifaru Bay (Table 5.1). Although simplistic in design, this methodology provided a robust index of the highly patchy (spatially and temporally) zooplankton community at the precise location where the manta rays were feeding during these aggregation events (Omori & Hamner 1982). Peak feeding time was determined as the one hour period when most feeding manta rays were present. During surveys where feeding behaviour occurred, the prevalent zooplankton

taxa which visually comprised the mantas' prey aggregations were also recorded, and observations on their behaviour and position in the water column noted.

Table 5.1 Zooplankton visual underwater density index at Hanifaru Bay in the Maldives.

| Zooplankton density | Description |
|---------------------|---|
| Low | Water is absolutely clear with a complete absence of zooplankton, or individual zooplankton are evident in the water but water visibility remains clear. |
| Medium | Thin layers or small patches of zooplankton appear in the water column and water clarity begins to drop, or thick layers or patches of zooplankton are present in the water column and water becomes cloudy. |
| High | Water is thick with a dense, cloudy concentration of zooplankton which can be felt on the skin, or water is thick and soup-like with zooplankton concentration and visibility is reduced to a couple of metres. |

During this study, manta rays were regularly observed to leap during feeding events (Fig. 5.4). A leaping event, hereafter termed a breach, was defined as a manta ray leaping completely clear of the water, sometimes by several metres, and then landing flat back onto the water on their belly or back. It is not known why manta rays exhibit this behaviour. The times of any observed manta breaches at Hanifaru Bay were recorded during the study between 2009 and 2014.



Figure 5.4 A *Manta alfredi* breaches in Baa Atoll, Maldives.

The characteristics of each feeding strategy observed remained consistent between all sites, seasons (monsoons), and throughout the entire study period. Feeding manta rays were classified as either group or individual feeders based on the orientation and proximity of the feeding rays to each another. Two or more manta rays travelling in the same direction, with their bodies orientated along the same axis, positioned closer than a body length apart, were considered to be group feeding. All other feeding configurations were considered as individual feeding strategies.

5.3.4 Data analysis

All images, videos and observations were collated and edited after the survey, and photo identification performed to determine each individual manta ray present, before all information was transferred to a Microsoft Excel database for analysis. Analyses of the difference in sighting frequencies between the sexes and maturity status at Hanifaru Bay throughout the study were conducted using Mann–Whitney U Tests because the data did not meet the assumptions of normality (Dytham 2010). Analyses of the feeding strategies recorded during May – Nov 2014 were conducted on the pooled feeding strategy types (group vs. individual) to test for strategy variation between the sexes, maturity status, and with differing zooplankton density. For this, a Pearson’s Chi-Square Test was performed because the expected values were greater than five for all instances (Dytham 2010). A Pearson’s Chi-Square Test was also performed on the manta ray breaching behaviour recorded between 2009 and 2014 to compare breach time with peak feeding time. As the data did not meet the assumption of normality, a Spearman’s Rank-Order Correlation was also performed on the breaching data to compare the time before and after peak feeding time and numbers of recorded breaches. Significance for all tests was accepted at $p < 0.05$. All statistical analysis were performed using SPSS version 19.0 (SPSS, Inc. 2011). Where applicable, data were plotted utilising t-distributions with 95% confidence intervals (CI) around the mean.

5.4 Results

Between January 2005 and December 2014 a total of 31,618 photo-ID sightings of 3,700 individual *M. alfredi* and 175 photo-ID sightings of 173 individual *M. birostris* were recorded in 22 of the Maldives’ 26 atolls at 224 different sites. Feeding activity was observed on 2,296 surveys at 150 different sites, resulting in 22,263 photo-ID sightings of the 2,733 individual manta rays feeding. Of these 2,733 individuals, 2,727 were *M. alfredi* (1,344 females, 1,358 males and 25 individuals for which sex could not be determined), and 6 were *M. birostris* (all males). 1,347 (49%) of these 2,733 feeding individuals were adults, 1,361 (50%) juveniles, and for the remaining 25 (1%) individuals the maturity status was not able to be determined (see Chapter 2).

During the study, based on swimming behaviour and number of individuals involved, the following eight feeding strategies were identified and an ethogram developed (Fig 5.5): (1) straight, (2) surface, (3) chain, (4) piggy-back, (5) somersault, (6) cyclone, (7) sideways, and

(8) bottom. Each of these feeding behaviours is described in detail below and the prevalent zooplankton taxa associated with each feeding strategy were identified (Table 5.2). All eight feeding strategies were observed being undertaken by *M. alfredi*, while only strategies 1, 2, 5 & 7 were observed for *M. birostris*.

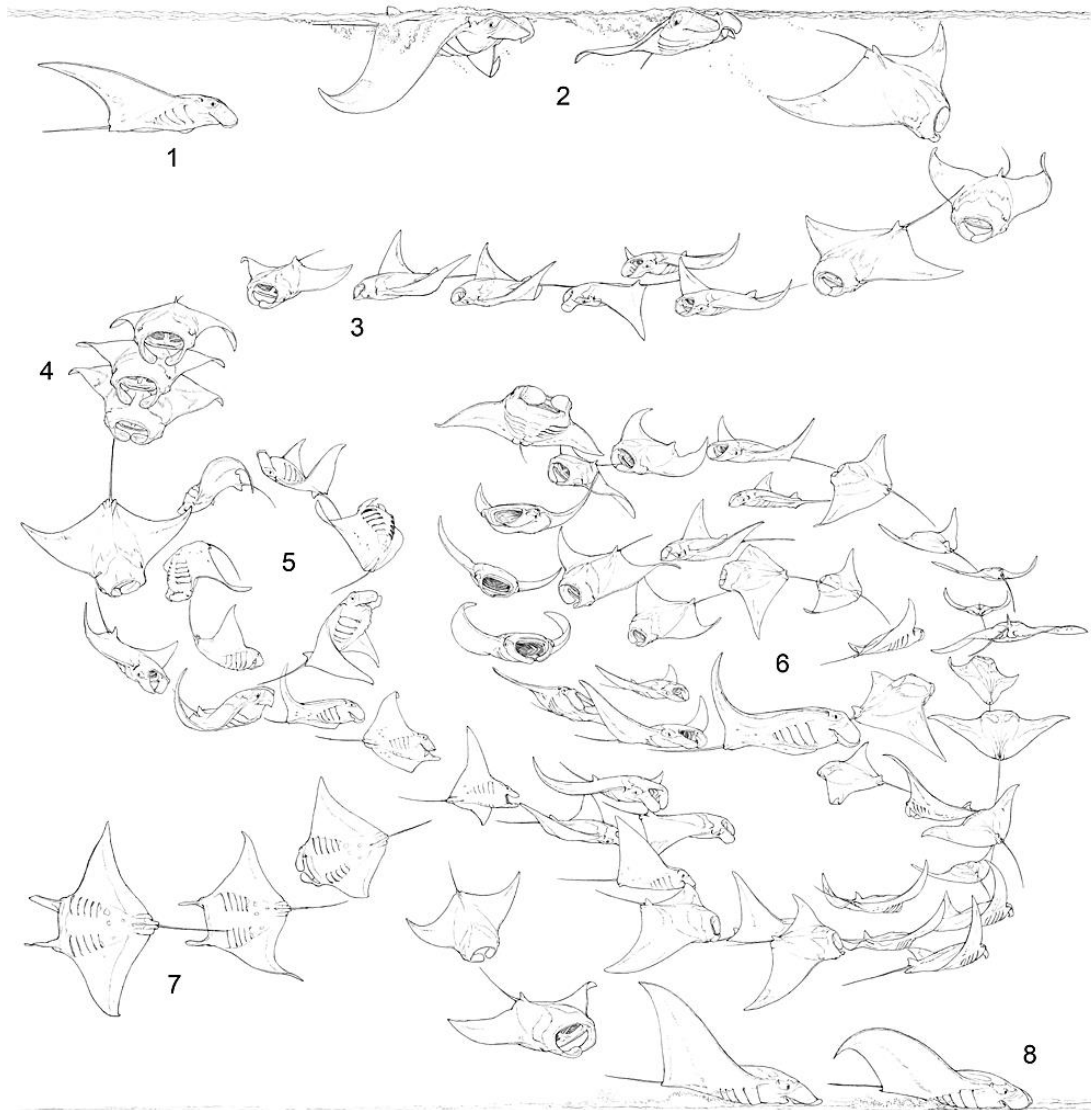


Figure 5.5 Illustration of the eight feeding strategies of manta rays: (1) straight, (2) surface, (3) chain, (4) piggyback, (5) somersault, (6) cyclone, (7) sideways, and (8) bottom (Illustrations © Marc Dando).

Table 5.2 Prevalent zooplankton taxa associated with the eight manta ray feeding strategies.

| Feeding strategy | Strategy type | <i>Manta alfredi</i> | <i>Manta birostris</i> | Prevalent zooplankton taxa |
|------------------|---------------|----------------------|------------------------|--------------------------------|
| (1) Straight | Individual | X | X | Copepoda, Mysida, Chaetognatha |
| (2) Surface | Individual | X | X | Ichthyoplankton, Chaetognatha |
| (3) Chain | Group | X | | Copepoda, Mysida, Chaetognatha |
| (4) Piggyback | Group | X | | Copepoda, Mysida, Chaetognatha |
| (5) Somersault | Individual | X | X | Copepoda, Mysida |
| (6) Cyclone | Group | X | | Copepoda, Mysida, Chaetognatha |
| (7) Sideways | Individual | X | X | Copepoda, Chaetognatha |
| (8) Bottom | Individual | X | | Ichthyoplankton, Mysida |

5.4.1 Description of manta ray feeding strategies

1. Straight feeding

In both manta ray species, straight feeding was the most frequently observed strategy. Individual mantas feed independently by swimming forward in a straight line with their cephalic fins held open in front of the mouth (Fig. 5.6A – C). These paddle-like appendages almost touch in the centre, forming a wide oval shape which funnels zooplankton into the manta's buccal cavity (Fig. 5.6D – F). Manta rays often feed in this manner horizontally along a current-line between converging bodies of water which concentrate prey. However, sometimes prey is concentrated along vertical gradients in the water column, such as along steep reef drop-offs, causing the mantas to feed vertically up and down. The mantas perform a sharp 180 degree turn at the end of each 'feeding run', before feeding in the opposite direction along the same plane. Feeding runs may extend from just a few dozen metres, to several hundred metres depending on the concentration and distribution of prey. In this fashion the animals feed repeatedly backwards and forwards through the same area of the ocean. During the few seconds it takes to turn at the end of the feeding run a manta usually stops feeding, closes its mouth, squeezing out the remaining water from the buccal cavity through its gills, before swallowing the captured prey.

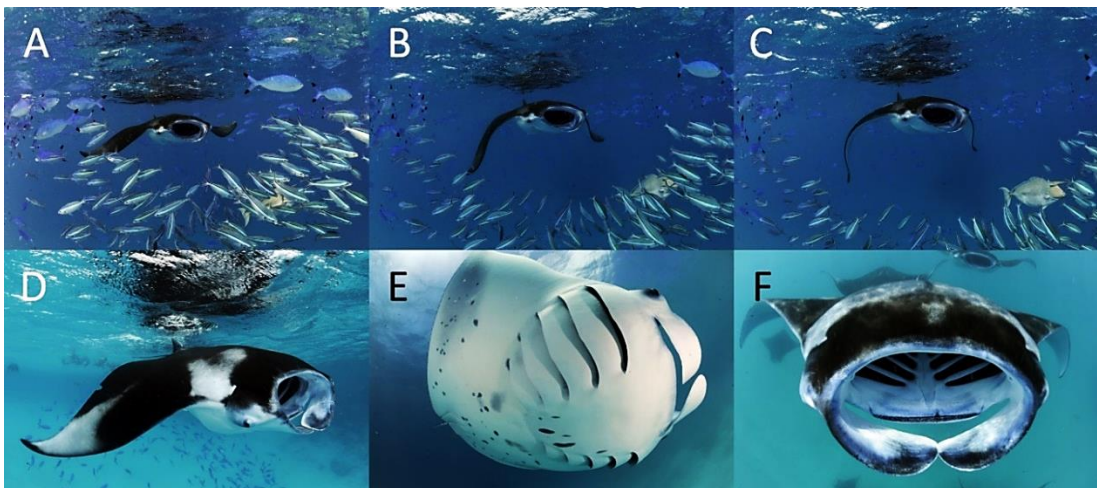


Figure 5.6 A straight feeding *Manta alfredi* in the Maldives feeds horizontally through a shoal of fusiliers (A – C) on a feeding run, positioning its unfurled cephalic fins together in alignment in front of the lowered bottom jaw (D – F). This positioning creates a wide oval shaped mouth, directing the manta's zooplankton prey into the buccal cavity and across the five pairs of feathery gill plates which encircle each of the gill slits.

