Project: Eyes on the Reef

Using remote cameras at cleaning stations to understand the seasonality and behavior of the reef manta rays (*Mobula alfredi*) of Baa atoll, Maldives.

Supervisors: Hannah Moloney, Beth Faulkner and Elspeth Strike

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Supervisor: Dr Phil Doherty

"I certify that this dissertation is entirely my own work and no part of it has been submitted for a degree or other qualification in this or another institution and give permission for a copy to be held by my supervisor and distributed at their discretion."

Henry Gould
Hg393@exeter.ac.uk
Student Number: 680023508
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Target Journal:
Aquatic Conservation marine and freshwater ecosystems

This is my targeted journal due to the range of topics which are covered, published papers are dedicated to the conservation of freshwater, brackish or marine habitats alongside promoting work within these ecosystems. The Eyes on the Reef project aims to better understand the temporal scales which influence *Manta alfredi* in the Maldives, as well as gain a better understanding of behaviour in the absence of human presence. This essential scientific research can advise and guide future conservation targets and marine protected areas, hence why this journal is selected. Presenting this studies finding in a forum which aims to better protect all aspects of aquatic biological life through co-operation and problem solving.
Abstract

Introduction

Reef manta rays (Mobula alfredi) are a marine planktivore within the family Mobulidae, recognised as the second largest species of ray. This ray is spread across a vast oceanic range, distributed within tropical and subtropical parts of the Indo-pacific. However, due to their inquisitive nature and limiting life history M.alfredi have become susceptible to anthropogenic threats. Global populations of reef manta rays have seen steep declines, resulting from exploitation and human influences such as habitat loss and degradation. As a result, research into this vulnerable species provides a better understanding of behavioural patterns, allowing for beneficial guidance for the creation of conservation strategies. This study in collaboration with the Manta Trust, aims to assess the influence of various environmental factors on Maldivian reef manta rays around Baa Atoll.

Methodology

This study ran from July 2019 to November 2021. Through the use of remote underwater time-lapse cameras which ran for up to thirteen hours in a single day, it allowed for monitoring the visitation rate of M.alfredi at selected cleaning stations around Baa Atoll. The abundance was then investigated against the following environmental factors: season (month and year), water temperature, moon phase, time of day, wind speed and wind direction.

Results

The only insignificant environmental drivers in this study appeared to be water temperature and tidal phase. Wind speed and direction both played an influential role in the abundance of reef manta rays, westwards winds have increased visitation rate, with it being suspected to increase foraging as plankton is blown westward into the atoll and upwelled. New and full moon phases show to have the largest effect as these lunar stages generate more favourable foraging conditions thus enhancing the need to cleanse post feed alternatively, cleaning conditions under selective lunar phases are more favourable due to the lack of prey aggregations. The results also revealed as the day gets later the visitation rate declines, with the evening being less favourable, potentially due to preferable foraging conditions at lower light levels. Overall, 146 different reef mantas were identified in three years, with 30.2% of these returning more than once, resembling site fidelity within individuals which choose to return to the same locations season after season.
Conclusion

Future work could encompass a greater variety of environmental factors, nevertheless, this study has shown the influence certain ecological drivers can have on the behavioural patterns of *M. alfredi*, and as a result, can be used to designate and design conservation strategies to better protect reef manta rays around Baa Atoll. Continual monitoring must be ongoing to ensure research remains robust and up to date, using remote underwater cameras in conjunction with in-water data collection offering insight into a better understanding of a vulnerable species requiring conservation, alongside revealing valuable information about a marine animal in the absence of human presence.
1 | Introduction

Anthropogenic threats to Mobulidae have increased in recent decades which has led to a declining global Mobulidae population, as a result, the International Union for Conservation of Nature (IUCN) Red List of Threatened Species classified several species including *M. alfredi* as ‘Vulnerable to extinction’ (Marshall et al., 2019). Both industrial and artisanal fisheries have targeted Mobulidae with demand rising since the 1990’s for their meat, skin, liver oil and gill plates, highly prized in the Asian market for their historic medicinal use (Croll et al., 2015; Bucair et al., 2021). Numerous studies around the globe show that urgent protection is needed for Mobulidae, with fishing activities both legal and illegal leading to sharp declines in populations of this threatened species (Bucair et al., 2021).

