Department of Environment and Geography

University of York

Dissertation Submission Cover Sheet

This cover sheet should be the first page of your Electronic Submission

Student Name: Elspeth Strike	**Module Code:** ENV00031M
Module Title: Summer Placement Dissertation	**Assessment Deadline: 25/09/2020**

I confirm that I have

- **conformed with University regulations on academic integrity**
- **please insert word count** 4,993
- **checked that I am submitting the correct and final version of my coursework**
- **saved my assessment in the correct format**
- **formatted my assessment in line with departmental guidelines**
    - I have converted my assessment to a pdf file and ensured the compatibility of any graphs or charts included in my submission
    - I have used 12pt font (preferably Arial or similar)
    - I have ensured all pages are clearly numbered using the system 1 of 6, 2 of 6 etc. Cover sheet, Template and Declaration are not included in the page numbering. Page 1 will be your abstract.

PLEASE TICK BOX TO CONFIRM

☑

Please note: if you have any questions please refer to the FAQs available on the VLE (Board of Studies community site).

Variations in sub-lethal injuries to manta rays in the Maldives

---

Elspeth Strike


Credit: Manta Trust

Summer Placement Dissertation is submitted in part fulfilment of the MSc in Marine Environmental Management, University of York

Exam Number: Y3876012

Supervised by Dr Guy Stevens and Dr Julie Hawkins

Department of Environment and Geography, University of York

25th September 2020

Word Count: 4,993

Declaration

I, Elspeth Strike, declare that the work submitted in this dissertation is the result of my own work and investigation and all the sources I have used have been indicated by means of completed references.

Signed:

Date: 25th September 2020

Acknowledgements

My first thanks go to Guy Stevens of the Manta Trust, for his guidance and expertise throughout this project, and particularly for his help with my analyses. Secondly, I would like to thank Julie Hawkins for her continued support, advice and feedback, which, as always, has been invaluable. I am extremely grateful for the skills and knowledge I have gained from working with you both on this project.

Thank you is extended to the Manta Trust and the Maldivian Manta Ray Project for providing me with data and images, and to Kirsty Ballard for her help with the categorisation of wounds and the dating of each injury event.

Finally, my biggest thank you goes to my family, friends and partner, Jasper, for always showing belief in me, and for listening to me talk about manta rays all day, every day.

Table of Contents

Abstract

Sub-lethal injuries to manta rays have rarely been studied in detail, yet populations have become increasingly affected by human activities. Maldivian manta rays are not directly exploited by fisheries but are known to sustain injuries from entanglement in fishing lines and boat strikes. To examine the frequency of sub-lethal injury events affecting *Mobula alfredi* and *Mobula birostris* from anthropogenic and natural origins, I analysed data from the Manta Trust's Maldivian Manta Ray Project (MMRP) database which contains almost 65,000 photo-identification sightings of the two species from 1987-2020. From this I calculated the rate of injury accumulation in *M. alfredi* and how injury event frequencies and origins varied for this species and *M. birostris.* Significantly more *M. alfredi* individuals were injured than *M. birostris*, with 26% of the population affected as opposed to 15% (χ^2 (1) = 41.9, p < .001). In both species, injuries were more likely to have been caused by predation than anthropogenic impacts. Although adult *M. alfredi* sustained more injury events than young rays (χ^2 (1) = 30.2, p < .001), the latter accumulated them at a higher rate (W = 12082, p < .001, r = -0.25). Atolls with the highest number of anthropogenic injury events per sighted *M. alfredi* were North Malé, Laamu, Addu, Baa and South Malé. Overall, this work will contribute to a growing understanding of the threats faced by manta rays in the Maldives, which will, in turn, highlight areas where management should be improved to protect them.

1. Introduction

Reef and Oceanic manta rays (*Mobula alfredi and Mobula birostris*), of the Mobulidae family (mobulids), are two of the ocean's most charismatic species (White et al., 2018). Populations of *M. alfredi* are widely distributed throughout the tropical and sub-tropical waters of the Indo-Pacific and Indian Oceans, while *M. birostris* occurs in temperate waters as well as the tropics (Kashiwagi et al., 2011; Couturier et al., 2012). *M. alfredi* mainly frequent the shallow, coastal reef habitats of islands and atolls; whereas *M. birostris* spend more of their time offshore and seasonally visit shallower coastal areas (Kashiwagi et al., 2011; Stewart et al., 2016a). Both species demonstrate long-term habitat fidelity within a home-range, and undergo seasonal aggregation (Dewar et al., 2008; Stewart et al., 2016b; Couturier et al., 2018; Harris et al., 2020).

As large-bodied, slow growing, late maturing animals, manta rays are among the least fecund of all vertebrates (Dulvy et al., 2014; Stevens, 2016). These life history traits create vulnerability at the population level, particularly in areas where manta numbers are small and fragmented (Kashiwagi et al., 2011; Dulvy et al., 2014). The predominant threat to manta rays worldwide is overexploitation by directed fisheries, which have been driven by the high demand for mobulid gill plates in traditional Asian markets (Ward-Paige et al., 2013; Croll et al., 2016; Lawson et al., 2017; O'Malley et al., 2017). As a result, *M. alfredi* and *M. birostris* are currently listed as 'vulnerable to extinction' on the IUCN Red List of Threatened Species (Marshall et al., 2018; Marshall et al., 2019). To address the threat of the gill plate trade, in 2013, both species were listed on Appendix II of the Convention on International Trade in Endangered Species (Lawson et al., 2017). However, incidental bycatch of manta rays in small-and large-scale fisheries remains a persistent threat (Croll et al., 2016), while climate change and reef degradation impact their food supply and suitable habitat (Richardson, 2008; Stevens and Froman, 2019).

The 26 coral atolls which form the Maldives archipelago in the Indian Ocean (**Figure 1**) support the largest known population of *M. alfredi* (Kitchen-Wheeler et al., 2012; Stevens, 2016). *M. birostris* are sighted less frequently there and are known to occur close to deeper water along outer atoll edges and around seamounts (Kashiwagi et al., 2011; Stevens, 2016). *M. alfredi* predictably migrate across the archipelago following areas of enhanced zooplankton availability which are driven by the bi-annual southwest and northeast monsoons (Anderson et al., 2011b; Harris et al., 2020). Aggregations of *M. alfredi* occur in places were food becomes seasonally abundant (Armstrong et al., 2016; Harris et al., 2020), and the animals collectively use cleaning stations for parasite removal (Stevens, 2016), and engage in courtship and mating activity there (Stevens et al., 2018a).

Manta rays are an economically important species in the Maldives, and, in 2011, it was estimated that manta ray diving and snorkelling trips there contributed ~US$8.1 million per year in tourist expenditure (Anderson et al., 2011a; Murray et al., 2020). However, increasing pressures from tourism activities can also result in habitat degradation, boat traffic injuries and can disrupt manta ray behaviour (Anderson et al., 2011a; Venables, 2013; Murray et al., 2020).