2. Surface feeding

Surface feeding occurs when prey is concentrated in the top few centimetres of the water column. While similar to horizontal straight feeding, the ray, feeding individually, tilts its head back so that the upper jaw of its mouth is just above the water (Fig 5.7A). The close proximity to the water's surface means it has to reduce the up-stroke of the pectoral fin to prevent its pectoral fins from lifting above the water's surface (Fig. 5.7C – F), although the tips of the pectoral fins regularly break the surface. Swimming speed is also slightly reduced due to the restricted swimming position. The cephalic fins are positioned in front of the mouth in the same manner as straight feeding; although the mouth is usually only three quarters open (Fig. 5.7B). Feeding runs follow the same pattern as straight feeding, although to turn at the end of each run the manta dips away from the water's surface. This surface feeding strategy is not to be confused with straight or chain feeding, which is sometimes undertaken close to the water's surface.

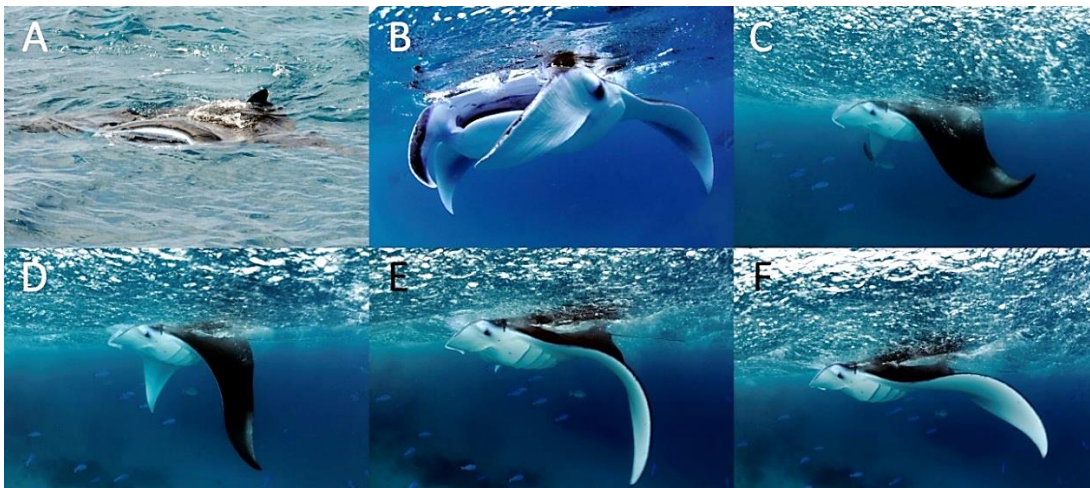


Figure 5.7 A surface feeding *Manta alfredi* in the Maldives feeds horizontally through the water with its head tilted upward so that its top jaw is positioned at, or above, the water surface (A – B). The close proximity to the water's surface means the manta has to reduce the up-stroke of the pectoral fin to prevent its pectoral fins from lifting above the water's surface (C – F).

3. Chain feeding

Feeding in groups, the manta rays line up head-to-tail to form strung out feeding chains of two to as many as several dozen individuals moving horizontally (Fig 5.8A – E). The feeding runs and cephalic fin positioning in chain feeding are the same as in straight feeding. At the end of the feeding run the chain of manta rays often continues to loosely hold the line which snakes around behind the leading animal. Creating an echelon formation, each of the following manta rays positions itself slightly above or below the individual in front, maintaining a distance less than a body length apart. During very large feeding events the chain feeding composition may expand to form multiple loosely interlinked chains several animals' wide, stretching back to form a tail of over 40 individuals (Fig. 5.8F).

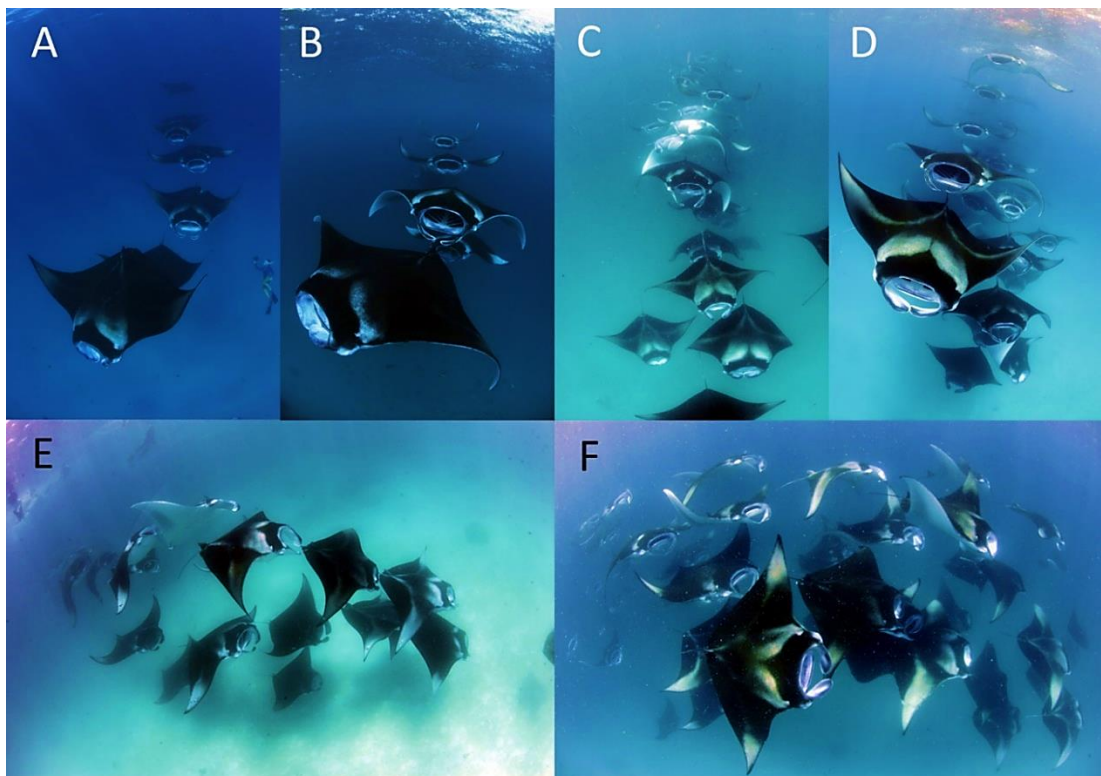


Figure 5.8 Chain feeding *Manta alfredi* in the Maldives line up head-to-tail to form strung out feeding chain of up to several dozen individuals (A – E). During very large feeding events the chain feeding composition may expand to form multiple loosely interlinked chains several animals' wide, stretching back to form a tail of over 40 individuals (F).

4. Piggyback feeding

Piggyback feeding involves multiple manta rays feeding together. A smaller individual, usually a male, positions itself directly on the back of a straight feeding larger individual, usually a female, matching the strokes of its pectoral fins to the beats of the larger individual (Fig. 5.9A – E). Occasionally several individuals will attempt to piggyback on top of one another, resulting in a stacked column of three, four, or even five rays swimming together horizontally (Fig. 5.9F). At the end of a feeding run when the lowest positioned individual turns back, the piggybacked individual/s are usually displaced.

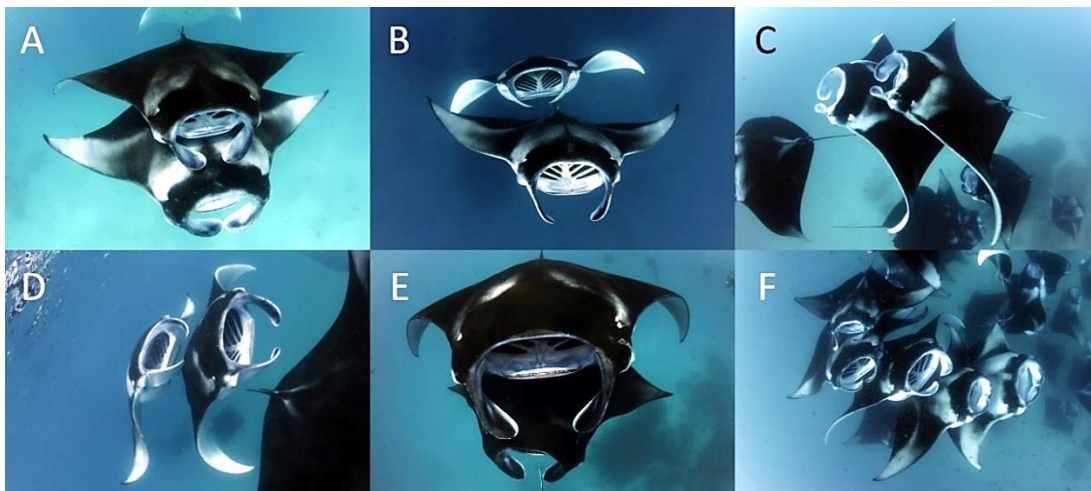


Figure 5.9 Piggyback feeding *Manta alfredi* in the Maldives. A following manta positions itself directly on the back of a straight feeding individual (A – B), matching its pectoral fin strokes to the other manta (C – E). Several individuals piggyback on top of one another, resulting in a stack of five rays feeding together (F).

5. Somersault feeding

Feeding individually the manta performs a tight backward somersault as if chasing its tail (Fig. 5.10A – D), completing a loop, the diameter of which is less than a body width. This behaviour is often performed when prey is concentrated in a dense patch. When targeting more mobile prey, such as a dense school of mysid shrimps, individuals may accelerate into a backward somersault. These rapid backward lunges are typically seen when prey is concentrated close to the surface, restricting prey movement and increasing feeding efficiency (Fig. 5.10C). Lunge backward somersaults are usually only performed once or twice before the manta resumes straight feeding. However, when a dense patch of less mobile prey is found (such as copepods), as many as several dozen continuous backward somersaults may be performed before the manta breaks the looping cycle and returns to straight feeding. Breaks between somersault feeding runs also enable prey to be swallowed. Mantas were never observed to somersault feed forwards. During somersault feeding the manta's mouth is held wide open and its unfurled cephalic fins are positioned just in front of the lower jaw (Fig. 5.10E – F).

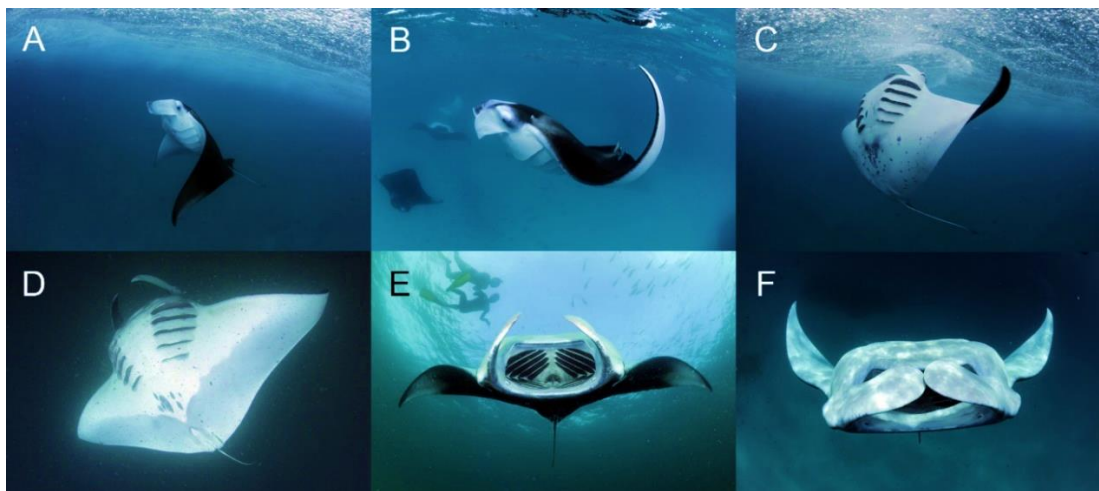


Figure 5.10 Somersault feeding manta rays [*Manta alfredi* (A – C, E – F) and *M. birostris* (D)] in the Maldives performing tight backward loops. During this mode of feeding the manta's mouth is held wide open and its unfurled cephalic fins are positioned just in front of the lower jaw (E – F).