Other anthropogenic threats reducing the population of marine elasmobranchs include habitat destruction and pollution. Habitat re-structuring through construction has led to large coastal areas of habitat loss, which are highly influential on marine organisms, including *M. alfredi* (Kessel et al., 2017). Reduction of coastal habitats impacts foraging grounds, cleaning stations and juvenile nursery grounds (Mazaris et al., 2009). Coastal sites have shown to hold valuable sites to *M. alfredi* such as cleaning stations, providing beneficial services to an organism, near shore habitats also provide nursery grounds which when disturbed induce a decline in recruitment for several species shown in previous studies (Rochette et al., 2010; Ayers et al., 2013). With Pate and Marshall (2020) study showing the impacts coastal development and anthropogenic influence has on manta rays, specifically juveniles in Florida, with many individuals, 27%, impacted by fishing line entanglement and vessel strikes. Pollution rates have increased in recent years threatening the marine environment, land usage leading to run-off and waste pollution gravely threatens the oceans (Vegter et al., 2014). Ingestion of plastic waste can lead to high mortality rates, with even small quantities of plastic leading to fatality, such as juvenile turtles requiring less than 1 gram of debris (Santos et al., 2015). Studies have looked at the plastic ingestion rate on *M. alfredi* in various coastal locations, with one study revealing on average a single reef manta can ingest ~63 pieces of plastic at local feeding sites in a single visit (Germanov et al., 2019).
Hence, anthropogenic threats in their various forms are a large driver for conservation concerns for not only reef manta rays but for global species, due to the large range of degradation inflicted upon marine ecosystems.

Changes in human perception over previous decades have led to the non-consumptive use of marine resources to becoming increasingly popular, the value of marine life alive can be far greater, providing longer-term benefits both environmentally and economically (Stronza et al, 2019). Marine species including elasmobranchs, pinnipeds and cetaceans are involved in a range of human-based activities, from in-water experiences such as diving or snorkelling to land or boat-based observations (Hoyte, 2001). With ever-increasing popularity since 1980, marine tourism and social interest has led to the expansion of these activities (Hoyte, 2001), providing a range of benefits from social, environmental, and economic (Catlin et al., 2010; Vianna et al, 2011).

Ecotourism has become the foremost example of non-consumptive marine use. It is the ideology of observing the natural environment with limited interactions and disruption, often being directed towards threatened or exotic animals, and is often intended to support conservation efforts (Stronza et al, 2019). There are challenges when managing wildlife-centred ecotourism ventures (Quiros, 2007), however well-managed models have shown to increase the conservation of species and generate sustainable livelihoods (Brunnschweiler, 2010).

The family Mobulidae consists of eleven marine species, comprised of single genera, *Manta* (White et al., 2017). Reef manta rays (*Mobula alfredi*) are a large filter-feeding planktivorous elasmobranch, with a widespread distribution in tropical and subtropical waters throughout the Indian and Pacific Oceans (Lawson et al., 2017). *M. alfredi* have conservative K-selected life-history traits, slow maturation rates, late sexual maturity, and infrequent litters and coupled with their inquisitive nature, large size and predictable movement patterns makes them susceptible to exploitation (Couturier et al., 2012; Braun et al., 2014).

The Republic of Maldives currently has the largest known population of *M. alfredi* globally, with 2012 estimates comprised of roughly 10,000 individuals (Kitchen-
Wheeler et al., 2012). Anderson et al (2011) generated a detailed report on the economic value of manta ray-based ecotourism, encompassing self-contained underwater breathing apparatus diving (SCUBA) and snorkelling within the Maldives, concluding manta ecotourism is worth an estimated ~US$8.1 million annually.

Recognising the value of Mobulidae within the Maldives has led to governmental implementation of management approaches based on the conservation of marine biota (Anderson et al., 2011). Strategies currently in place are bans on exporting all ray species in addition to any products derived from their body (Anderson et al., 2011). With all ray species now being protected under the Environmental Protection Agency’s 2014 law, whereby the action of harming, capturing, or keeping any species of the ray is illegal as set out in the Batoidea Maldives Protection Gazette No. (IUL) 438-ECAS/438/2014/81 (Maldives EPA, 2014). Other conservation approaches include the creation and implementation of effective marine protected areas with strict regulations and enforcing sustainable practise, for both industrial and artisanal fishing as well as other marine users such as eco-tourism charters (Andrzejaczek et al., 2020).