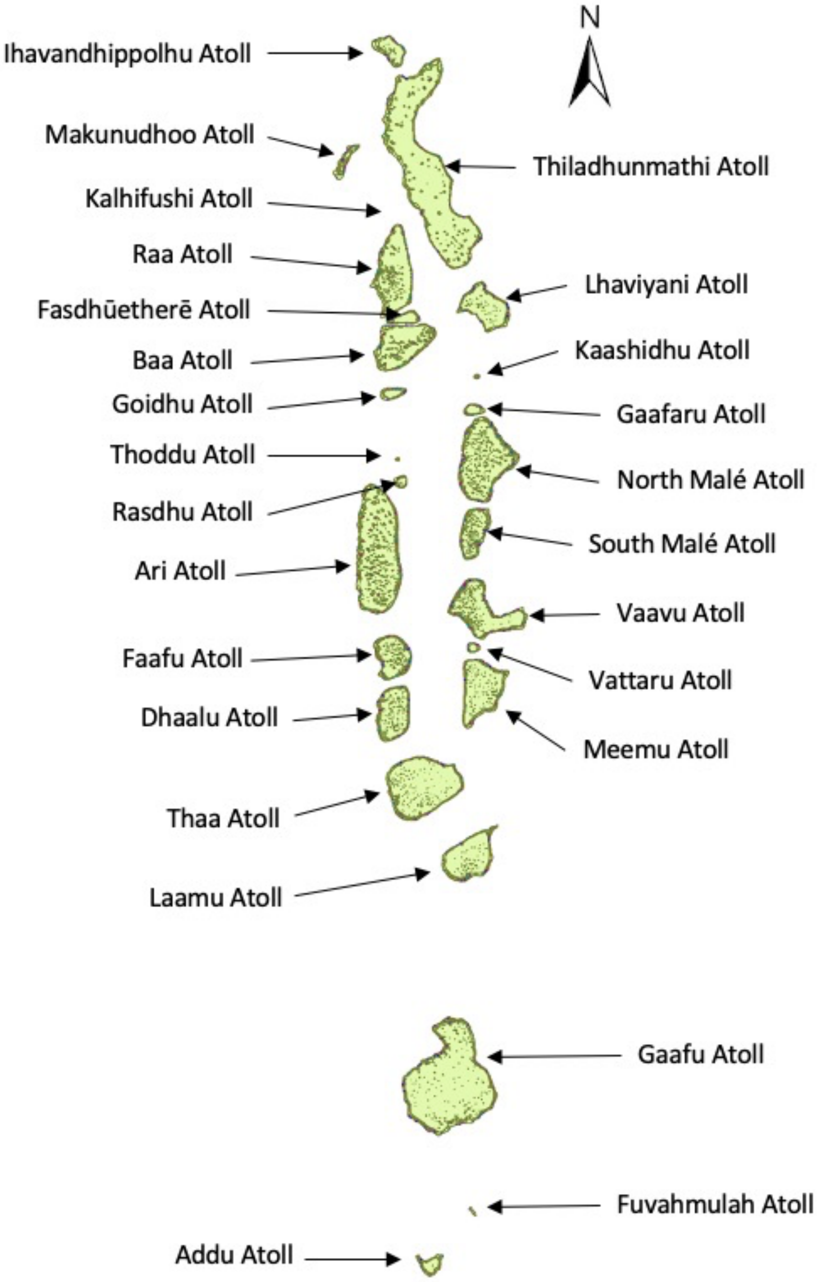

Figure 1. Map of the Maldives archipelago showing the 26 geographical atolls in green. Image adapted from the original shapefile which was sourced from Revolutionary GIS (2014).

Maldivian manta rays represent an isolated and historically unfished population, and, in 2014, the Maldives declared all species of ray protected (Stevens, 2014; Stevens, 2016). Nevertheless, these animals face threat from a number of natural and anthropogenic issues (Couturier et al., 2012). Unsuccessful predation attempts by large sharks (e.g. Tiger

Galeocerdo cuvier and Bull *Carcharhinus leucas*) and some cetaceans can leave manta rays with permanent injuries ranging from quickly-healing flesh wounds and tissue loss, to severe bites which truncate or disfigure the pectoral fins (Marshall and Bennett, 2010; Stevens et al., 2018b). Entanglement in lost or discarded fishing line, nets and mooring ropes presents an indirect threat (Couturier et al., 2012; Carpentier et al., 2019). For example, if monofilament fishing line gets wrapped around a manta's cephalic fins, it can cause cuts, reduce their functioning, or amputate them (Deakos et al., 2011). Manta rays are also susceptible to boat strikes in the surface waters, through which propellers can cause severe injuries (McGregor et al., 2019).

Sub-lethal injuries from predation, entanglement or vessel strikes affect manta rays across the world, and do so in the form of missing tissue, scars, and disfigurements (Stevens et al., 2018b). The fitness cost of these injuries to individuals, and populations, is currently unclear and these impacts have been identified as an important knowledge gap in mobulid research (Couturier et al., 2012; Stewart et al., 2018). Researchers have investigated sub-lethal injuries observed in *M. alfredi* in Mozambique, Hawaii, French Polynesia and Australia, but no published work has examined their occurrence in *M. birostris* (Marshall and Bennett, 2010; Deakos et al., 2011; Carpentier et al., 2019; McGregor et al., 2019). Previous studies have analysed only a single injury origin in detail, for example predatory bites (Marshall and Bennett, 2010), or provided only a limited quantification of injuries observed (e.g. Deakos et al., 2011). Moreover, temporal and spatial trends in the frequency and origins of sub-lethal injuries have rarely been examined.

In 2015, Stark (unpublished) investigated injury occurrence in *M. alfredi* and *M. birostris* in the Maldives, reporting that natural impacts (e.g. predation) posed a greater threat to the population than anthropogenic. Using more recent data with a larger identified population, this project will update and develop this work to provide a more comprehensive analysis of the sub-lethal impacts affecting Maldivian manta rays. In doing so, it will describe the frequency of injuries observed in *M. alfredi* and *M. birostris* and the predominant causes of these. My work will assist conservation researchers to understand how manta rays are affected by human activities in the Maldives. This knowledge will highlight areas where management should be improved to protect these animals.

Key questions I examine in this study are: (1) does injury event frequency or origin vary between manta ray sexes, maturity statuses, or species; (2) at what rate do *M. alfredi* accumulate injuries, and do females or adults incur different injury rates to males and young rays; (3) are anthropogenic injuries becoming more prevalent over time in *M. alfredi*; (4) at which atolls in the Maldives are *M. alfredi* more likely to be injured, and by which causes?

2. Methods

The Manta Trust's Maldivian Manta Ray Project (MMRP) monitors sightings of *M. alfredi* and *M. birostris* using photographic-identification and *IDtheManta* software (see Stark, 2015; Stevens, 2016). Photo-ID is widely used as a non-intrusive method for studying elasmobranchs, which enables individuals to be reliably distinguished then easily re-identified over time (Marshall and Pierce, 2012). This works for manta rays because they have unique ventral spot patterns which remain unchanged (Kitchen-Wheeler, 2010; Marshall et al., 2011; Stevens, 2016).

From January 2005 to December 2018, and intermittently between 1987 and 2004, the MMRP has logged 64,011 photo-ID sightings of 4,662 individual *M. alfredi* at 21 Maldivian atolls. For *M. birostris,* 778 sightings of 714 individuals were logged between January 1996 and April 2020 at 14 atolls. The MMRP's database also records an individual's: sex, size, state of maturity, age class, whether it has any visible injuries and if so where, and which atolls the animal has been sighted at. Manta rays of unknown sex and maturity were excluded from all analyses, and subadult and juvenile mantas were combined into a single maturity status of 'young rays'.

2.1 Injury Identification

Once healed, sub-lethal wounds on manta rays can leave permanent scars, disfigurements and missing tissue that remain for the rest of an animal's life (**Figure 2; Figure 3**) (Marshall and Bennett, 2010). As the focus of this study is on permanent injury, small cuts and events like the presence of a lightly embedded fishing hook were excluded from analyses. Injury events were categorised according to their origin which was determined by the characteristics and placement of the wound(s) on a manta ray's body (**Table 1; Figure 2; Figure 3**). The categories were classified as either natural or anthropogenic and when the specific origin of a wound could not be determined, for example if tissue regeneration masked the injury characteristics used for categorisation, the injury event was recorded as "unknown" and excluded from analyses (Stark, 2015). When multiple scars clearly originated from a single injury event, this was given a score of "one" on the database. For example, if a piece of monofilament line had damaged both the cephalic fins and gills, then this would be treated as a single injury event.

Before analysis, I first reviewed the photo-ID images of every injured manta ray in the MMRP's database to ensure that injury event frequencies, origin categories and wound location(s) on the body had been recorded correctly. I identified the date a wound on a manta ray first became evident in the photographs and, by entering the injury event into the relevant sighting log in the database, I could then see which atoll the wound was first observed at.

Table 1. Description of the anthropogenic and natural injury event categories used in the analysis with the type of wound characteristics used to determine them.