6. Cyclone feeding

When zooplankton is extremely dense in a limited area, a line of chain feeding mantas may begin to loop around on itself until the lead animal joins the trailing mantas to form a large feeding circle. As increasing numbers of animals join the circle, the column of mantas builds through the water to resemble an underwater cyclone of mantas approximately 15 – 20 m in diameter (Fig 5.11A – B). The spiralling mass, which has included up to 150 individuals in Hanifaru Bay, circles around for as long as 30 minutes, although on average it only lasts for a few minutes. The rotating cyclone always turns anticlockwise when viewed from above, possibly due to lateralisation. The mantas' movement appears to create a vortex as the rotating animals' movement combines to create a current which is strong enough to draw a diver positioned just outside the wall of feeding mantas towards them. This current pulls plankton rich water inwards towards the filter feeding rays. Sometimes the cyclone becomes compressed in height, forming more of a spiralling circle of mantas close to the seabed (Fig. 5.11C – F).

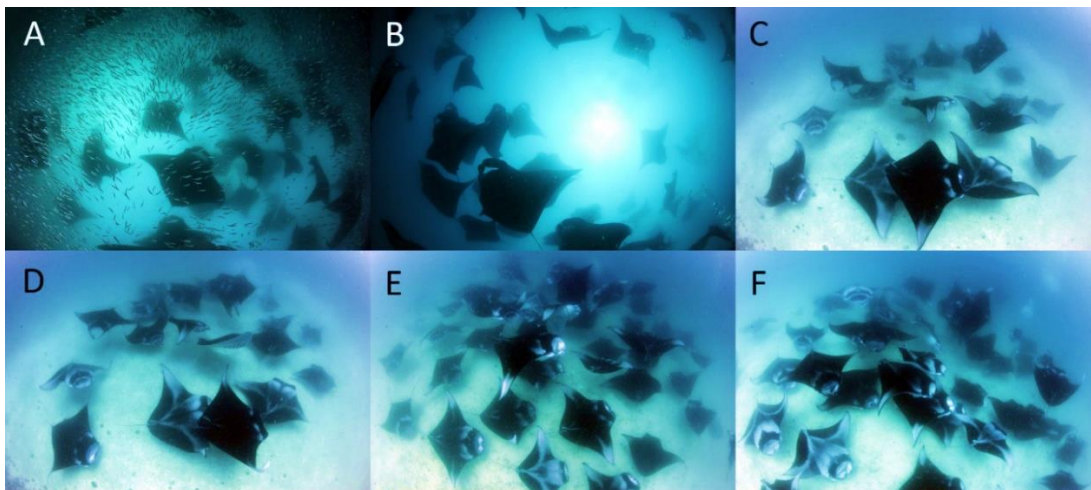


Figure 5.11 A cyclone feeding group of *Manta alfredi* in the Maldives spiral anticlockwise, resembling an underwater cyclone approximately 15 metres in diameter (A – B). If the mantas' prey is denser close to the seafloor the cyclone becomes compressed in height, forming more of a spiralling circle of mantas close to the seabed (C – F).

7. Sideways feeding

Sideways feeding is like straight feeding, except the manta flips sideways, rotating 90 degrees from the normal horizontal position (Fig. 5.12A – B, E). The cephalic fins are held perpendicular to the plane of the body, away from the manta's head (Fig. 5.12D). This position is very different to the cephalic fin position in straight feeding, where they are held together below the mouth. In this position the manta feeds backwards and forwards along a concentrated line of plankton, returning to the horizontal only during turns. Although sideways feeding individuals predominantly feed alone, sometimes they may form a loosely grouped sideways chain (Fig. 5.12C), or feed among a group of chain feeding manta rays (Fig. 5.12F – G).

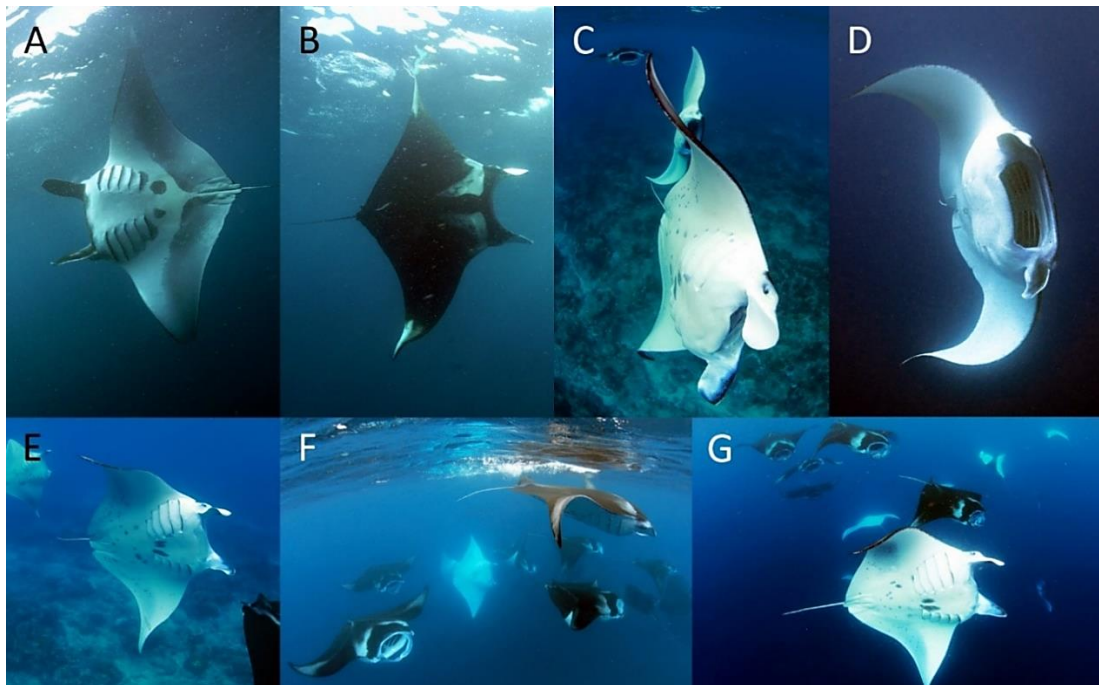


Figure 5.12 A sideways feeding *Manta birostris* in the Maldives swims through the water with its body rotated 90 degrees from horizontal (A – B). Sideways feeding mantas hold their cephalic fins perpendicular to the plane of the body, away from the head (D). Two sideways feeding individuals form a chain (C, E), while others feed among a group of chain feeding manta rays (F – G). Images C – G show *Manta alfredi* in Palau (Images © Mandy Etpison).

8. Bottom feeding

Bottom feeding is undertaken independently by individuals. The manta swims along the seabed with its open mouth positioned a few centimetres above the bottom (Fig. 5.13A – C). Like surface feeding, the seabed forms a natural barrier to escape for zooplanktonic prey, so the manta's unfurled cephalic fins are usually splayed apart, positioned away from the mouth to funnel plankton towards the centre of the mouth (Fig. 5.13D). During bottom feeding the cephalic fins may also be held in the central position adopted in most other feeding strategies. Bottom feeding usually occurs over areas of open sand, enabling mantas to manoeuvre without significant injury. However, contact between the seabed and the cephalic fins and lower jaw, especially between the gill slits, causes red abrasions (Fig. 5.13E). It appears that as a result of regular bottom feeding, the tips of the cephalic fins of some individuals become permanently scarred and worn down (Fig. 5.13F).

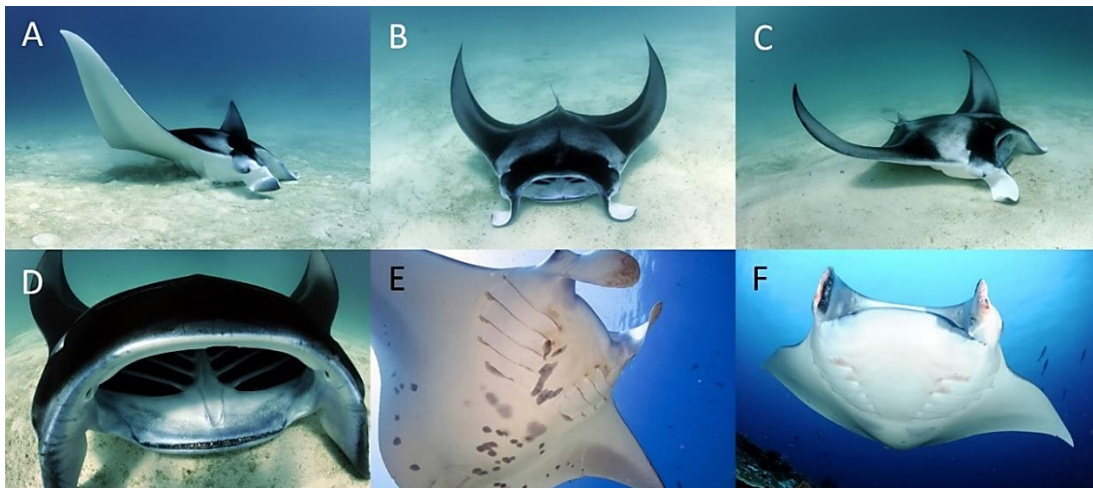


Figure 5.13 Bottom feeding *Manta alfredi* swim along the seabed with their open mouth positioned within a few centimetres of the seafloor (A – C). Unfurled cephalic fins are splayed apart to funnel plankton inwards (D). Regular contact of the manta ray's cephalic fins and gill slits with the seabed during bottom feeding causes red abrasions (E) and can rub away the tips of the cephalic fins (F). Images A – D show *Manta alfredi* in the Maldives, E – F in Raja Ampat, Indonesia.

5.4.2 Zooplankton composition, location and behaviour

The zooplankton prey of manta rays is not a homogenous mix (Mackas et al. 1985; Omori & Hamner 1982) and during the study the prey species composition and location changed from one hour to the next, and between days and months. Larval fish (Ichthyoplankton) often hugged the seabed or water surface in thin layers, while copepods (Copepoda) and opossum shrimps (Mysida) actively clumped together in cloudy patches in the middle of the water column, forming dense concentrations (Fig 5.14). At the start of a feeding event, as the concentration of copepods and arrow worms (Chaetognatha) began to rise (often

interspersed with low densities of decapods and ostracods), *M. alfredi* would begin straight feeding. As the prey density increased, usually along converging current lines, mantas would switch to chain and piggyback feeding.



Figure 5.14 A *Manta alfredi* straight feeds through dense patches of copepods which have clumped together to form elongated clouds in Baa Atoll, Maldives.

Where denser pockets of zooplankton formed, some individuals would begin somersault feeding, while others switched to surface feeding if larval fish fry or arrow worms had accumulated at the water's surface. If concentrations of prey, primarily copepods, reach extremely dense levels a cyclone feeding event may form. Often towards the end of a feeding event, as prey concentrations in the water column dropped, mantas would switch to bottom feeding, targeting prey that had sunk to the seabed.