Hanifaru Bay is one marine protected area designed to protect the annual mass aggregations of feeding mantas on the eastern edge of Baa Atoll, from May to December (Stevens, 2016). Due to the cul-de-sac reef structure with a surrounding shallow (<1m) reef, when strong lunar tides overcome the monsoon currents, plankton-rich water is upwelled from deep ocean water and drawn back over the shallower atoll water (Stevens, 2016). When this phenomenon occurs the atoll pass joining Hanifaru Bay, called Dharavandhoo Kanduolhi, leads to the formation of a back eddy concentrating plankton in shallow water drawing in a vast aggregation of *M. alfredi* however *M. birostris* have also been observed (Stevens, 2016).

While Hanifaru Bay has become vastly protected, continual monitoring and research is ongoing at this site, whilst also limiting human influence to maintain protection for marine biota utilising the planktonic concentrations. There are other reef structures that attract megafauna with one of the most important being cleaning stations (O'Shea, Kingsford and Seymour, 2010). Cleaning stations are highly diverse, attracting a range of marine biota which utilise the stations to remove parasitic organisms, bacterial growth, and general cleansing via cleaner fish (Ashe, 2016).
M. alfredi are often recorded visiting cleaning stations for numerous reasons (O'Shea, Kingsford and Seymour, 2010; Armstrong et al., 2021), Stevens (2016) studied the social interactions between manta rays, concluding the majority of mating and courtship behaviours exhibited by mantas occurred at cleaning stations thus deemed an essential gathering point, Stevens et al (2018) later concurred this further this with a study showing ninety per cent of manta courtship events occur at cleaning sites. Mantas along with other marine fauna also use cleaning stations for body regulation, performing vertical migrations to aid in metabolic and physical functioning after spending prolonged periods foraging below the thermocline (Andrzejacze, 2021; Armstrong et al., 2021).

A variety of environmental drivers have shown to influence the behaviour of manta rays; wind speed and direction (Harris and Stevens, 2021), water temperature (Peel et al., 2019), moon and tidal phase (Jaine et al., 2012) and the season (month) including time of day (Knochel et al., 2022). Further research is required to fill gaps in scientific knowledge pertaining to manta behaviour which will be crucial for M. alfredi conservation around Baa Atoll. Identifying a range of drivers which influence an animal's behaviour is key in developing an inclusive conservation strategy, with a greater understanding of how the environment impacts the movements of M. alfredi allowing for a more comprehensive designation of a marine protected area.

Behavioural studies on M. alfredi are further limited by the lack of long-term studying (Marshall, Dudgeon and Bennett, 2011), with limiting data collection methods (SCUBA and free diving) only offering a brief insight into manta ray movements.

The Manta Trust’s project “Eyes on the Reef” has been developed to better understand Maldivian manta ray behaviour around three hot-spot atolls (Baa, Raa and Laamu). This long-term study will focus on the manta ray populations surrounding Baa atoll between 2019 and 2021, looking at a range of environmental factors; season, wind speed and direction, tidal phase, moon phase and water temperature which have all shown to influence planktivorous elasmobranchs (Peel et al., 2019; Armstrong et al., 2021; Harris and Stevens, 2021). Objectives of this study are (1) to identify determining environmental factors on Baa atolls M. alfredi populations and (2) to use
a remote long-term study to monitor the behaviour of *M.alfredi* at cleaning stations in the absence of human presence, to inform conservation planning.

2 | Methodology
2.1 | Study area

During the Baa atoll field seasons of 2017 to 2019, short-term remote underwater video surveys were carried out, up to four hours long, positioned at particular sites frequently visited by manta rays. This data allowed for site selection for the “Eyes on the Reef” project, choosing three coral bommies around Baa atoll; Veyofushi Gaa (VG), Magoodhoo Gaa (MG) and Olhu Kolhu (OK). These three sites have been classified as a cleaning station and major aggregation sites for *M.alfredi* which are situated on the east side of Baa Atoll in the Maldives (Figure 1). The specific locations of the sites remain anonymous for conservation reasons such as one site only being accessible to scientific researchers and thus has very little anthropogenic influence.