Classification	Injury Event Category	Characteristics
Anthropogenic	Boat Strike	Lacerations on the dorsal surface, often in distinctive parallel lines from a propeller; tissue missing from trailing edge of pectoral fins with angular straight edges
	Fishing Line / Hook	Straight-edged cuts or slices to cephalic fins or leading/ trailing edge of pectoral fins; thin line scars on ventral or dorsal surface or around mouth from entanglement; damage from embedded hook such as scarring to gill slits; deformed or amputated cephalic fins with evidence of line damage
	Net Entanglement	Straight-edged cuts or slices in a distinctive regular pattern, usually to leading/ trailing edge of pectoral fins
	Rope Entanglement	Thicker cuts or slices than fishing line where entangled rope has cut into flesh, e.g. around cephalic fins
Natural	Predatory Bite	Distinctive semi-circular bite marks or portions of missing tissue, most commonly on trailing edge of pectoral fins; truncated pectoral fins; healed bites with semi-circular scar tissue
	Deformity	Natural deformities such as a bent, wavy or marked tail
	Infection / Disease / Parasite	Inflammation, lesion or scarring on gill slits from infection or remora living inside gill cover; fleshy tumorous growth on manta ray's body

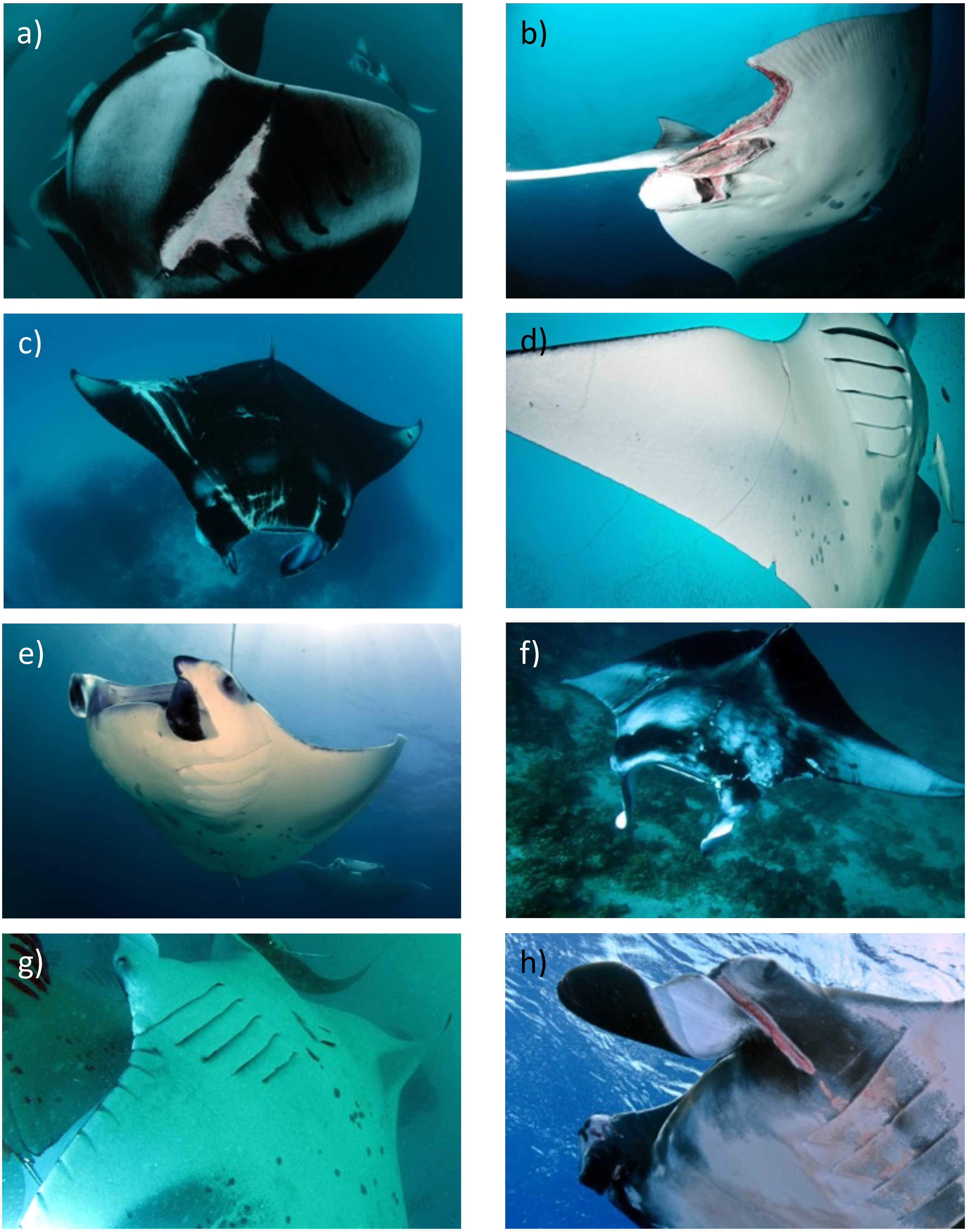

Figure 2. Examples of anthropogenic originated injuries affecting *Mobula alfredi* and *Mobula birostris* in the Maldives. **a)** fresh dorsal lacerations from a boat propeller; **b)** missing tissue with angular straight edges, characteristic of vessel strike; **c)** scarring to dorsal surface, mouth and right pectoral fin from entanglement in fishing line; **d)** straight-edged slices on leading and trailing edge of right pectoral fin from embedded fishing line; **e)** partially severed left cephalic fin from fishing line entanglement; **f)** dorsal scarring from entanglement in drift net; **g)** distinctive regular slices to leading edge of right pectoral fin from fishing net; **h)** amputated right cephalic fin and laceration on left cephalic from rope entanglement. Images courtesy of the Manta Trust.

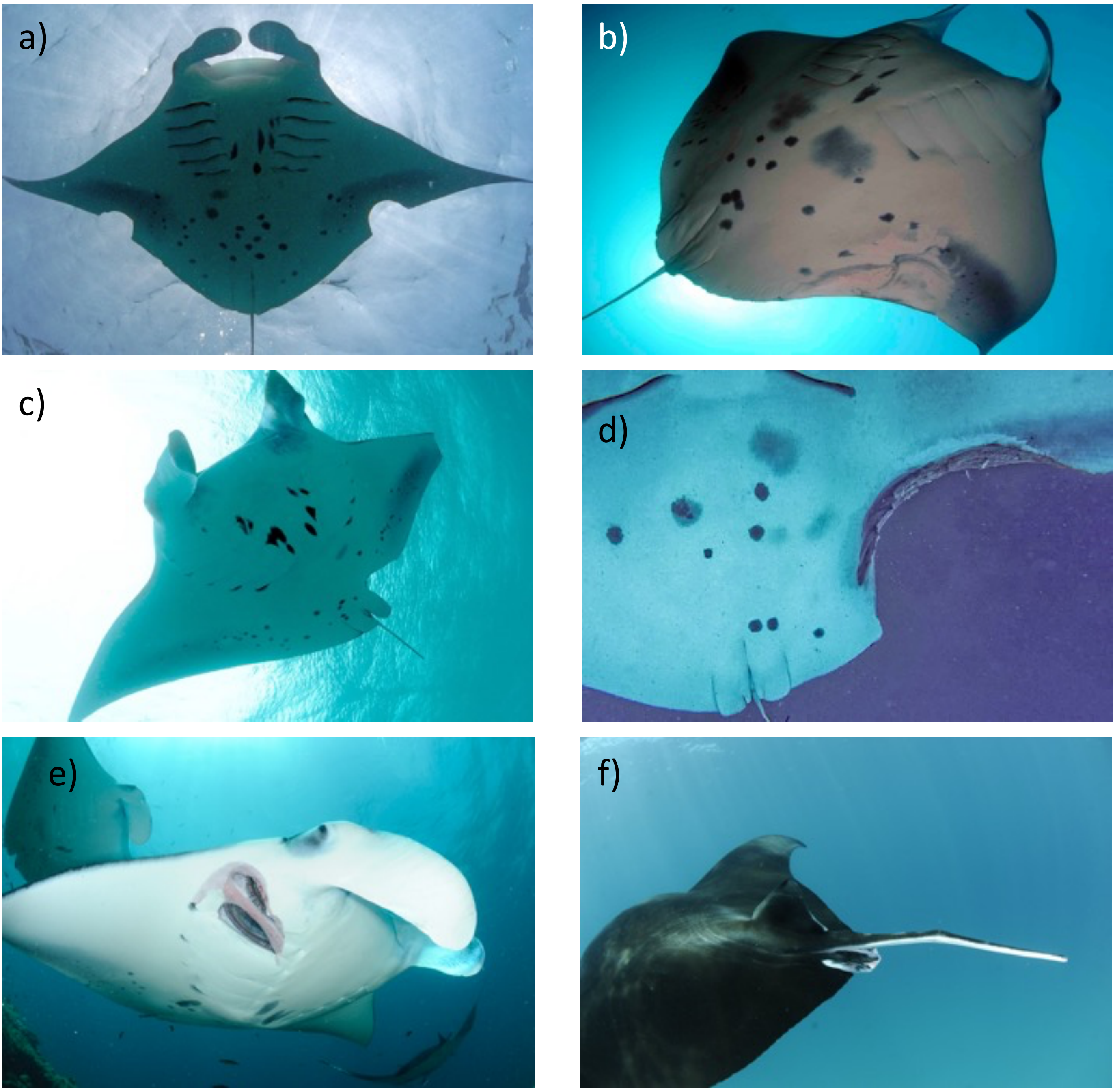

Figure 3. Examples of natural originated injuries affecting *Mobula alfredi* and *Mobula birostris* in the Maldives. **a)** predatory bites resulting in characteristic semi-circular portions of missing tissue on trailing edge of both pectoral fins; **b)** healed predatory bite with distinctive semi-circular scar tissue; **c)** truncated left pectoral fin from predatory bite; **d)** fresh bite wound, red and unhealed; **e)** red, inflamed and damaged first and second right gill slits from infection; **f)** natural deformity resulting in bent tail. Images courtesy of the Manta Trust.