5.4.3 Primary study site - Hanifaru Bay, Baa Atoll

Sighting variations

A total of 12,852 photo-ID sightings of 1,427 individual feeding manta rays were recorded during the 954 surveys at Hanifaru Bay. There were only four sightings of *M. birostris*, all males, each observed only once. The 12,848 sightings of 1,423 individual *M. alfredi* were comprised of 793 (56%) females, 625 (44%) males and 5 (<1%) individuals for which sex was unable to be determined. Just over half of these individuals were adults (N = 814 or 57%), while 604 (43%) were juveniles. Adult *M. alfredi* accounted for 63% (N = 8,085) of the total sightings, of which 57% (N = 4,570) were adult females. Each adult female was recorded at Hanifaru 13.4 times on average throughout the study, while each adult male averaged 7.5 sightings. Although not seen as frequently as adults, juvenile female *M. alfredi* also frequented Hanifaru Bay and were more abundant than juvenile males, with an average of 9.5 and 3.1 sightings respectively. When adult and juvenile sightings are combined, each

female recorded in this study was seen on average almost twice as often in Hanifaru Bay as a male (Fig. 5.15A), while adults were more frequently sighted than juveniles (Fig. 5.15B). These observed sighting variations were significantly different between both females and males ($U = 200664$, $N = 1,418$, $p < 0.001$), and between adults and juveniles ($U = 204245$, $N = 1,418$, $p < 0.001$).

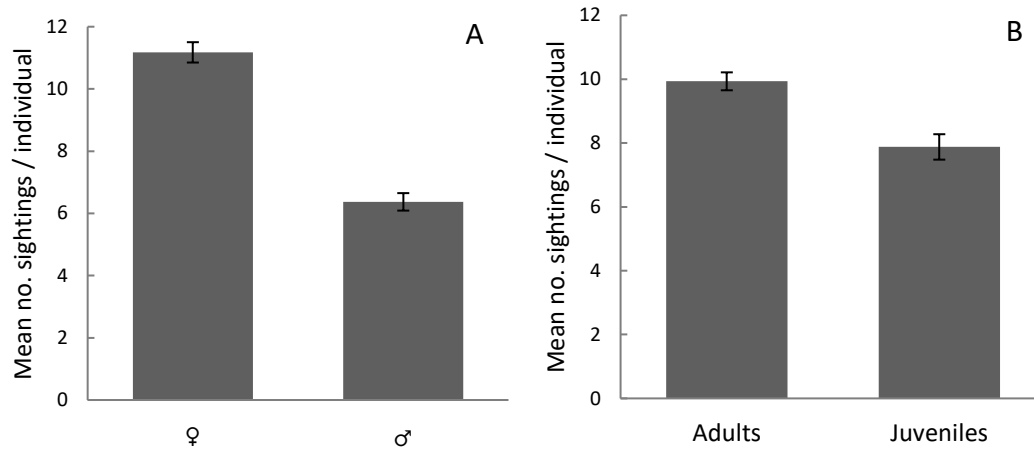


Figure 5.15 Mean number of sightings recorded in Hanifaru Bay for each identified *Manta alfredi* throughout the study; (A) males ($N = 625$) vs. females ($N = 793$), (B) adults ($N = 814$) vs. juveniles ($N = 604$). Mean number of female sightings were 11.18 ± 0.33 (95% CI, $N = 8,862$), males 6.37 ± 0.28 (95% CI, $N = 3,981$), adults 9.34 ± 0.28 (95% CI, $N = 8,085$), juveniles 7.88 ± 0.40 (95% CI, $N = 4,758$).

Feeding strategies

Throughout May – November 2014 a total of 455 individual feeding *M. alfredi* were identified from a total of 1,367 sightings at Hanifaru Bay. 179 of these mantas were randomly selected during 24 separate feeding events (each on a different day) and the predominant feeding strategy employed by each individual was identified during each observation period. Each of the selected individuals was continually observed for an average of 02:52 min. The shortest observation was 00:10 sec., the longest 14:45 min. Total observation time was 22:40:37 hrs. None of the 179 selected mantas was observed more than once during each feeding event. On average, each individual was observed 1.6 times across all 24 events, and the maximum number of observations was 8.

A total of 285 observations were recorded of the 179 randomly selected mantas, 116 (65%) were females, and 63 (35%) males, 85 (48%) of which were adults, and 94 (52%) juveniles. Bottom feeding was the only feeding strategy not recorded in this sample, although it was recorded at Hanifaru Bay (and elsewhere) at other times during this study. When combined, chain, somersault, and straight feeding accounted for the majority (94%) of the total sightings

between May – November 2014 (Table 5.3). *M. alfredi* of both sexes were observed straight, surface, chain, piggyback, somersault and cyclone feeding, but only one adult male and a juvenile female were observed surface feeding, and no adult females were observed piggyback feeding.

Sideways feeding across the entire study period was the least frequently observed behaviour, with just one *M. alfredi* and four *M. birostris* seen feeding in this manner. Indeed, although there were very few encounters with feeding *M. birostris* (N = 6) throughout the entire study, this sideways feeding strategy was the prevalent feeding behaviour seen. During the other three observations of feeding *M. birostris* these individuals were primarily either somersault or straight feeding. During the three feeding events in Hanifaru Bay when *M. birostris* were present, two individuals employed sideways feeding, while not one of the *M. alfredi* present (10 and 56 individuals) at the same time were observed to feed in this way.

Table 5.3 Frequency of feeding strategy employed by 179 *Manta alfredi* during a seven month study (May – November 2014) at Hanifaru Bay in the Maldives.

| Feeding strategy | Strategy type | Sightings | Percent |
|------------------|---------------|-----------|---------|
| (1) Straight | Individual | 69 | 24.21% |
| (2) Surface | Individual | 2 | 0.70% |
| (3) Chain | Group | 116 | 40.70% |
| (4) Piggyback | Group | 7 | 2.46% |
| (5) Somersault | Individual | 84 | 29.47% |
| (6) Cyclone | Group | 6 | 2.11% |
| (7) Sideways | Individual | 1 | 0.35% |
| (8) Bottom | Individual | 0 | 0.00% |
| Total | | 285 | 100.00% |

Group vs. individual strategy

Due to the small sample size during the May – November 2014 study it was not possible to determine if there was any discernible preference for a specific feeding strategy between the sexes, or between adults and juveniles. Instead, observations were pooled into either individual or group strategy type (Table 5.1) for further analysis. For this, no clear differences emerged between the proportions of group or individual feeding observations for either sex ($\chi^2 = 0.00$, $df = 1$, $N = 285$, $p = 0.99$), or maturity status ($\chi^2 = 0.41$, $df = 1$, $N = 285$, $p = 0.52$) (Fig. 5.16). However, due to the relatively small number of observations, the strength of comparisons for determining any predilection for any specific feeding strategy, between both the sexes and maturity status, was weak. Therefore, further data collection is recommended.

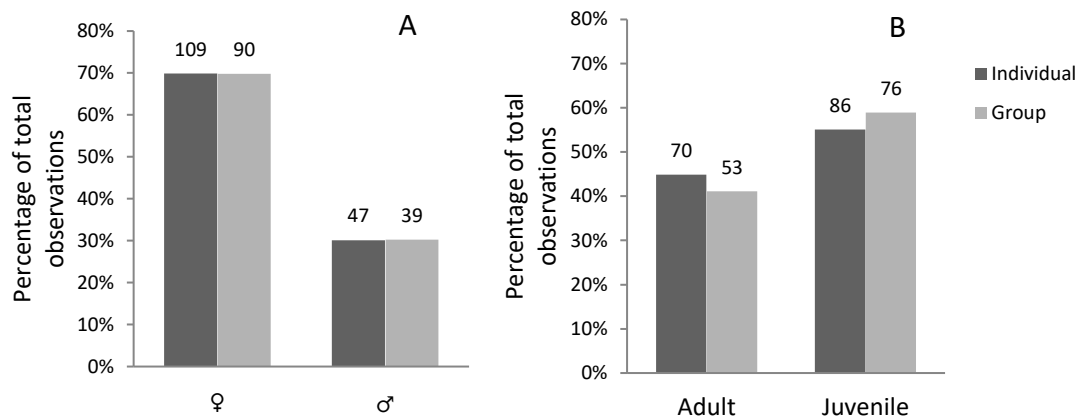


Figure 5.16 Occasions 179 individual feeding *Manta alfredi* engaged in either a type of group or individual feeding strategy during 285 observations throughout a seven month study (May – November 2014) at Hanifaru Bay, Maldives. A (N = 116 female, N = 63 males), B (N = 85 adults, N = 94 juveniles). Actual numbers presented at the top of bars.

Predilection for either group or individual feeding strategy type in relation to zooplankton density (low, medium or high) was also investigated. The results showed there was a significant shift from the majority of individuals feeding individually when the zooplankton density was low, to the majority engaging in group feeding as zooplankton levels rose to medium and high (Fig. 5.17) ($\chi^2 = 19.46$, $df = 2$, $N = 285$, $p < 0.001$).

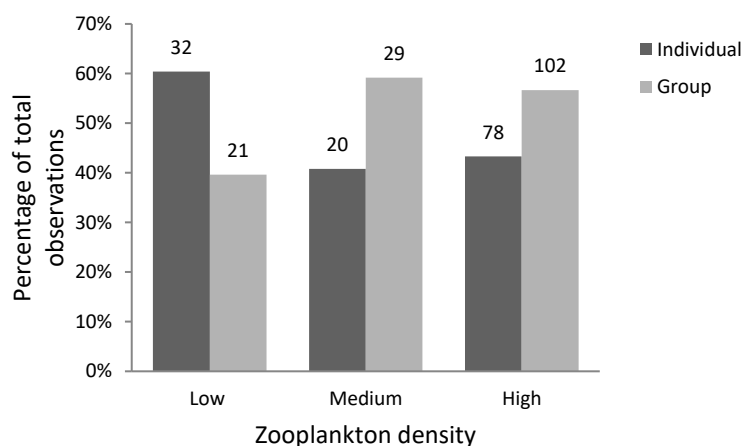


Figure 5.17 Visual estimates of zooplankton density (low, medium, high) during 285 observations of 179 feeding *Manta alfredi* employing either group or individual feeding strategies throughout a seven month study (May – November 2014) at Hanifaru Bay in the Maldives. Actual numbers presented at the top of bars.

A confounding factor for the increase in group feeding behaviour with increasing zooplankton density may be that as plankton density increases, so too does the number of individuals present at the manta's feeding aggregation site (Fig. 5.18), creating more opportunities to feed in groups. However, even during the feeding events where zooplankton density was low the mean number of individuals present was 21. Although this was less than the mean number of feeding individuals present during events where the zooplankton density was medium (42) or high (32), in the confined aggregation area of Hanifaru Bay, this amount of individuals still provides ample opportunity for group feeding strategies to be employed by the individuals present.

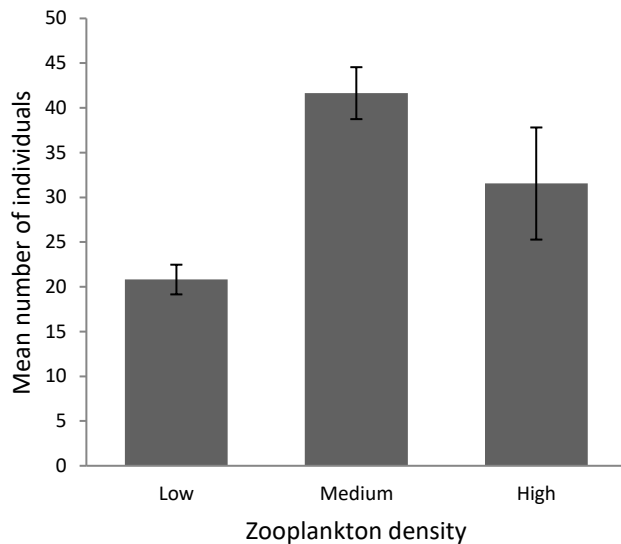


Figure 5.18 Variation in the mean number of feeding *Manta alfredi* present with varying visual estimates of zooplankton density (low, medium, high) during the seven month study (May – November 2014) at Hanifaru Bay in the Maldives. Mean number of individuals when zooplankton density was low (20.82 ± 1.66 , 95% CI, N = 99), medium (41.65 ± 2.90 , 95% CI, N = 164), and high (31.55 ± 6.27 , 95% CI, N = 22).