Each study site has variable environmental conditions, altering current strength and direction alongside depths, VG around seven meters which in this study is the most studied site, MG reaching eight meters and finally OK being the shallowest with five meters. The sites also range in biodiversity, such as Reef manta ray (*Mobula alfredi*), Blacktip reef shark (*Carcharhinus melanopterus*), Humphead wrasse (*Cheilinus undulatus*) and Hawksbill turtles (*Eretmochelys imbricata*) all sighted. Consistently between all the locations is the majority of the cleaner fish species, with the most dominant being Bluestreak cleaner wrasse (*Labroides dimidiatus*).
A time-lapse recording set-up was used on each site to monitor manta rays, with the location of the camera placement reported prior to deployment using a Global Positioning System, as well as the direction of the camera. The camera used was a GoPro Hero 4 (resolution 1080p; frames per second 30; wide-angle mode) (Figure 2) and set up to turn on before sunrise and turn off after sunset, which was configured to take a single image every sixty seconds throughout the day. The recording times differ day to day however the time frame was between 05:45 and 18:45, allowing for recording periods of up to 13 hours per day. The camera was placed in an underwater housing measuring 31.5 x 20 x 16.5cm (Figure 2), with the camera lens facing outwards through an acrylic pane. The housing was consistently positioned two meters

Figure 1: The boxed area on the right shows where Baa atoll is situated within the Maldivian Archipelago. The labelled sites on the left being approximate locations of the cleaning stations studied within this project by the Manta Trust, as well as Hanifaru Bay annotated due to aggregation importance for *M. alfredi* locally.

2.2 | Remote data collection

A time-lapse recording set-up was used on each site to monitor manta rays, with the location of the camera placement reported prior to deployment using a Global Positioning System, as well as the direction of the camera. The camera used was a GoPro Hero 4 (resolution 1080p; frames per second 30; wide-angle mode) (Figure 2) and set up to turn on before sunrise and turn off after sunset, which was configured to take a single image every sixty seconds throughout the day. The recording times differ day to day however the time frame was between 05:45 and 18:45, allowing for recording periods of up to 13 hours per day. The camera was placed in an underwater housing measuring 31.5 x 20 x 16.5cm (Figure 2), with the camera lens facing outwards through an acrylic pane. The housing was consistently positioned two meters...
away from the cleaning station in the same location facing the same direction (SSW), with the rear submerged into the substrate to angle the camera upwards more providing a greater view.

Observations were carried out between the 4th of July 2019 to the 26th of November 2021. In 2019 there was a total of 92 days’ worth of data recorded spread across the three sites between July 4th and November 24th, 52 days at Veyofushi Gaa, the next 23 days at Magoodhoo Gaa and the remaining 17 days at Olhu Kolhu, generating 57,376 images. 2020 data was sampled from a single site Veyofushi Gaa between the 10th of October and to 23rd of November, totalling 38 days and 24,239 photos. Finally, 2021 has 140 days sampled from June 5th to November 26th, all sampled from Veyofushi Gaa recording a total of 88,084 photos.

Figure 2: (A) Placement of the camera and the angle captures photographs of the manta rays when swimming through the cleaning station. (B) Manta Trust researcher positioning the camera system 2m away at the edge of the cleaning station. Images (C) and (D) show the self-made camera housing from the front and sides, with (D) a water temperature probe attached.
2.3 | Environmental data collection

The moon phase has been recorded from an online moon phase calendar recording; full moon, first quarter, third quarter and new moon (https://www.timeanddate.com/moon/phases/maldives/male). The water temperature data were recorded using a HOBO Water Temperature Pro v2 Data Logger, which was attached to the underwater housing containing the GoPro (Figure 2). Hourly water temperature recordings of each day were taken, and for analysis, a daily average was taken. Tide charts have been provided by a Maldivian diving company called Moto and Moosa. Wind data has been gathered by a weather station onsite called Landaa along with some historical data from the Maldivian meteorological society.

2.4 | Photo analysis

When analysing the images, the number of manta rays present was counted per image, however, due to an issue with time-lapse data collection, the 60-second period between an image being taken results in a limited field of view (Charfi, Wakamiya and Murata, 2009), meaning if manta rays are at the site, it is unlikely to be present in all images. To mitigate this issue ‘sighting events’ are recorded for each day, consisting of the start time of the event, the duration of which manta rays are present and the maximum number of manta rays in a single image (MaxN). The events start when the first individual(s) appear and are continuous until a period of 10 minutes has passed without a single sighting, at which point it is assumed that the manta rays have vacated the cleaning station and the next sighting will be a new sighting event (Peel, 2019).