2.2 Injury Rates

The rate at which manta rays accumulated injuries in the Maldives was only calculated for *M. alfredi* because most *M. birostris* individuals were only sighted once. If an animal had an existing injury at its first sighting, this was not included in the analysis as it was not possible to tell when these had occurred. This meant injuries to manta rays which were only sighted once were also excluded. To calculate injury rates for the remaining 445 individuals, I divided the number of injury events an individual had sustained after its initial sighting, by the number of years in which a sighting of that manta had occurred. This accounted for the fact that not all manta rays were seen every year. Multiple sightings of an individual during the same year were not counted.

2.3 Spatial and Temporal Trends

Spatial trends in *M. alfredi* injury event frequencies (n=1353) between 1987-2018 were investigated by comparing the number of injury events recorded at each of the 21 Maldivian atolls where mantas were sighted. To account for higher numbers of animals in certain atolls, I divided the total number of injury events at each atoll by the number of individual manta rays sighted there. When investigating temporal trends in injury event frequencies (n=505), existing injury events recorded on initial sightings were excluded as the specific year these events occurred could not be determined. The number of injury events that occurred subsequent to first sightings was calculated for each year from 2004-2018. To account for higher sampling efforts in later years, each total was divided by the number of individual manta rays sighted within that year. For spatial and temporal analyses, injury event frequencies were split by origin category to investigate trends in each type of impact.

2.4 Statistical Analyses

After collating the number and origin of injury events to Maldivian manta rays, Pearson's chi-square (χ^2) tests were used to determine whether the proportion of injured manta rays differed between species, sex and maturity status (Field, 2012). While most injured manta rays had just one injury event recorded, some had two or more from a variety of impacts. The proportions of individuals with anthropogenic injuries and those with natural injuries could not be compared as some mantas had injuries from both origins. To account for this, the frequencies of injury events were used for further analyses, rather than the number of individuals. Moreover, chi-square (χ^2) goodness-of-fit tests were used to compare the proportions of injury events affecting each sex and maturity status. Expected values were calculated from the total population ratios (e.g. adults:young) to ensure the population bias towards adults did not affect the test. Additionally, chi-square tests of independence (χ^2) were used to examine whether injury event origins (natural or anthropogenic) were associated with sex, maturity and species. For analyses where one or more expected values were less than 5, a Fisher's Exact test was conducted (Dytham, 2011). Finally, *M. alfredi* injury rates were compared between sexes and maturity statuses. This was done using Wilcoxon rank-sum (W) tests as the data could not be normalised (Field, 2012). Significance was accepted at $p < 0.05$ and statistical analyses were completed using R (Version 3.6.1).

3. Results

The sex ratio for the Maldivian population of *M. alfredi* was 49.6% (n=2312) female and 49.7% (n=2316) male, with identification not possible for 0.7% (n=34) of animals in the database (**Table 2**). Of the 4628 individuals of known sex, 63% (n=2928) were adults, of which 36%

(n=1053) were female and 64% (n=1875) male. 1700 (37%) rays were young, of which 74% (n=1259) were female and 26% (n=441) male. For *M. birostris*, the sex ratio was 43% (n=304) female and 51% (n=363) male, whilst the sex of 7% (n=47) of animals could not be identified (**Table 3**). Of the 667 individuals of known sex, 82% (n=546) were adults, of which 38% (n=210) were female and 62% (n=336) male. 117 (18%) individuals were young, of which 78% (n=91) were female and 22% (n=26) male. The maturity status of <1% (n=4) of these animals could not be determined.

Table 2. Description of the distribution of sex and maturity status recorded for *Mobula alfredi* in the Maldives, based on information in the Maldivian Manta Ray Project's photographic identification database.

Sex	Maturity Status	No. Individuals	Percentage
Female	Adult	1053	22.6%
	Young	1259	27.0%
	Sub-total	2312	49.6%
Male	Adult	1875	40.2%
	Young	441	9.5%
	Sub-total	2316	49.7%
Unknown	Young	16	0.3%
	Unknown	18	0.4%
	Subtotal	34	0.7%
	Grand Total	4662	100.0%

Table 3. Description of the distribution of sex and maturity status recorded for *Mobula birostris* in the Maldives, based on information in the Maldivian Manta Ray Project's photographic identification database.

Sex	Maturity Status	No. Individuals	Percentage
Female	Adult	210	29.40%
	Young	91	12.70%
	Unknown	3	0.40%
	Sub-total	304	42.60%
Male	Adult	336	47.10%
	Young	26	3.60%
	Unknown	1	0.10%
	Sub-total	363	50.80%
Unknown	Adult	19	2.70%
	Young	5	0.70%
	Unknown	23	3.20%
	Sub-total	47	6.60%
	Grand Total	714	100.00%

3.1. Injury profile for *Mobula alfredi*

Of the 4628 *Mobula alfredi* of known sex, 1368 (30%) individuals had permanent scars from one or more sub-lethal injury events. In total these came from 1553 injury events, of which 200 (13%) were described as "type unknown" and excluded from analyses. Accordingly, 1353 classified injury events were examined for 1206 individuals which equates to 26% of the Maldivian population. Of these injured animals, 53% (n=640) were female and 47% (n=566) male, and, when grouped by maturity status, 69% (n=835) were adults and 31% (n=371) were young rays. Significantly more females (28%) were injured than males (24%) (χ^2 (1) = 6.3, p = .01) and significantly more adults (29%) than young rays (22%) (χ^2 (1) = 25.01, p < .001).

The number of injury events per injured individual ranged from one to five, with a mean of 1.12 (SD 0.36), while this figure for the population as a whole was 0.29 (SD 0.53). 11% (n=136) of injured rays had two or more injury events recorded in the database. Females were affected by significantly more injury events than males (χ^2 (1) = 9.1, p = .003), with the figure being 54% (n=732) compared to 46% (n=621). Adults had significantly more injury events than young rays (χ^2 (1) = 30.2, p < .001), sustaining 70% (n=950) and 30% (n=403) of events respectively.

Sub-lethal injuries affecting *M. alfredi* were significantly more likely to be from natural causes than anthropogenic (χ^2 (1) = 94.2, p < .001). Of the 1353 classified injury events, 37% (n=498) were of anthropogenic origin and 63% (n=855) of natural origin. The most common anthropogenic induced injury originated from entanglement with fishing line or hooks (89%), with boat strikes (9%) and net (2%) and rope (<1%) entanglement accounting for the rest. Among natural injury events, predatory bites (86%) were most common, with natural deformities (e.g. bent tail) accounting for 10% and scars attributable to infection, disease or parasites occurring in 4% of cases.

An animal's sex did not affect the type of impact it suffered (χ^2 (1) = 0.4, *p* = .54), with 36% (n=264) of injury events to females being anthropogenic and 64% (n=468) natural, and 38% (n=234) and 62% (n=387) respectively for males. However, a manta ray's maturity status significantly affected the type of injury event sustained (χ^2 (1) = 6.3, *p* = .01). Adults had more anthropogenic originated injuries than young rays, with the figure being 39% (n=370) compared to 32% (n=128). Incidence of natural injury was higher in young rays than adults, with the figures being 68% (n=275) and 61% (n=580) respectively.