Breaching behaviour

In total 286 breaches were recorded between 2009 and 2014 inside Hanifaru Bay during 89 surveys of feeding events. Although usually few or no breaches were observed, on some occasions dozens occurred within just a few hours. For example, on the 22nd June 2012 47 breaches were recorded between 10:26 and 16:10. Each recorded breach was grouped into an hourly slot leading up to, or away from, the peak feeding time. The results revealed a significant difference in the number of breaches recorded between the different grouped slots ($\chi^2 = 319.25$, $df = 9$, $N = 286$, $p < 0.001$). Indeed, during the three hours leading up to the peak feeding time 209 (73%) of the total 286 breaches occurred, with breaches occurring 232% more times than would be expected at random during the hour before peak feeding time ($N = 228$, $r_2 = 0.79$, $p < 0.001$). Conversely, during the three hour period after the peak feeding time, only 57 (11%) of the total breaches recorded took place ($N = 153$, $r_2 = -1.00$, $p = 0.01$) (Fig. 5.19).

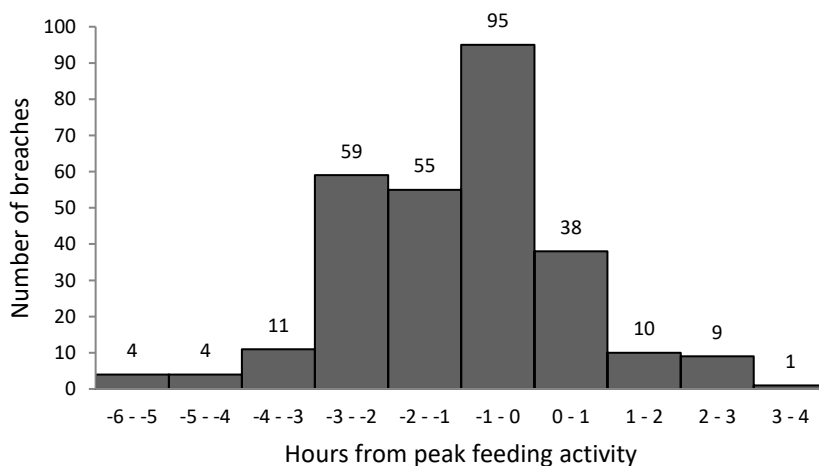


Figure 5.19 Number of *Manta alfredi* breaches recorded at Hanifaru Bay in the Maldives between 2009 and 2014 during each of the hours before and after the peak feeding time.

5.5 Discussion

This paper presents, for the first time, a record of all known feeding behaviours exhibited by both species of manta ray. Described in detail, the eight identified feeding strategies: (1) straight, (2) surface, (3) chain, (4) piggy-back, (5) somersault, (6) cyclone, (7) sideways, and (8) bottom, are presented with the aid of an ethogram and photographs. It is hoped that these descriptions of manta feeding strategies will form the base from which a clearer understanding of how these evolutionary adaptive traits contribute to the fitness of these species. By understanding which variables have driven the evolution of the different feeding strategies manta rays employ, we can better understand these species, their fascinating interactions with one another, and their environment. As our ability to observe manta rays throughout their entire habitat increases with the development of technology, it is probable new feeding behaviours will emerge, allowing further insight into these behaviours to be characterised.

Although only a few *M. birostris* were seen to feed during this study in the Maldives, these animals conformed to behaviour seen off the Pacific coast of Mexico (pers. obs.). There, as in the Maldives, this species was not observed to engage in group feeding, only in straight, surface, somersault, and sideways feeding. However, researchers studying *Manta c.f. birostris* in the Caribbean Sea and Gulf of Mexico, as well as observing all of the above *M. birostris* feeding strategies, have occasionally observed this species to chain and piggyback feed (K. Fuentes, pers. comm.). Although there are no records of *M. birostris* bottom feeding, individuals have been observed in Raja Ampat, Indonesia (pers. obs.) and in the Yucatán, Mexico (K. Fuentes, pers. comm.) with similar red abrasions to their cephalic fins and lower jaw as those regularly observed on *M. alfredi* in the Maldives, suggesting *M. birostris* may also undertake bottom feeding (Fig 5.20).

M. alfredi have also been observed on occasion to intentionally rub, both their ventral and dorsal surfaces, on sandy substrate while in the vicinity of cleaning stations (pers. obs.) (Fig 5.21), presumably in an effort to rid themselves of parasites (Côté 2000; Feder 1966; Losey Jr 1972). It is possible that this scratching behaviour, if repeated regularly, may cause similar injuries to those caused by bottom feeding. However, the observed scratching behaviour was usually brief, lasting only a few seconds, while the bottom feeding observed during this study would continue for many minutes, or even several hours, interspersed with other feeding strategies. It is therefore more likely the red abrasion injuries often observed on the ventral

surfaces of both manta species are from repeated and sustained rubbing along the seabed while bottom feeding.

M. birostris have never been observed to cyclone feed, yet whether the conclusion that *M. birostris* engage in less group feeding behaviour than *M. alfredi* is correct, requires further research. However, given the oceanic nature of *M. birostris* it will be difficult to acquire this information.



Figure 5.20 Red abrasions, most likely caused as a result of regular bottom feeding, cover the lower jaw and ventral surface of this *Manta birostris* in Raja Ampat, Indonesia.



Figure 5.21 A *Manta alfredi* scratches itself on the sandy seabed at a cleaning station in the Maldives, presumably to dislodge itchy parasites or irritable cleanerfish.

The observed increase in group feeding behaviour of *M. alfredi* with increasing prey density may be driven by improved prey capture, improved hydrodynamic efficiency, collision avoidance (as more manta rays aggregate at the feeding site, to avoid collisions, they form ordered feeding groups), or a combination of all three. For each of the eight strategies described, improved prey capture especially appeared to be an important influencing factor

on the foraging behaviour employed. The mantas appeared to adjust their feeding strategy type to the changing prey species composition and the prey's position in the water column.

While prey species composition and location are likely to be determining factors in the feeding strategy employed, observations suggest the escape response of prey may also be a factor. Fast-swimming swarms of opossum shrimp, which are known to use schooling behaviour to avoid predation (O'Brien & Ritz 1988), often appeared capable of avoiding the gaping mouth of a straight feeding manta. The escape response of the schooling shrimps caused the mantas to employ lunge somersault feeding, accelerating rapidly into the school of prey and often attempting to trap the prey close to the surface. During the study at Hanifaru Bay occasionally extremely dense schools of anchovy (*Engraulidae*) fry would tempt a few individual *M. alfredi* to undertake swift somersault lunges into the densely shoaling fish. By closely observing this behaviour it appeared that all the feeding attempts were unsuccessful as the agile fish fry were able to easily avoid the manta's mouth. After a few attempts these individuals ceased feeding, and although several dozen other individual mantas were present, the majority of the mantas did not attempt to capture the fish, focusing their efforts instead on other prey species. On the few occasions when these dense schools of anchovy fry were observed, groups (usually 5 – 10 individuals) of the manta rays' close relatives, the short-fin pygmy devil rays (*Mobula kuhlii*), employed a rapid attack strategy to capture the fry. Much faster than the manta rays, the *M. kuhlii* lunged rapidly together through the dense shoals of fry with mouths agape, scattering their prey (Fig. 5.22A).

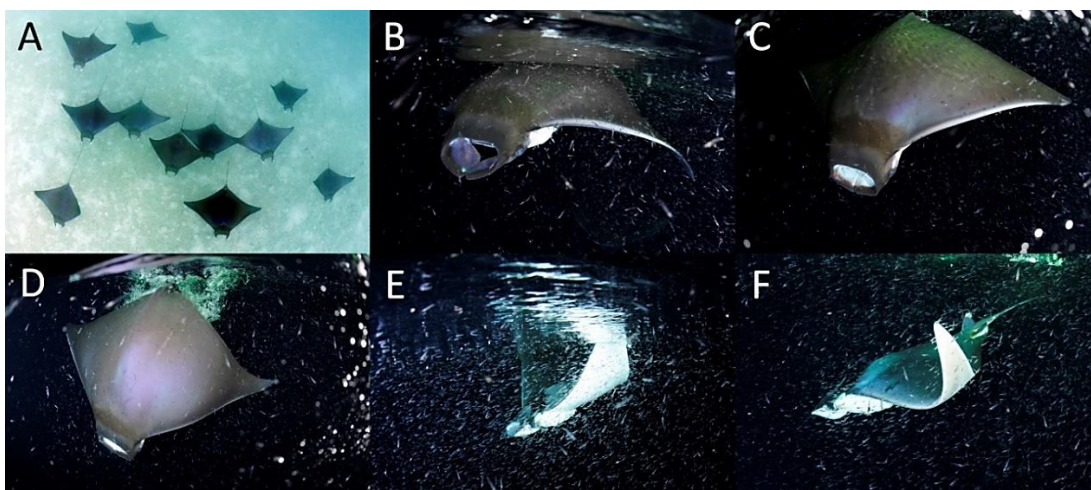


Figure 5.22 A shoal of the *Mobula kuhlii* work together in groups to splinter and chase down their fast moving prey in Hanifaru Bay in the Maldives (A). At night in the Maldives bright lights attract zooplankton which in turn attract *M. kuhlii* (B – D) and *Mobula japonica* (E – F) to lunge feed, accelerating rapidly through dense schools of opossum shrimps and larval fish fry at the surface.

Similar rapid lunge feeding behaviour in this species was also recorded at night in the Maldives and in Komodo National Park, Indonesia, when bright lights attached to the back of a research vessel attracted small groups (2 – 5 individuals) of *M. kuhlii* to feed on dense patches of opossum shrimps and larval fish fry at the surface (Fig. 5.22B – D). This species was also observed on several occasions in the Maldives feeding singularly or in small groups (2 – 5 individuals) close to the seabed, their mouths positioned just above the sand. Similar bottom feeding behaviour has also been observed in two other pygmy devil ray species; Munk’s pygmy devil ray (*M. munkiana*) in the Sea of Cortez, Mexico (O. Aburto, pers. comm.), and the Atlantic pygmy devil ray (*M. hypostoma*) in Florida, US (K. Hull, pers. comm.). When bottom feeding, often in extremely shallow water (<1 m) along the shoreline, these pygmy mobulas swim in a line, or in echelon formation, similar to the chain feeding observed by *M. alfredi* in this study (Fig. 5.23).



Figure 5.23 A group of *Mobula hypostoma* feed in echelon formation along the shoreline in Florida, US (Image © Kim Hull).

On two separate occasions during this study spine-tail devil rays (*M. japonica*) were also attracted at night to feed on zooplankton concentrated by lights positioned at the back of a research vessel. Feeding in a similar fashion to *M. kuhlii*, this species also lunged rapidly through the dense schools of prey, although only solitary individuals of this species were encountered (Fig. 5.22 E – F). Juvenile Munk’s pygmy devil rays (*M. munkiana*), often in groups of 50 – 100 individuals, have also been recorded feeding at night behind dive vessels

in the Sea of Cortez, Mexico (pers. obs.). The *M. munkiana* were recorded straight, lunge and bottom feeding (Fig. 5.24A – E) similar to *M. alfredi* and *M. kuhlii* in the Maldives, however the large number of rays also resulted in a spiralling feeding formation similar to the cyclone feeding recorded for *M. alfredi* in this study (Fig. 5.24F). Long-finned pygmy devil rays (*M. eregoodootenkee*) have also been observed hunting in groups of approximately 6 – 12 individuals, preying upon shoals of adult anchovies in Raja Ampat, Indonesia (pers. obs.). Similar group hunting behaviour has also been observed in the larger sickle-fin devil rays (*M. tarapacana*), preying upon shoals of mesopelagic lanternfish (Myctophidae) off the coast of Costa Rica (E. van der Poll, pers. comm.). This is the first time the feeding strategies of mobula rays have been documented.