Manta rays are all unique, their ventral surface markings alter between individuals and can be used for identification. Photos which clearly depict the ventral surface of the elasmobranch are used for later identification. As the rays age, the marking on the ventral skin remains unchanged, this distinctive marking for each individual allows for identification (Stevens, 2016). The Manta Trust has built a database of identifiable Maldivian manta rays, with all photo identification from this study done manually by the manta trust and matched to an individual. The ventral spot pattern is taken from two locations, the primary being around the gill slits and the secondary being spot patterns on the underside of the pectoral wings (Figure 3a).
2.5 | Manta ray abundance

There are three different measures of manta ray abundance which could be used within analysis: MaxN, the estimated number of manta rays which is calculated per day of recording and represents the number of individual manta rays identified, finally a calculated proportion of manta ray presence in photos, using the number of photos containing manta rays divided by the total number of photos taken that day. MaxN was the determinant variable used when analysing data due to its consideration for showing a conservative estimate (Sherman et al., 2018).

2.6 | Data analysis

All data was recorded and edited in Microsoft Excel 2022 and saved as CSV files. The statistical test generalised linear model (GLM) has been conducted to analyse the data for this study with significance testing via a chi-square test. R-studio (2022) is used as the analysis software where significance values (p-values) were <0.05. The generalised linear model analyses the influence that the environmental variables (season both month and year, wind speed and direction, moon phase, tidal condition, and water temperature) have on manta ray’s abundance.

Figure 3: Underwater photographs depicting the unique identification for each *M. alfredi* on the ventral side. (A) shows the zonation of how a manta ray is identified with the primary ID area in yellow, with secondary zones along with the wings in red (Stevens, 2016). (B) An image captured by a manta trust GoPro on the Veyofushi Gaa cleaning station in 2021.
The GLM used quasipoisson error correction due to the overdispersion and lack of normal distribution. Due to the variable sampling effort between years, a weights argument has also been used, this is based on years to account for the variation. This method of analysis accounts for the range of environmental variables over the three years, allowing for an in-depth look at factors influencing manta ray abundance around Baa atoll. MaxN has been selected as the unit for manta ray abundance as it is widely considered as a conservative estimate for an abundance of a species (Campbell et al., 2015; Sherman et al., 2018). The minimum adequate model was achieved using stepwise selection reducing the model accordingly. The significance of each variable was examined using one-way ANOVA, with significant categorical variables further examined using Tukey’s post-hoc Test via the package ‘eemeans’ (Searle, Speed and Milliken, 1980).

3 | Results
Sampling across the three years varied, there were 92 days sampled in 2019, 38 days sampled in 2020 and 137 days sampled in 2021, for a total 267 sampling days in total. The daily means recording duration was 651.3 minutes per day and an average of 631 photos taken per day. The max number of manta ray photos taken in one day was 193, with the mean number being 23, however the estimated number of manta rays had an average of 2.7 per day with the confirmed number being lower with a daily average of 1.2 rays.

3.1.1 | Representation of manta ray abundance
Whilst MaxN was used during the analysis checking the correlation of all representation of the manta ray abundance was performed via three Pearson’s correlation coefficient tests on all three indices (MaxN and estimated, MaxN and proportion score, proportion score and estimated). The results indicated a high correlation to one another (Table 1).
Table 1: The three different methods of indicating manta ray abundance compared to one another using Pearson’s correlation coefficient, with the r and p-values indicated.

<table>
<thead>
<tr>
<th>MaxN</th>
<th>Estimated</th>
<th>Proportion Score</th>
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<tbody>
<tr>
<td>MaxN</td>
<td>r</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estimated</td>
<td>r</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proportion</td>
<td>r</td>
<td>1.0000</td>
</tr>
<tr>
<td>Score</td>
<td>p</td>
<td></td>
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3.1.2 | Environmental influence
Using a generalised linear model, it revealed that of the seven environmental variables, five were significant. These five were year, month, moon phase, wind direction and wind speed, leaving high tide time and water temperature to have an insignificant impact on the abundance of *M. alfredi*.