3.2. Injury profile for *Mobula birostris*

Of the 663 *M. birostris* individuals of known sex and maturity, 122 (18%) had permanent injuries. In total, 131 sub-lethal injury events were recorded, of which 30 (23%) were classified as "unknown" and excluded from analyses. Therefore, 101 classified injury events were examined for 96 individuals, which equates to 15% of *M. birostris*. Of these injured animals, 40% (n=38) were female and 60% (n=58) were male, and, when grouped by maturity status, 79% (n=76) were adults and 21% (n=20) were young rays. The proportions of injured females (13%) and males (16%) were not significantly different (χ^2 (1) = 1.5, *p* = .22), and there was no significant difference between the proportions of adults (14%) and young rays (17%) with injuries (χ^2 (1) = 0.8, *p* = .38).

The number of injury events per injured individual was either one or two, with a mean of 1.05 (SD 0.22), and 0.15 (SD 0.38) for the entire population. Only 5% (n=5) of injured rays had two injury events recorded. Injury event frequency was not significantly different between females and males (χ^2 (1) = 1.2, *p* = .28), with 40% (n=40) of injury events affecting females and 60% (n=61) affecting males. The proportions of injury events affecting adults (80%, n=81) and young rays (20%, n=20) were not significantly different (χ^2 (1) = 0.2, *p* = .64).

M. birostris were significantly more likely to be affected by natural injury events than anthropogenic (χ^2 (1) = 10.8, *p* = .001). Of the 101 classified injury events, 34% (n=34) were of anthropogenic origin and 66% (n=67) were of natural origin. The most common anthropogenic injury event was entanglement with fishing line or hooks (91%), whilst rope (6%) and net (3%) entanglement were much less frequent, and no boat strikes were recorded. Predatory bites (86%) were the most common natural injury events, with natural deformities and scars from infection, disease or parasites only occurring in 6% and 1% of cases respectively.

An individual's sex did not affect the type of injury event it sustained (χ^2 (1) = 0.4, *p* = .54), as 30% (n=12) of injury events affecting females were anthropogenic and 70% (n=28) were natural, and 36% (n=22) and 64% (n=39) respectively for males. The type of impact an animal suffered was independent of its maturity status (χ^2 (1) = 0.5, *p* = .50), with 32% (n=26) of injury events to adults being anthropogenic and 68% (n=55) natural, while the figures were 40% (n=8) and 60% (n=12) respectively for young rays.

3.3. Comparison of injury occurrence in *Mobula alfredi* and *Mobula birostris*

Significantly more *M. alfredi* (26%) were injured than *M. birostris* (15%) (χ^2 (1) = 41.9, *p* < .001). An animal's species did not affect the origin of their injuries (natural or anthropogenic) (χ^2 (1) = 0.4, *p* = .53). However, the two species differed significantly in the type of anthropogenic injury events that individuals suffered (Fisher's Exact, *p* = .01), because no boat strikes were recorded for *M. birostris* (**Figure 4**). The type of natural impact sustained was unrelated to species (Fisher's Exact, *p* = .39)

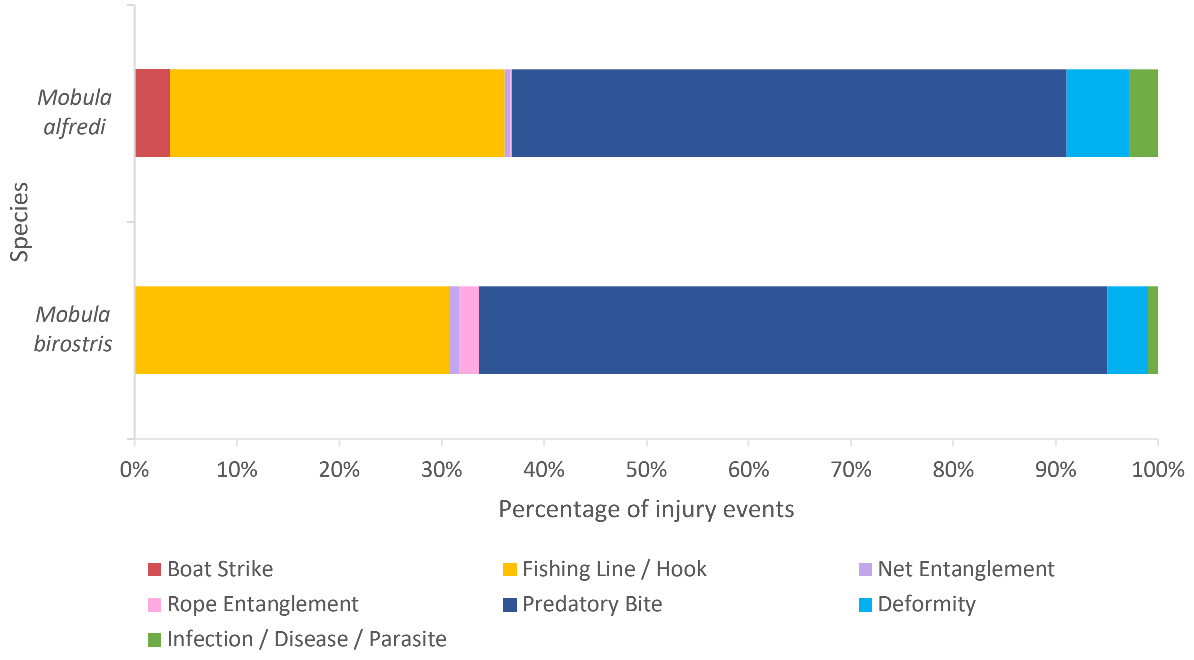

Figure 4. Comparison of the proportions of each injury event origin recorded for *Mobula alfredi* (n=1353) and *Mobula birostris* (n=101) in the Maldives.

3.4. Injury Rates: *Mobula alfredi*

The mean injury rate for *M. alfredi* individuals (n=445) was 0.26 (SD 0.18), and the median was 0.2. Injury rates of female manta rays (*Mdn* = 0.2) did not differ from males (*Mdn* = 0.2) ($W = 24696$, $p = .73$, $r = -0.02$). However, young manta rays had significantly higher injury rates (*Mdn* = 0.25) than adult rays (*Mdn* = 0.17) ($W = 12082$, $p < .001$, $r = -0.25$).

3.5. Trends in *Mobula alfredi* injuries between 2004-2018

Overall, the number of injury events recorded per sighted manta ray increased by a factor of 2.6 between 2004 and 2018, increasing from 0.014 to 0.036 (**Figure 5**). In total, anthropogenic injuries remained fairly constant, but there was a decrease in the number of fishing line impacts per sighted individual from 0.014 in 2004 to 0.005 in 2018, and a slight increase in boat strike injuries from 0 to 0.006. Boat strikes were of comparatively low frequency and fluctuated throughout the period. An increase in predatory bites also occurred from 0 in 2004 to 0.023 in 2018. However, in 2006, 2007 and 2008, the number of injury events recorded per sighted individual fluctuated from 0.033, to 0.021 and 0.029 respectively. This was predominantly due to a decrease in predatory bites by a factor of 1.9 from 0.015 in 2006 to 0.008 in 2007, and 0.012 in 2008. Another fluctuation which occurred between 2011-2013 was largely due to a decline in fishing line impacts by a factor of 2.8 from 0.011 in 2011 to 0.004 in 2012, before the figure rose again to 0.013 in 2013. Net entanglements were only recorded between 2008-2013 at very low frequencies. Natural deformities and scars attributed to infection, disease or parasites also accounted for very few injury events in each year.