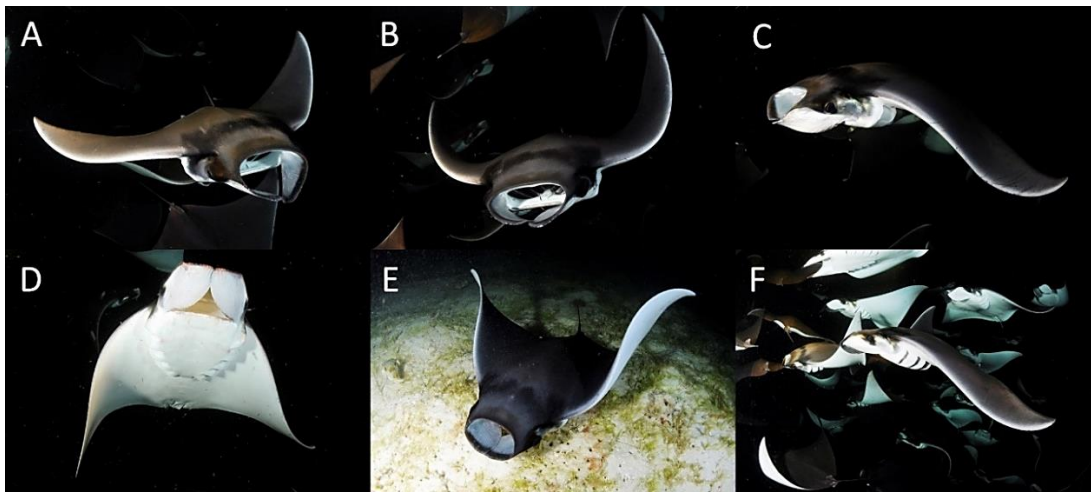


Figure 5.24 At night in the Sea of Cortez, Mexico bright lights attract dense schools of opossum shrimps which are eaten by groups of juvenile *Mobula munkiana*, which can number up to 100 individuals. The pygmy rays straight (A – C) lunge (D), bottom (E), and cyclone (F) feed beneath the lights.

Observations of the copepods (*Undinula sp.*) in the water at Hanifaru Bay during this study, which comprised the majority of the zooplankton available to the mantas at this site (Richardson et al. in prep.), revealed the copepod's escape response may have helped shape the various group feeding strategies employed by mantas. Planktonic copepods are consumed by a wide range of predators, leading to the evolution of a variety of adaptations to reduce risk of predation (Buskey 1994; Gliwicz 1986; Herring 1988). One adaptation is an escape response triggered by mechanoreception of hydrodynamic disturbance (Buskey et al. 2002; Fields & Yen 1997), which involves rapid acceleration using a short series of escape jumps or kicks (Burdick et al. 2007). Upon detecting the hydrodynamic disturbance of an approaching ray, the copepods may try to escape by jumping away from the manta's mouth (pers. obs.). However, because the escape kicks become less vigorous at the end of each escape response (Lenz & Hartline 1999), which is typically sustained for 2 – 3 kick cycles over

a short distance of a few centimetres (Lenz & Hartline 1999), a proportion of the escaping copepods will jump into the feeding path of any chain, piggyback or cyclone feeding mantas. Therefore, each following manta could gain an advantage in food intake by positioning themselves slightly off-line and behind the leading manta.

Given that prey is highly patchy and often densely concentrated along current lines, chain and cyclone feeding may also in part be a simple function of the necessity to coordinate movements to reduce collisions, thus saving energy and time costs otherwise spent in avoiding other mantas. Another contributing factor which may explain this group behaviour, at least in chain feeding events, could be the effect of the turbulence created by passing manta rays upon zooplankton. As the leading manta passes through the water its motion forms a series of wake eddies in its path (Dewey 2013), which, due to the copepods small size and similar density to seawater (Hopcroft et al. 2001; Knutsen et al. 2001), may prevent them from escaping because they will tend to be concentrated into the centre of the eddy vortex and channelled into the path of the following ray (Green 1995).

Feeding in groups may also improve hydrodynamic efficiency, the following animals gaining an energy saving advantage by slip streaming behind the leading manta, much like birds often fly in echelon or v-shaped formations to save energy (Lissaman & Shollenberger 1970). Feeding bowhead whales (*Balaena mysticetus*), which predominantly prey upon copepods and euphausiids (Lowry & Burns 1980), also swim in echelon formations, with the trailing whales positioned slightly behind and to the side (Würsig et al. 1985). Speculation upon the cause of this feeding behaviour has also been linked to improved prey capture (Würsig et al. 1985), and improved hydrodynamics as a result of the whales swimming in a close organised formation (Fish et al. 2012). Although surface feeding manta rays have only ever been seen to feed individually in this study, there is photographic evidence of surface feeding *M. alfredi* in Dugonab Bay, Sudan in the 1980s group feeding together in echelon formation (Fig. 5.25). It is therefore possible similar factors to those described above in relation to chain feeding may also apply to surface feeding manta rays, and indeed the echelon shoreline feeding *M. hypostoma* observed in the Atlantic.

Sideways feeding was the least observed of all the feeding strategies throughout the study across all sites. Although this behaviour was recorded in both *M. alfredi* and *M. birostris*, it was more prevalent in the latter. Due to the infrequency of observations, it is difficult to discern what factors influence choice of this strategy. Observations of *M. alfredi* sideways feeding in Palau over many years have revealed that of the 275 individuals photographically

identified there, only 11 were recorded sideways feeding (M. Etpison, pers. comm.). Interestingly, these 11 animals, many of which have been seen dozens of times over many years, fed exclusively in this way, regardless of location and prey availability. While these individuals always sideways feed there were often other feeding *M. alfredi* in close proximity, employing one or more of the other feeding strategies (Fig. 5.12).



Figure 5.25 *Manta alfredi* surface feeding together in group echelon formation at Dugonab Bay, Sudan in the 1980s (Image © Pierfranco Dilenge).

Anecdotal observations over many years during this study suggest some individual *M. alfredi* appear to predominantly utilise a somersault feeding strategy at Hanifaru Bay, regardless of the plankton density. This behaviour suggests, like those sideways feeding individuals in Palau, that some manta rays may have a preference for a specific feeding strategy, possibly choosing to target a sub-section of the available prey species available to them. Indeed, populations of predators often consist of individuals with preferences for different prey (Pettorelli et al. 2011). For example, a seven year study of ten sea otters (*Enhydra lutris*) at the Monterey Peninsula, California found extreme inter-individual variation in the otter's diet which persisted throughout the study and could not be explained by prey availability (Estes et al. 2003). A study of the common kestrel (*Falco tinnunculus*) around Rome in Italy also reported long term individual preferences in prey selection for this species, with no obvious correlation to phenotypic attributes (Costantini et al. 2006). However, preferences can often be linked to a variety of phenotypic attributes, such as sex (Cooper et al. 2007), age (Field et al. 2007; Rutz et al. 2006), reproductive status (Pierce et al. 2000), group size (Packer et al. 1990), family structure (Gaydos et al. 2005; Maniscalco et al. 2007), or social status (Saulitis et al. 2000). While directed studies are needed to test the hypothesis of prey specialisation

or preference between individual manta rays, observations of somersault and sideways feeding *M. alfredi* in the Maldives and Palau respectively lend support to this hypothesis.

Cooperative hunting is probably the most widely distributed form of cooperative behaviour in animals (Benoit-Bird & Au 2009; McMahon & Evans 1992; Pitman & Durban 2012; Rossbach 1999; Schmitt & Strand 1982; Similä & Ugarte 1993; Stander 1992), and may be an important evolutionary selective force for sociality (Bednarz 1988; Macdonald 1983; Packer & Rutten 1988). The evolution of cooperative hunting strategies are most likely to occur under conditions where close kin relationships are maintained (Clutton-Brock et al. 2002; Hamilton 1964). Given the social systems of manta rays (i.e., promiscuous breeding, no parental care, single birth offspring, and high dispersal of juveniles) (Chapters 3 and 4), the group feeding strategies observed in this study are therefore unlikely to have arisen as a result of kinship among the feeding individuals. However, sociality may also arise when there is a mutual advantage of cooperation resulting in the per capita rate of food intake within a hunting group exceeding that of a solitary individual (Bednarz 1988; Mangel & Clark 1986). If group feeding in manta rays results in an increase in the per capita rate of food intake within a hunting group, those individuals which most frequently visit the same location are more likely to develop a higher degree of sociality, leading to more frequent group feeding between these individuals, resulting in cooperation through reciprocal mutualism. Philopatry will also favour the evolution of cooperative traits between members of the sedentary sex (Greenwood 1980). In this study, the adult females were significantly more likely to visit Hanifaru Bay than either the adult males or juveniles. However, they were no more likely to feed in groups than either the males or juveniles, suggesting by-product mutualism instead of real cooperation is the more likely explanation for this behaviour.

Cooperative behaviour has been proposed as a hypothesis for repeated associations between group feeding humpback whales (*Megaptera novaeangliae*) (Weinrich 1991), which have similar social and reproductive systems to manta rays (Clapham 1996; Chapters 3 and 4.). However, reciprocity or by-product mutualism as a driver for the group bubble-net feeding behaviour which humpback whales undertakes has also been hypothesised (Wiley et al. 2011), similar to the explanation for the hunting behaviour observed in sailfish (*Istiophorus sp.*), which corral their prey by working together in groups (Domenici et al. 2014).

Reciprocal mutualism may also help to explain the manta ray breaching behaviour observed throughout this study at Hanifaru Bay during feeding events. Breaching behaviour has been reported across a number of marine groups: dolphins (Fish et al. 2006; Lusseau 2006), whales

(Carroll & Smithhisler 1980; Clapham et al. 1995; Taber & Thomas 1984), sharks (Curtis & Macesic 2011; Klimley 1994; Martin et al. 2005; Sims et al. 2000), rays (Marshall & Bennett 2010a; Weir et al. 2012), and billfishes (Romeo et al. 2009). There are numerous proposed theories to explain breaching, including energetics (Au & Weihs 1980; Taber & Thomas 1984), play and socializing (Lusseau 2006; Norris et al. 1994; Würsig et al. 1989), parasite removal (Fish et al. 2006), courtship and reproduction (Marshall & Bennett 2010a; Pyle et al. 1996; Romeo et al. 2009; Sims et al. 2000; Whitehead 1985), predation (Curtis & Macesic 2011; Klimley 1994; Martin et al. 2005), and communication (Würsig & Whitehead 2009).

Sound travels a lot faster and further in water than air and a breaching manta ray splash landing creates a large sound wave underwater which is likely to travel a great distance. On several occasions I have been in the water when a manta ray breached within 20 metres of me and the result felt and sounded like a small explosion. Furthermore, breaching mantas in this study always landed either flat on their dorsal or ventral surface upon re-entry to the water, further increasing the impact. Feeding events at Hanifaru Bay are often ephemeral, the currents sweeping zooplankton prey out of the bay after a few hours. Feeding events therefore are often limited not by the time it takes the plankton feeders in the vicinity to consume all of the food, but by turning tides; i.e. mantas do not compete for food against one another but against time. By feeding in groups manta rays are able to maximise the food they can consume in a finite period. In this hypothesis, breaching is an intentional form of signalling to attract other mantas to enable increased group feeding, reinforcing social interactions and improving feeding success for individuals. Given the peak in manta breaching behaviour observed in this study occurred during the few hours building up to the peak feeding time, this behaviour may alert other mantas in the area to the feeding opportunity. If communication is driving some of the breaching behaviour observed, it would suggest a much higher degree of social interaction than has previously been recorded for this species.

Mobula rays also regularly breach (pers. obs.; Weir et al. 2012), often leaping several metres out of the water in spectacular displays of aerial acrobatics. Mobula breaching events regularly occur during mass aggregations (G. Notarbartolo-di-Sciara, pers. comm.). In the Sea of Cortez in Mexico these aggregations occur in the summer when thousands of individual *M. munkiana* come together (O. Aburto, pers. comm.). *M. munkiana* most likely form these mass aggregations in order to mate, the leaping individuals slapping their pectoral fins down flat on the water's surface, possibly trying to create the loudest splash in an attempt to signal

their fitness to other rays in the area. Breaching behaviour during courtship events has also been observed in manta rays (Marshall & Bennett 2010a; Chapter 3), and during feeding aggregations of *Manta c.f. birostris* in the Gulf of Mexico (K. Fuentes, pers. comm.) and *Manta birostris* in the Paranaguá Estuarine Complex in Southern Brazil (Medeiros et al. 2015).