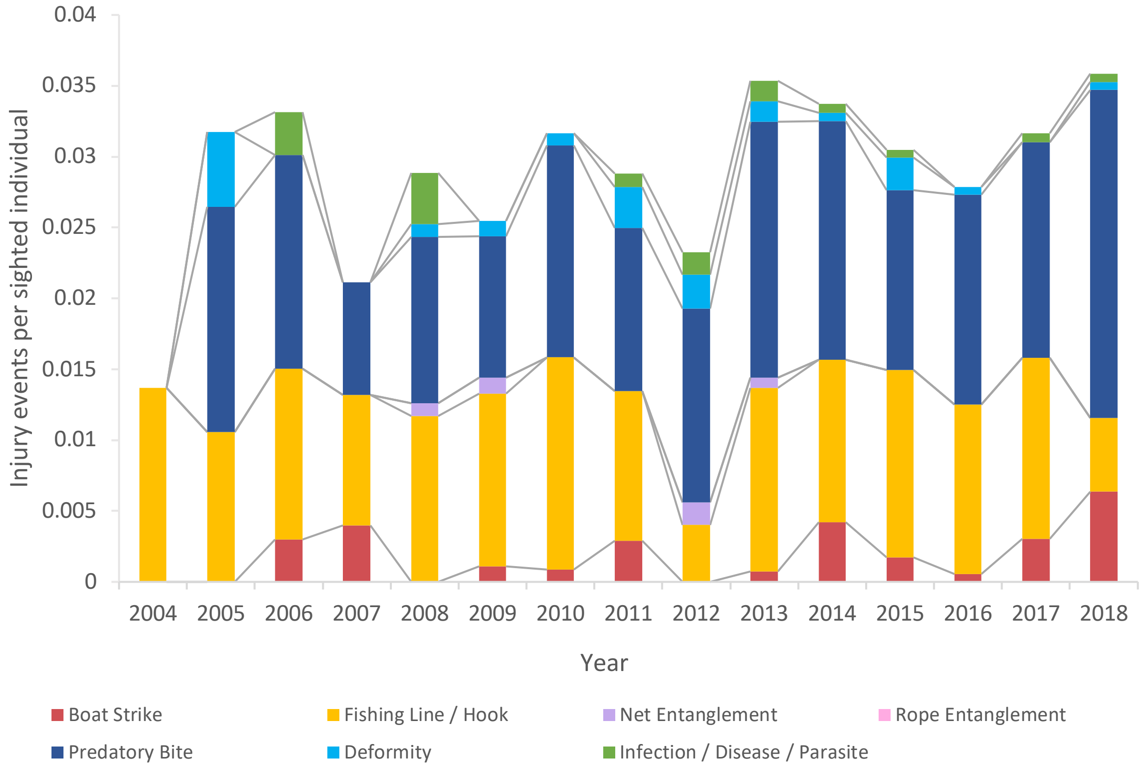

Figure 5. Distribution of categories used to describe anthropogenic and natural injury events (n=505) per individual *Mobula alfredi* sighted in each year between 2004-2018. Series lines have been added to show trends in each category over time.

3.6. Trends in *Mobula alfredi* injuries at Maldivian atolls

Between 1987-2018, injury events were recorded at 18 of the 21 Maldivian atolls examined. North Malé atoll had the highest number of injury events per sighted individual (0.32), followed by Laamu (0.30), Lhaviyani (0.27), Thiladhunmathi (0.25) and Baa atolls (0.25) (**Figure 6**). Gaafu atoll had the highest number of predatory bites per sighted individual (0.20), although only 5 manta rays were sighted there, and only one predatory bite observed (**Table 4**). Disregarding Gaafu, the atolls with the most predatory bites recorded were North Malé (0.18), Lhaviyani (0.18), Ihavandhippolhu (0.17) and Thiladhunmathi atoll (0.17). Whereas, Laamu (0.11) and Addu (0.11) atolls had the most fishing line impacts, followed by North Malé (0.10) and Baa (0.09). Boat strikes were recorded at 8 atolls, with South Malé atoll having the highest number of boat strike events per sighted individual (0.04), followed by Addu (0.014), North Malé (0.011) and Baa atoll (0.011). At very low frequencies, injuries from entanglement in fishing nets were recorded at 5 atolls and rope entanglement at just one. Natural deformities were infrequent but recorded at 14 atolls; whilst scars from infection, disease or parasites were recorded at 7 atolls.

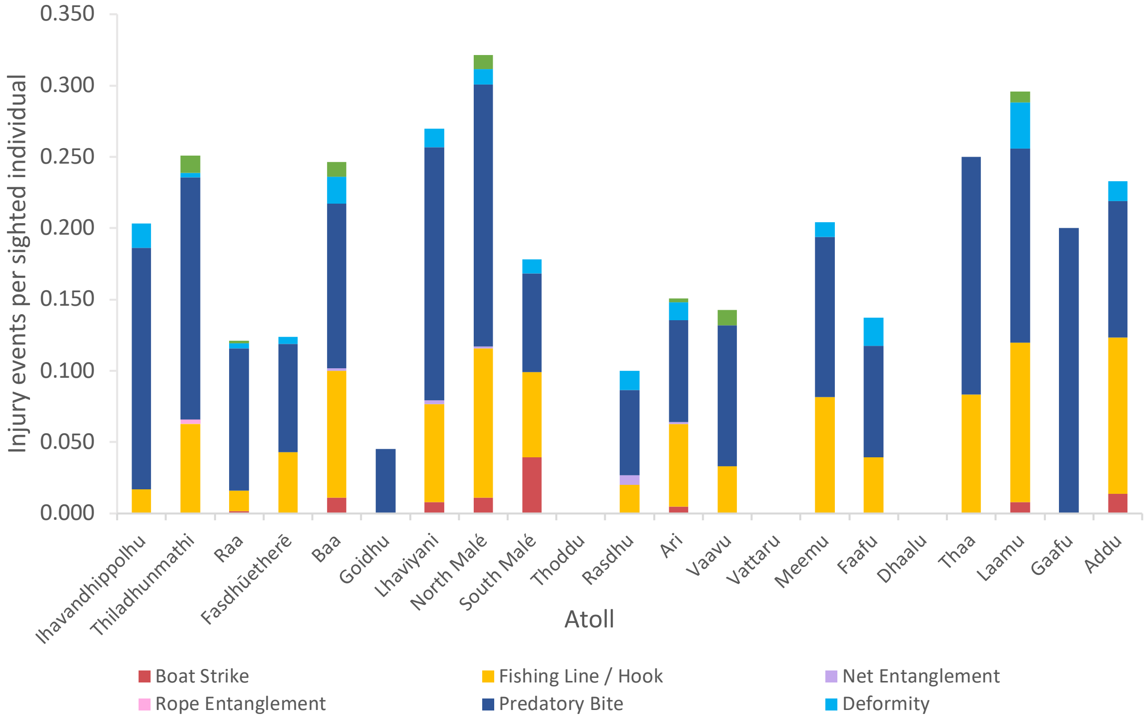

Figure 6. Distribution of injury events (n=1353) originating from anthropogenic and natural causes per individual *Mobula alfredi* observed. Records came from 21 of the Maldives' 26 atolls between 1987-2018. Atolls are listed north to south (left to right).

Table 4. Description of the distribution of anthropogenic and natural injury events across 21 Maldivian atolls, showing the number of individual *M. alfredi* sighted at each atoll between 2005-2018, and intermittently between 1987 and 2004. Atolls are listed north to south and atoll size information was compiled from Stevens (2016).

Atoll name	Atoll size	No. individuals sighted	No. anthropogenic injury events	No. natural injury events
Ihavandhippolhu Atoll	Small	59	1	11
Thiladhunmathi Atoll	X-Large	335	22	62
Raa Atoll	Large	562	9	59
Fasdhūetherē Atoll	Small	210	9	17
Baa Atoll	Large	2055	209	297
Goidhu Atoll	Small	22	0	1
Lhaviyani Atoll	Medium	378	30	72
North Malé Atoll	Large	725	85	148
South Malé Atoll	Medium	101	10	8
Thoddu Atoll	V-Small	15	0	0
Rasdhu Atoll	Small	150	4	11
Ari Atoll	X-Large	1261	81	109
Vaavu Atoll	Large	91	3	10
Vattaru Atoll	Small	19	0	0
Meemu Atoll	Large	98	8	12
Faafu Atoll	Medium	51	2	5
Dhaalu Atoll	Medium	5	0	0
Thaa Atoll	Large	12	1	2
Laamu Atoll	Medium	125	15	22
Gaafu Atoll	X-Large	5	0	1
Addu Atoll	Small	73	9	8
Total			498	855

4. Discussion

Examination of the MMRP's photo-ID database for *Mobula alfredi* and *M. birostris* showed that significantly more *M. alfredi* (26%, n=1206) were injured than *M. birostris* (15%, n=96) (χ^2 (1) = 41.9, *p* < .001), but that both species exhibited more injuries from natural causes (63% versus 66%) than anthropogenic (37% versus 34%). The two species differed significantly in the type of anthropogenic injury events suffered (Fisher's Exact, *p* = .01) because no boat strikes were recorded for *M. birostris*. Although adult *M. alfredi* sustained more injury events than young rays (χ^2 (1) = 30.2, $p < .001$), the latter accumulated them at a higher rate (W = 12082, $p < .001$, r = -0.25). Adult *M. alfredi* (39%, n=370) sustained more anthropogenic injuries than young (32%, n=128), while young rays (68%, n=275) were affected by more natural injury events than adults (61%, n=580). Between 2004-2018, injury frequency per sighted *M. alfredi* increased by a factor of 2.6, with a decrease in fishing line injuries, but an increase in predatory bites and boat strikes. Of the 21 atolls examined, North Malé, Laamu, Addu, Baa and South Malé atolls had the highest number of anthropogenic injury events per sighted manta ray.