Breaching behaviour in basking sharks (*Cetorhinus maximus*) is also regularly observed during feeding aggregations of these giant zooplankton grazers around the coast of the United Kingdom (C. Speedie, pers. comm.). Sims et al. (2000) attributed this breaching behaviour to courtship, suggesting the feeding aggregations provided an opportunity for these habitually dispersed creatures to court and mate. Furthermore, they proposed the observed 'close-following' behaviours, in which one or several sharks closely followed one another as they fed at the surface, to courtship behaviour. These close-following behaviours were only observed when large sharks aggregated in relatively high numbers in rich zooplankton patches. Furthermore, when social interactions between basking sharks were observed, they occurred more frequently when aggregations formed in zooplankton patches along fronts. Given that *C. maximus* feed upon similar zooplankton copepod prey to manta rays, it might be that the close-following echelon behaviour observed in *C. maximus* may also be a group feeding adaptation in response to their prey's escape response or hydrodynamic efficiencies, similar to the group behaviour observed in manta rays and bowhead whales. *C. maximus* breaching may also be linked to social communication in relation to feeding or courtship, or both.

Future studies to investigate the possible existence of more complex social structure among the individuals within populations of manta rays, and the construction of hydrodynamic models to determine water flow around feeding mantas (which also incorporate prey behaviour), would together enable more detailed questions to be investigated about the reasons for manta feeding aggregations. If manta rays are signalling to one another using sound to improve feeding success, these animals have a much greater level of social communication than is currently appreciated.

Chapter 6

Summary and conclusions

This multi-decade study reports on an isolated and unfished population of manta rays - the world's largest known population of *M. alfredi* - advancing the knowledge of key life history traits, reproductive strategies, population demographics and habitat use. The findings will help to shape management decisions and drive effective conservation of this vulnerable species, as outlined below. This study also elucidates the reproductive and feeding behaviour of both manta species, providing insight into the selective pressures which shape behaviour. The study underscores the importance of long-term research on long-lived species with conservative life history strategies if important ecological and evolutionary questions are to be answered.

One of the most important contributions to the conservation ecology of *M. alfredi* from this study is the elucidation in Chapter 2 of the age at maturity of this species (11 and 15 years for males and females respectively), which was estimated to be up to 7 – 8 years higher than previously reported for females and males respectively. Estimates in Chapter 2 of size at birth, maturity and maximum disc widths, and maximum longevity estimates, were consistent with more limited data from previous studies. Chapter 2 also characterised habitat use, hypothesising that use of shallow coral reefs by *M. alfredi* may be a function of behavioural thermoregulation and predator avoidance, with cleaning stations acting as focal gathering points where social behaviour is undertaken, such as courtship and mating. Indeed it appeared that the function of cleaning for parasite removal, or wound healing, may often be a secondary or tertiary driver of site use. The frequent use of shallow protected lagoons by juvenile *M. alfredi* suggests these sites may act as nursery areas.

Chapter 3 significantly adds to knowledge of manta ray mating behaviour, with over 100 courtship events recorded during the study. Eighty three percent of these events were at cleaning sites, suggesting that these are important focal sites for reproductive activity and may also function as leks. This study was the first to photographically document the entirety of manta courtship and mating, enhancing knowledge of these events. My findings confirm that both *M. alfredi* and *M. birostris* engage in elaborate courtship rituals, exhibiting similar behaviours during all stages of the courtship and mating process. The results imply that female manta rays invest heavily in mate choice, which shapes the reproductive strategies of these species, providing insight into the selective pressures on this species and their relatives.

The second major contribution of this study to the conservation of manta rays in the Maldives are the findings of Chapter 4, in which I estimate *M. alfredi* to be two to more than three times less fecund than the current literature indicates, making this species one of the world's

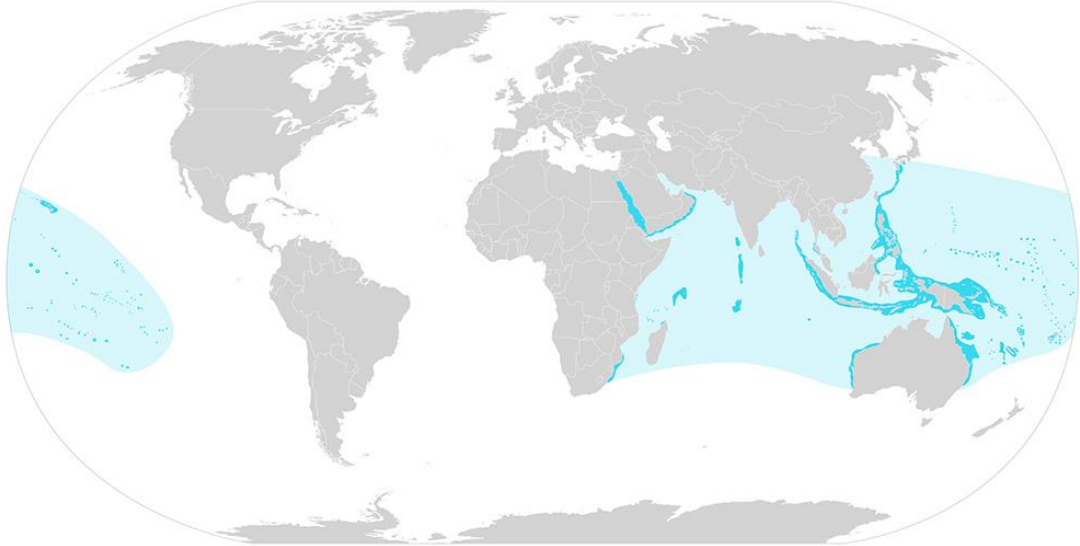
least fecund vertebrates. With such low fecundity and generally low population densities, coupled with the increased estimates of age at maturity in Chapter 2, there is no possibility of *M. alfredi* supporting sustainable fisheries anywhere in the world. The species needs complete protection from exploitation throughout its range. Fecundity varied considerably among years and appeared related to variability in abundance of the manta's planktonic food, which in turn may be linked to large-scale weather patterns such as the Indian Ocean Dipole and ENSO. Within years, reproductive activity was linked to the seasonal changes, with *M. alfredi* exhibiting a partially defined annual reproductive cycle with one or two peaks in reproductive activity each year occurring prior to transitions between the monsoons. Intra-annual variations in fecundity between the female sub-populations studied may be linked to social cues among females associated with mate choice, and thereby individual fitness.

Chapter 5 described for the first time all of the different feeding strategies exhibited by both manta species, also revealing significant variations in the degree of site fidelity at feeding sites between the sexes and maturity status, and some correlations between prey densities and the feeding strategy employed. This study also speculates that manta breaches may be a mechanism for communication, attracting other rays to a feeding site to aid group feeding, and that predator escape responses of the manta's prey, hydrodynamic efficiency, and collision avoidance techniques, may all influence the feeding strategy adopted. However, more work is needed to investigate these hypotheses. It is hoped this study will form the basis for such work.

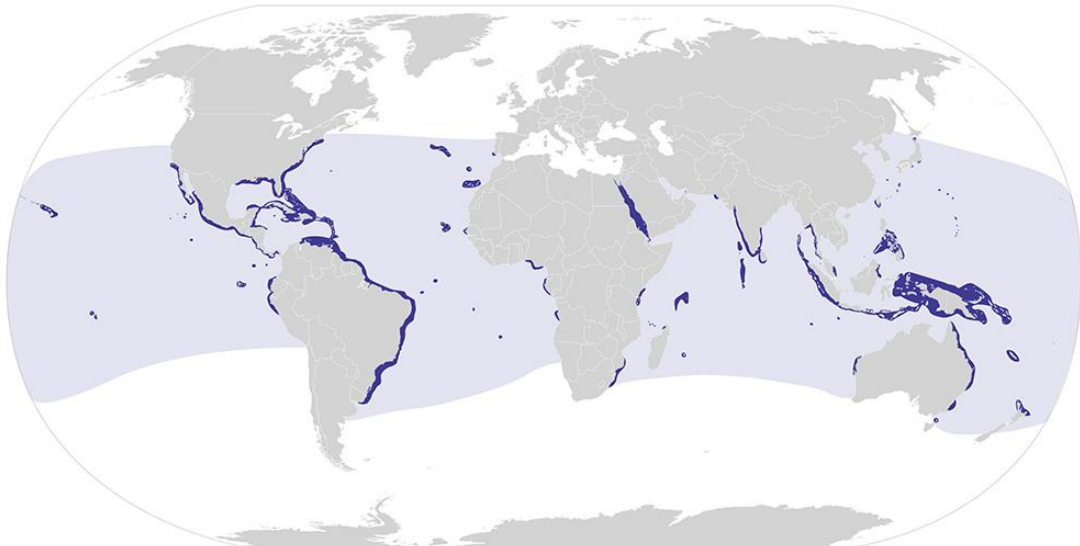
Finally, my research reinforces the benefits of using photo-ID studies to compile a database on a population of wild marine megafauna which can be used to gather detailed life history information which traditionally has been acquired from fisheries data. To gain further benefit from my dataset, it needs to be continually built upon so that estimates of age at maturity (especially for females), longevity, and fecundity can continue to be refined, and other avenues of research pursued. Future studies should also incorporate more accurate methods, such as stereo-video photogrammetry, to calculate accurate growth rates for both manta species. Further research into the more elusive and less studied *M. birostris* population in the Maldives should also be undertaken in the southern Maldivian atolls, where a number of potentially important aggregation sites for them were documented towards the end of this study.

Appendices

Appendix I: Manta ray global distribution maps (Source: Manta Trust, 2016)



Distribution of the reef manta ray (*Manta alfredi*) throughout the Indo-West Pacific within 32° of latitude north and south. Dark green = confirmed distribution, light green = expected range.



Distribution of the oceanic manta ray (*Manta birostris*) throughout the throughout the tropical to temperate oceans within 41° of latitude north and south. Dark blue = confirmed distribution, light blue = expected range.

Appendix II: Manta ray aggregation sites in the Maldives

| Atoll no. | Atoll | Site name | Primary site function | Manta species | Predominant demographic | Site location | Site seasonality |
|-----------|------------|---------------------|-----------------------|----------------------|-------------------------|---------------|------------------|
| 1 | I-hippo | Huvahandhoo Corner | Cleaning | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 1 | I-hippo | Ihavandhoo | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 1 | I-hippo | Manafaru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 1 | I-hippo | Vagaaru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 2 | Haa Alifu | Alidhoo Finolhu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 2 | Haa Alifu | Baarah Thila | Cleaning | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 2 | Haa Alifu | Dhonakulhi Kandu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 2 | Haa Alifu | Dhonakulhi North | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 2 | Haa Alifu | Hanimaadhoo Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 2 | Haa Alifu | Le Bocal | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 2 | Haa Alifu | Mulidhoo Aquarium | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 2 | Haa Dhaalu | Bodu Thila Haa | Cleaning | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 2 | Haa Dhaalu | Maavaidhoo Thila | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 2 | Noonu | Christmas Rock | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Noonu | Fodhdhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 2 | Noonu | Huivani Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 2 | Noonu | Manadhoo Corner | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 2 | Noonu | Minaavaru Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Noonu | Orimas Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Noonu | Orivaru Giri | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Noonu | Randheli Falhu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Noonu | Snow White | Cleaning | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 2 | Shaviyani | Delidhoo | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 2 | Shaviyani | Dhikomandhoo Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 2 | Shaviyani | Ekasdhoo Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 2 | Shaviyani | Eriyadhoo Faru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 2 | Shaviyani | Firubaidhoo Thila | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 2 | Shaviyani | Gaakoshinbi | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 2 | Shaviyani | Kabaalifaru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 2 | Shaviyani | Kuda Lhaimendhoo | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 2 | Shaviyani | Miyaru Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 5 | Raa | Beriyani Giri | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 5 | Raa | Boomerang Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 5 | Raa | Fainu Beryu | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 5 | Raa | Fuggiri | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 5 | Raa | Kadoogadu Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 5 | Raa | Kinolhas Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 5 | Raa | Kottefaru Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 5 | Raa | Kottefaru Thila | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 5 | Raa | Kotti Kanduolhi | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 5 | Raa | Kukuludhoo Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 5 | Raa | Kuroshigiri Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 5 | Raa | Maa Faru Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | NE monsoon |
| 5 | Raa | Maafaru Beyru | Feeding | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 5 | Raa | Maamunagau Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 5 | Raa | Meedhupparu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 5 | Raa | Neyo Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 5 | Raa | Sola Corner | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 5 | Raa | Vandhoo Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 6 | Fasdhu | Bathalaa Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 6 | Fasdhu | Bathalaa Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | SW monsoon |
| 6 | Fasdhu | Vinaneiy Finolhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 6 | Fasdhu | Voavah Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 7 | Baa | Ahivahfushi Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 7 | Baa | Aidhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 7 | Baa | Andhagiri | Feeding | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Anga Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Bodu Thila | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 7 | Baa | Dhandhoo Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 7 | Baa | Dharavandhoo Corner | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 7 | Baa | Dharavandhoo Thila | Cleaning | <i>Ma & Mb</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Dhigu Thila | Cleaning | <i>Ma & Mb</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Dhonfanu Faru | Cleaning | <i>Ma & Mb</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Dhonfanu Thila | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Dhunikulhu Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Dhunikulhu Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | SW monsoon |
| 7 | Baa | Donut Falhu | Cleaning | <i>Manta alfredi</i> | Adults | Lagoonal Reef | SW monsoon |
| 7 | Baa | Eboodhoo Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 7 | Baa | Finolhoss Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Funadhoo Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Hanifaru Bay | Feeding | <i>Ma & Mb</i> | Adults | Channel Reef | SW monsoon |

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|----|-----------|------------------------|----------|------------------------|-----------|---------------|------------|
| 7 | Baa | Hanifaru Beyru | Feeding | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 7 | Baa | Hanifaru Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Hanifaru Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 7 | Baa | Horubadhoo Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 7 | Baa | Hurai Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 7 | Baa | Hurai Thila | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 7 | Baa | Kamadhoo Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 7 | Baa | Kihaadhuffaru Thila | Cruising | <i>Ma & Mb</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Kudadhoo Corner | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 7 | Baa | Kudadhoo Faru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 7 | Baa | Landaa Giraavaru Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 7 | Baa | Maa Faru Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 7 | Baa | Maamaduvvari West | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 7 | Baa | Maaneigaa | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Maarogaali | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Maavaru Beyru | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 7 | Baa | Maavaru Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | NE monsoon |
| 7 | Baa | Miriandhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 7 | Baa | Muthaafushi Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 7 | Baa | Muthaafushi Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 7 | Baa | Nelivaru Thila | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 7 | Baa | Reethi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Thulhaadhoo Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 7 | Baa | Vandhoomaa Faru | Feeding | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 7 | Baa | Veyofushi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Veyofushi Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 7 | Baa | Wellafaru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 8 | Goidhu | Doru Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 8 | Goidhu | Innafushi | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Aliha Giri | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Anemone Thila | Feeding | <i>Manta alfredi</i> | Adults | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Dhanifaru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Dhidhdhoo Beyru | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | SW monsoon |
| 9 | Lhaviyani | Faadhoo Falhu | Cleaning | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 9 | Lhaviyani | Felivaru Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 9 | Lhaviyani | Felivaru Thila | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Fushifaru Kandu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 9 | Lhaviyani | Hinnavaru Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 9 | Lhaviyani | Huravalhi Finolhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Kanifushi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Kanuhuraa Corner | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 9 | Lhaviyani | Kudadhoo Kandu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 9 | Lhaviyani | Kuredu Caves / Express | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | SW monsoon |
| 9 | Lhaviyani | Kuredu Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 9 | Lhaviyani | Madivaru Kandu/Thila | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | NE monsoon |
| 9 | Lhaviyani | Mashura Beyru | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 9 | Lhaviyani | Mashura Etere | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 9 | Lhaviyani | Olhukolhu Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | NE monsoon |
| 9 | Lhaviyani | Vavvaru Beryu | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 9 | Lhaviyani | Veligadu Falhu | Feeding | <i>Manta alfredi</i> | Adults | Inner Reef | NE monsoon |
| 9 | Lhaviyani | Veyvah Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 12 | N-Malé | Boduhithi Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 12 | N-Malé | Bodufinolhu Beyru | Cruising | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 12 | N-Malé | Boduhithi Thila | Cleaning | <i>Ma & Mb</i> | Adults | Channel Reef | NE monsoon |
| 12 | N-Malé | Dhiya Adi Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 12 | N-Malé | Dhiya Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 12 | N-Malé | Dhonveli Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 12 | N-Malé | Furana Thila | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 12 | N-Malé | Gaamadhoo | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 12 | N-Malé | Gasfinolhu Beyru | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | SW monsoon |
| 12 | N-Malé | Helengeli Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 12 | N-Malé | HP Thila | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 12 | N-Malé | Hudhuveli Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 12 | N-Malé | Kani Corner | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | SW monsoon |
| 12 | N-Malé | Kuda Huraa Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 12 | N-Malé | Lankan Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 12 | N-Malé | Lhofifushi Aquarium | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | SW monsoon |
| 12 | N-Malé | Madi Thila | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 12 | N-Malé | Madivaru Beyru | Cruising | <i>Ma & Mb</i> | N/A | Outer Reef | NE monsoon |
| 12 | N-Malé | Madivaru Corner | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 12 | N-Malé | Maska Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 12 | N-Malé | Nassimo Thila | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 12 | N-Malé | Olhahali Falhu | Cruising | <i>Manta alfredi</i> | N/A | Lagoonal Reef | SW monsoon |
| 12 | N-Malé | Rasfari North | Cleaning | <i>Ma & Mb</i> | Adults | Outer Reef | NE monsoon |
| 12 | N-Malé | Rasfari South | Cleaning | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 12 | N-Malé | Sunlight Faru | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 12 | N-Malé | Thulaagiri Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 12 | N-Malé | Thulusdhoo Beyru | Cleaning | <i>Ma & Mb</i> | Adults | Outer Reef | SW monsoon |

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|----|---------|-----------------------|----------|------------------------|-----------|---------------|------------|
| 13 | S-Malé | Embudu Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 13 | S-Malé | Guraidhoo Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 13 | S-Malé | Guraidhoo Kandu | Cruising | <i>Ma & Mb</i> | N/A | Channel Reef | SW monsoon |
| 13 | S-Malé | Kandhooma Thila | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 13 | S-Malé | Olhuvelli Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 13 | S-Malé | Ranikan Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 13 | S-Malé | Rannalhi Falhu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 13 | S-Malé | Vadoo Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 14 | Thoddu | Twin Towers | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 15 | Rasdhu | Kuramathi Beyru | Feeding | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 15 | Rasdhu | Rasdho Madivaru | Feeding | <i>Ma & Mb</i> | Adults | Channel Reef | NE monsoon |
| 15 | Rasdhu | Veligandu Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Angothi Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 16 | Ari | Ariyadhoo Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 16 | Ari | Bathalaa Kandu Ari | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Camel Rock | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |
| 16 | Ari | Dhangethi Falhu | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 16 | Ari | Dhiffushi Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 16 | Ari | Dhiggaru Kandu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 16 | Ari | Dhigurah Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 16 | Ari | Dhigurah Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 16 | Ari | Dhigurah Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 16 | Ari | Dhonkalo Thila | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Dhuvan Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 16 | Ari | Emas Thila | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Etheremadivaru | Cleaning | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 16 | Ari | Fenfushi Beyru | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 16 | Ari | Fesdu Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 16 | Ari | Gangehi Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 16 | Ari | Gangehi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 16 | Ari | Gangehi Kandu | Feeding | <i>Manta alfredi</i> | Juveniles | Channel Reef | NE monsoon |
| 16 | Ari | Genburugau Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 16 | Ari | Himendhoo Rock | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Himendhoo Thila | Cleaning | <i>Ma & Mb</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Hukurudhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 16 | Ari | Huravalhi Falhu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 16 | Ari | Huruelhi Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Innafushi Faru | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Kalhahandi Huraa | Cleaning | <i>Ma & Mb</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Kandolhu Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 16 | Ari | Kandolhu Maaha | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 16 | Ari | Kiru Thila | Cleaning | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Maalhoss Thila | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | NE monsoon |
| 16 | Ari | Maamigili Beyru | Feeding | <i>Ma & Mb</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Maavaru Beyru Ari | Feeding | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 16 | Ari | Maavaru Corner | Cleaning | <i>Manta alfredi</i> | Juveniles | Outer Reef | NE monsoon |
| 16 | Ari | Maavaru Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 16 | Ari | Maayafushi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | SW monsoon |
| 16 | Ari | Mathiveri Beyru | Feeding | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Mathiveri Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 16 | Ari | Mathiveri Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | NE monsoon |
| 16 | Ari | Moofushi Corner | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Moofushi Denagili | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Mushimasmingili Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 16 | Ari | Nalaguraidhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | NE monsoon |
| 16 | Ari | Radhdhiggaa Falhu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 16 | Ari | Rangali Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 16 | Ari | Rangali Finolhu | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | NE monsoon |
| 16 | Ari | Rangali Madivaru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 16 | Ari | Ranveli Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 16 | Ari | Thundufushi Falhu | Feeding | <i>Manta alfredi</i> | Juveniles | Lagoonal Reef | NE monsoon |
| 16 | Ari | Thundufushi Thila | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 16 | Ari | Vihamaafaru Falhu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | NE monsoon |
| 16 | Ari | Vihamaafaru Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | NE monsoon |
| 16 | Ari | Vilamendhoo Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 16 | Ari | Vilamendhoo Thila | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 17 | Vaavu | Madhi Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 17 | Vaavu | Medhu Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 17 | Vaavu | Miyaru Kandu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 17 | Vaavu | Uthuru Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 18 | Vattaru | Vattaru Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 19 | Meemu | Kurali Kandu | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 19 | Meemu | Medhufushi Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 19 | Meemu | Muli Kandu | Cleaning | <i>Ma & Mb</i> | Adults | Channel Reef | SW monsoon |
| 19 | Meemu | Vanhuravalhi Kandu | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 20 | Faafu | Cliffhanger | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 20 | Faafu | Filathi Kandu | Cruising | <i>Manta birostris</i> | N/A | Inner Reef | NE monsoon |
| 20 | Faafu | Kuda Falhu | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |

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|----|------------|----------------------|----------|------------------------|-----------|--------------|------------|
| 21 | Dhaalu | Kudahuvadho Corner | Cleaning | <i>Manta alfredi</i> | Juveniles | Outer Reef | SW monsoon |
| 22 | Thaa | Fahala Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 22 | Thaa | Gunnar's Rock | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 22 | Thaa | Guraidhoo Kandu Thaa | Cruising | <i>Manta alfredi</i> | N/A | Channel Reef | SW monsoon |
| 22 | Thaa | Maalifushi Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | SW monsoon |
| 22 | Thaa | Madifushi Faru | Feeding | <i>Manta alfredi</i> | Juveniles | Inner Reef | SW monsoon |
| 22 | Thaa | Mathidhoo Beyru | Cruising | <i>Manta alfredi</i> | N/A | Outer Reef | SW monsoon |
| 23 | Laamu | Fushi Kandu | Feeding | <i>Manta alfredi</i> | Adults | Channel Reef | SW monsoon |
| 23 | Laamu | Hithadhoo Corner | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | SW monsoon |
| 23 | Laamu | Isdhoo Point | Cleaning | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 23 | Laamu | Munnafushi Kandu | Cleaning | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 24 | Gaafu | Falhuma Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 24 | Gaafu | Hahahaa Faru | Cruising | <i>Manta alfredi</i> | N/A | Inner Reef | NE monsoon |
| 24 | Gaafu | Kuredhoo Kandu | Cruising | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 24 | Gaafu | Nilandhoo Kandu | Cruising | <i>Manta birostris</i> | N/A | Outer Reef | NE monsoon |
| 25 | Fuvahmulah | North Point | Cruising | <i>Manta birostris</i> | Adults | Outer Reef | NE monsoon |
| 25 | Fuvahmulah | Southern Spur | Cruising | <i>Manta birostris</i> | Adults | Outer Reef | NE monsoon |
| 26 | Addu | Koathey Beyru | Cleaning | <i>Ma & Mb</i> | Adults | Outer Reef | NE monsoon |
| 26 | Addu | Maradhoo Beyru | Cleaning | <i>Manta alfredi</i> | Adults | Outer Reef | NE monsoon |
| 26 | Addu | Meedhoo Beyru | Cleaning | <i>Manta birostris</i> | Adults | Outer Reef | NE monsoon |
| 26 | Addu | Mudakan | Cleaning | <i>Ma & Mb</i> | Adults | Channel Reef | NE monsoon |
| 26 | Addu | Mulikolhu Faru | Cleaning | <i>Manta alfredi</i> | Adults | Inner Reef | SW monsoon |

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