In a study in French Polynesia, most injured manta rays had sustained their wounds from boat propellers or fishing gear (Carpentier et al., 2019). This differed from my finding that the majority of injuries to both *M. alfredi* and *M. birostris* in the Maldives were predatory bites. In a study from Mozambique, 76% of *M. alfredi* showed signs of shark bites (Marshall and Bennett, 2010), while, in my study, predatory bites were exhibited by 15% of *M. alfredi* in the databse and 9% of *M. birostris.* Entanglement in fishing nets is considered a key threat to manta rays worldwide (Stewart et al., 2018), however, net entanglement events were of very low frequency in my study. This suggests the net fishing ban in the Maldives has been successful (Nizar and Ibrahim, 2019).

The reason no boat strike injuries were recorded for *M. birostris* is likely because the species rarely occurs in the nearshore reefs frequented by *M. alfredi*, where boat traffic is busiest (Kashiwagi et al., 2011). While vessel strikes have been identified as a major concern for *M. alfredi* as a species (Stewart et al., 2018), these kinds of injuries were not common in my study. However, quantifying any anthropogenic and natural impacts is challenging because those causing fatal injuries cannot be detected and are rarely observed in the field (Deakos et al., 2011).

My finding that more adult *M. alfredi* were injured than young is consistent with previous research (Deakos et al., 2011; Stark, 2015). Adult manta rays have lived longer so have been exposed to more threats over their lifetime than young rays (Deakos et al., 2011). However, as my study found young *M. alfredi* accumulated injuries at a faster rate than adults, this suggests manta rays are most vulnerable to injury before they are mature. Previous studies have suggested young rays use shallow lagoons to avoid predators, which can increase their vulnerability to human activities (McCauley et al., 2014; Stevens, 2016). However, my findings that young *M. alfredi* sustained more natural injuries than adults, while adults had more anthropogenic injuries than young rays, do not accord with these suggestions. One reason for this may be that 50% of natural deformities recorded for *M. alfredi* were exhibited by young rays, but these were of low frequency compared with predation injuries.

A study by Stark (2015) found a decrease in ‘fresh’ anthropogenic originated injuries to Maldivian *M. alfredi* in 2012, which is consistent with my results. Stark attributed it to successful enforcement measures in Hanifaru Bay marine protected area (MPA) following the implementation of a management plan there in 2011 (MMRP, 2013). While the overall decrease in fishing line injuries I found could be due to enforcement of protective measures, this is considered lacking at most Maldivian MPAs (MMRP, 2017). Moreover, although the increase in boat strike injuries was small, as tourism, boat traffic and demand for seafood increase in the Maldives (The World Bank, 2020), it will be important to continue monitoring trends in anthropogenic impacts over time. A limitation of this analysis is that the exact dates injury events occur are unknown. While existing injuries recorded on a manta ray's initial sighting were excluded from my analysis to address this limitation, their exclusion also means the resulting trends are not an exact representation of the injuries observed in each year.

Tourism and human development in the Maldives are generally focused around the central atolls, with North Malé containing the country's capital and the international airport. My finding that *M. alfredi* sighted at North Malé, Laamu, Addu, Baa and South Malé atolls had more anthropogenic injuries than those at other atolls, corresponds to where tourism activities, fishing (commercial and leisure) and boat traffic are more concentrated. This is consistent with a study in French Polynesia which found manta rays were more likely to be injured around inhabited islands with more marine traffic than at remote uninhabited areas (Carpentier et al., 2019). In my study, boat strike injuries were highest per sighted manta at South Malé atoll, where there is a lot of speedboat activity. This is concerning as manta rays, primarily juveniles, are known to frequent shallow lagoons there (MMRP, 2017). Although 75% of boat strikes at South Malé, and 70% of all boat strike events recorded, were to adult rays, given that propeller injuries are often severe, more boat strikes to juveniles may prove fatal (Deakos et al., 2011; McGregor et al., 2019). A limitation of this analysis is that the locations where injury events occurred are unknown. However, as manta rays show a high fidelity to locations (Couturier et al., 2018), where injuries were recorded is likely to be where or near to where the event occurred.

Although injured manta rays have high wound healing capacities (McGregor et al., 2019), sub-lethal injuries have the potential to affect their long-term health and fitness. For example, damage to a manta ray's sexual organs can impair, or even prevent, reproductive success

(Marshall and Bennett, 2010) and severe injuries to the cephalic fins may reduce feeding efficiency (Deakos et al., 2011). While the Maldives contains 42 MPAs, these only cover 0.5% of the country's total area (Stevens and Froman, 2019) and only 3 of 48 key manta ray aggregation sites fall within an MPA with active enforcement (Harris et al., 2020). Establishment of no-take fishing zones in areas of critical manta ray habitat (e.g. known feeding sites), would help reduce the frequency these animals become entangled in fishing line, and are hit by vessels (Carpentier et al., 2019; Stevens and Froman, 2019).

Word Count: 4,993

5. References

Anderson, R. C., Adam, M. S., Kitchen-Wheeler, A.-M. and Stevens, G. (2011a). Extent and Economic Value of Manta Ray Watching in Maldives. *Tourism in Marine Environments,* 7**,** 15-27.

Anderson, R. C., Adam, M. S. and Goes, J. I. (2011b). From monsoons to mantas: seasonal distribution of Manta alfredi in the Maldives. *Fisheries Oceanography,* 20**,** 104-113.

Armstrong, A. O., Armstrong, A. J., Jaine, F. R. A., Couturier, L. I. E., Fiora, K., Uribe-Palomino, J., Weeks, S. J., Townsend, K. A., Bennett, M. B. and Richardson, A. J. (2016). Prey Density Threshold and Tidal Influence on Reef Manta Ray Foraging at an Aggregation Site on the Great Barrier Reef. *PLOS ONE,* 11**,** e0153393.

Carpentier, A. S., Berthe, C., Ender, I., Jaine, F. R. A., Mourier, J., Stevens, G., De Rosemont, M. & Clua, E. (2019). Preliminary insights into the population characteristics and distribution of reef (Mobula alfredi) and oceanic (M. birostris) manta rays in French Polynesia. *Coral Reefs,* 38**,** 1197-1210.

Couturier, L. I. E., Marshall, A. D., Jaine, F. R. A., Kashiwagi, T., Pierce, S. J., Townsend, K. A., Weeks, S. J., Bennett, M. B. and Richardson, A. J. (2012). Biology, ecology and conservation of the Mobulidae. *Journal of Fish Biology,* 80**,** 1075-1119.

Couturier, L. I. E., Newman, P., Jaine, F. R. A., Bennett, M. B., Venables, W. N., Cagua, E. F., Townsend, K. A., Weeks, S. J. and Richardson, A. J. (2018). Variation in occupancy and habitat use of Mobula alfredi at a major aggregation site. *Marine Ecology Progress Series,* 599**,** 125-145.

Croll, D. A., Dewar, H., Dulvy, N. K., Fernando, D., Francis, M. P., Galván-Magaña, F., Hall, M., Heinrichs, S., Marshall, A., Mccauley, D., Newton, K. M., Notarbartolo-Di-Sciara, G., O'malley, M., O'sullivan, J., Poortvliet, M., Roman, M., Stevens, G., Tershy, B. R. and White, W. T. (2016). Vulnerabilities and fisheries impacts: the uncertain future of manta and devil rays. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 26**,** 562-575.

Deakos, M. H., Baker, J. D. and Bejder, L. (2011). Characteristics of a manta ray *Manta alfredi* -population off Maui, Hawaii, and implications for management. *Marine Ecology Progress Series,* 429**,** 245-260.

Dewar, H., Mous, P., Domeier, M., Muljadi, A., Pet, J. and Whitty, J. (2008). Movements and site fidelity of the giant manta ray, Manta birostris, in the Komodo Marine Park, Indonesia. *Marine Biology,* 155**,** 121-133.

Dulvy, N. K., Pardo, S. A., Simpfendorfer, C. A. and Carlson, J. K. (2014). Diagnosing the dangerous demography of manta rays using life history theory. *PeerJ,* 2**,** e400.

Dytham, C. (2011). *Choosing and Using Statistics: A Biologist's Guide,* Hoboken.

Field, A. P. (2012). *Discovering statistics using R,* Los Angeles, Calif. London: SAGE.

Harris, J. L., Mcgregor, P. K., Oates, Y. and Stevens, G. M. W. (2020). Gone with the wind: Seasonal distribution and habitat use by the reef manta ray (Mobula alfredi) in the Maldives, implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 30**,** 1649-1664.

Kashiwagi, T., Marshall, A. D., Bennett, M. B. and Ovenden, J. R. (2011). Habitat segregation and mosaic sympatry of the two species of manta ray in the Indian and Pacific Oceans: *Manta alfredi* and *M. birostris*. *Marine Biodiversity Records,* 4**,** e53.

Kitchen-Wheeler, A.-M. (2010). Visual identification of individual manta ray (*Manta alfredi)* in the Maldives Islands, Western Indian Ocean. *Marine Biology Research,* 6**,** 351-363.

Kitchen-Wheeler, A.-M., Ari, C. and Edwards, A. J. (2012). Population estimates of Alfred mantas (*Manta alfredi*) in central Maldives atolls: North Male, Ari and Baa. *Environmental Biology of Fishes,* 93**,** 557-575.

Lawson, J. M., Fordham, S. V., O'Malley, M. P., Davidson, L. N. K., Walls, R. H. L., Heupel, M. R., Stevens, G., Fernando, D., Budziak, A., Simpfendorfer, C. A., Ender, I., Francis, M. P., Notarbartolo Di Sciara, G. and Dulvy, N. K. (2017). Sympathy for the devil: a conservation strategy for devil and manta rays. *PeerJ,* 5**,** e3027.

Maldivian Manta Ray Project. (2013). *Maldivian Manta Ray Project 2013 Season Summary.* Manta Trust. [Online]. Available at: https://static1.squarespace.com/static/5a196500914e6b09132e911f/t/5ce40b08474fc70001a5d152/1558448915246/Maldivian-Manta-Ray-Project-2013-Report-for-Maldivian-Government.pdf. [Accessed 14 Sept 2020]

Maldivian Manta Ray Project. (2017). *North and South Malé atoll report 1987-2017.* Manta Trust. [Online]. Available at: https://static1.squarespace.com/static/5a196500914e6b09132e911f/t/5dc54d1c6f569d05a9724bdd/1573211591533/MT_MMRP_North+and+South+Male+Atoll+Report_1987-2017_FINAL.pdf. [Accessed 14 Sept 2020].

Marshall, A. D. and Bennett, M. B. (2010). The frequency and effect of shark-inflicted bite injuries to the reef manta ray Manta alfredi. *African Journal of Marine Science,* 32**,** 573-580.

Marshall, A. D., Dudgeon, C. L. and Bennett, M. B. (2011). Size and structure of a photographically identified population of manta rays *Manta alfredi* in southern Mozambique. *Marine Biology,* 158**,** 1111-1124.

Marshall, A. D. and Pierce, S. J. (2012). The use and abuse of photographic identification in sharks and rays. *Journal of Fish Biology,* 80**,** 1361-1379.

Marshall, A., Bennett, M.B., Kodja, G., Hinojosa-Alvarez, S., Galvan-Magana, F., Harding, M., Stevens, G. & Kashiwagi, T. (2018). *Mobula birostris* (amended version of 2011 assessment). *The IUCN Red List of Threatened Species* 2018 e.T198921A126669349. [Online]. Available at: https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T198921A126669349.en. [Accessed 22 August 2020].

Marshall, A., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Pacoureau, N., Rigby, C.L., Romanov, E. & Sherley, R.B. (2019). *Mobula alfredi*. *The IUCN Red List of Threatened Species* 2019: e.T195459A68632178. [Online]. Available at: https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T195459A68632178.en. [Accessed 22 August 2020].

Mccauley, D. J., Desalles, P. A., Young, H. S., Papastamatiou, Y. P., Caselle, J. E., Deakos, M. H., Gardner, J. P. A., Garton, D. W., Collen, J. D. and Micheli, F. (2014). Reliance of mobile species on sensitive habitats: a case study of manta rays (*Manta alfredi*) and lagoons. *Marine Biology,* 161**,** 1987-1998.

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

Murray, A., Garrud, E., Ender, I., Lee-Brooks, K., Atkins, R., Lynam, R., Arnold, K., Roberts, C., Hawkins, J. and Stevens, G. 2020. Protecting the million-dollar mantas; creating an evidence-based code of conduct for manta ray tourism interactions. *Journal of Ecotourism,* 19**,** 132-147.

Nizar, H. R. and Ibrahim, M. *Fishermen's Forum 2019:* On the occasion of the 39th Fishermen's Day of the Maldives*.* Ministry of Fisheries, Marine Resources and Agriculture, Malé, Maldives. [Online]. Available at: https://www.gov.mv/en/files/report-fishermens-forum-2019-maldives.pdf. [Accessed 10 Sept 2020].

O'Malley, M. P., Townsend, K. A., Hilton, P., Heinrichs, S. and Stewart, J. D. (2017). Characterization of the trade in manta and devil ray gill plates in China and South-east Asia through trader surveys. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 27**,** 394-413.

Revolutionary GIS. (2014). Maldives Shapefile Data. 15 August 2014. *Revolutionary GIS*. [Online]. Available at: https://revolutionarygis.wordpress.com/2014/08/15/maldives-shapefile-data/. [Accessed 23 Sept 2020].

Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science,* 65**,** 279-295.

Stark, J. (2015). *Natural or anthropogenic: threats faced by Maldivian manta rays.* Unpublished: University of York. MSc Thesis.

Stevens, G. M. W. (2014). *All Ray Species Now Protected in the Maldives.* [Online]. Save Our Seas Foundation. Last updated: 6 June 2014. Available at: https://saveourseas.com/update/all-ray-species-now-protected-in-the-maldives/. [Accessed 23 Sept 2020].

Stevens, G. M. W. (2016). *Conservation and Population Ecology of Manta Rays in the Maldives.* Unpublished: University of York. PhD.

Stevens, G. M. W. and Froman, N. (2019). Chapter 10 - The Maldives Archipelago. *In:* Sheppard, C. (ed.) *World Seas: An Environmental Evaluation (Second Edition).* Academic Press.

Stevens, G. M. W., Hawkins, J. P. and Roberts, C. M. (2018a). Courtship and mating behaviour of manta rays Mobula alfredi and M. birostris in the Maldives. *Journal of Fish Biology,* 93**,** 344-359.

Stevens, G., Fernando, D., Dando, M. and Di Sciara, G. N. (2018b). *Guide to the Manta and Devil Rays of the World*, Plymouth: Wild Nature Press.

Stewart, J. D., Hoyos-Padilla, E. M., Kumli, K. R. and Rubin, R. D. (2016a). Deep-water feeding and behavioral plasticity in Manta birostris revealed by archival tags and submersible observations. *Zoology,* 119**,** 406-413.

Stewart, J. D., Beale, C. S., Fernando, D., Sianipar, A. B., Burton, R. S., Semmens, B. X. and Aburto-Oropeza, O. (2016b). Spatial ecology and conservation of Manta birostris in the Indo-Pacific. *Biological Conservation,* 200**,** 178-183.

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

Venables, S. (2013). *Short term behavioural responses of manta rays, Manta alfredi, to tourism interactions in Coral Bay, Western Australia.* Unpublished: Murdoch University. Honours Thesis.

Ward-Paige, C. A., Davis, B. and Worm, B. (2013). Global Population Trends and Human Use Patterns of Manta and Mobula Rays. *PLOS ONE,* 8**,** e74835.

White, W. T., Corrigan, S., Yang, L., Henderson, A. C., Bazinet, A. L., Swofford, D. L. and Naylor, G. J. P. (2018). Phylogeny of the manta and devil rays (Chondrichthyes: mobulidae), with an updated taxonomic arrangement for the family. *Zoological Journal of the Linnean Society,* 182**,** 50-75.

The World Bank. (2020). *Maldives Development Update, In Stormy Seas.* The World Bank. [Online]. Available at: http://documents1.worldbank.org/curated/en/336011591808041663/pdf/Maldives-Development-Update-In-Stormy-Seas.pdf. [Accessed 1 Sept 2020].