3.1.3 | Year and month significance
The year had a significant impact (F=17.942, p<0.0001) as well as month (F=5.345, p<0.0012) influencing the abundance of manta rays. Using post hoc testing to reveal significance of the levels within variables revealed that abundance was significantly different between 2019 and 2020 (z=-2.409, 95% CI [0.0106,0.94], p<0.04) and 2020 and 2021 (z=2.358, 95% CI [1.0179, 387.17], p<0.04), however insignificant between 2019 and 2020 (z=0.853, 95% CI [0.3036, 0.853], p<0.66). With the greatest abundance in 2019 for unit of effort and the lowest in 2020 (Figure 4). Over the course of the eighteen months (the same six months from three years), sampled the results showed that the lowest month over the three years was October, with a declining abundance each year sampled (Figure 5), this was confirmed using Tukey Testing, with October having a significantly lower abundance compared to any other month sampled.
**Figure 4:** The abundance of manta rays using MaxN between each of the years sampled and the estimated number per month indicates a rise and fall with the greatest abundance in 2019, despite a decreased number of images taken compared to the final year.

**Figure 5:** How the individual months alter the abundance of *M.alfredi*
3.1.4 | Moon phase
The moon phase did significantly impact the abundance of manta rays \( (F=6.258, \ p<0.0019) \) around Baa. When the influence of moon phase was removed from the model, and tested, it revealed to make the model significantly worse \( (F=6.103, \ p<0.0022) \). The Tukey test showed the significant states to be a new moon with both third quarter and first quarter. Figure 6 also shows a peak abundance in years 2020 and 2021 when a full moon is present.

![Figure 6: Influence of the four categorised moon phases on the mean abundance of reef manta rays across the three years (mean ± SE).](image)

3.1.5 | Wind
Wind direction \( (F=3.303, \ p<0.0024) \) and daily average wind speed \( (F=6.567, \ p<0.0155) \) both on continuous scales were significant environmental influencing factors on the abundance of manta rays. Wind direction, containing a westerly direction revealed a higher abundance of manta rays recorded compared to other wind directions. This was a pattern over all three years of data with a straight westerly wind having the highest recorded abundance in two (2019 and 2021) of the three years. Daily average wind speed \( (F=6.567, \ p<0.0155) \) therefore plays a significant role in the
appearance of manta rays. We can see that over the three-year study the number of sightings occurring declined as wind speeds increase, with very few sighting events occurring above wind speeds of 15 knots per hour or greater. The predominant wind speeds where the greatest number of M. alfredi are sighted is between 5 and 13 knots per hour, with the lower winds speeds producing the greatest sighting rates (Figure 7).

Figure 7: 7(A) the wind direction compared to the mean abundance of reef mantas. 7(B) shows the influence wind speed has over the mean abundance of reef mantas. Both of these are averages across all months over the three-year study period.
3.2 | Behavioural analysis

3.2.1 | Duration at cleaning station

Over the three years there was a total of 618 sighting events, totalling a duration of 9993 minutes (166.55 hours) of manta rays captured at the cleaning stations around the atoll, with an average sighting event lasting 16.17 minutes. Of this time the number of sightings under one minute was 195 which is 31.55% of the total number of sightings, however less than 2% of the total time spent on the site. Comparing this to the number of sighting events that lasted longer than one hundred minutes was only 12 occasions (1.9% of sighting events) but totals 1971 minutes of time, one fifth of the time for manta rays captured.

Figure 8: The mean duration of which the manta rays sited spent on the cleaning station, alongside the mean the number of sighting events captured during the entire month.
3.2.2 | Time of day

The time of day was investigated to see when the most abundant periods could be for the rays, with results showing it was significant on the abundance of Maldivian reef manta rays (F=4.8094, p<0.001). Recording time was split into four three-hour periods; dawn (record start– 09:00), morning (09:01 – 12:00), afternoon (12:01 – 15:00) and dusk (15:01 – record stop). The data showed that the most abundant period for sighting events was in the dawn period, with a total of 231 events occurring during this time, with morning closely following with 217 sighting events. These two periods total 72.5% of the sighting events, with the remaining 27.5% occurring in the afternoons and evening periods. This trend followed over the three years and within the months, with the afternoon and dusk periods having lower sightings when compared, revealing closer to evening periods that the abundance of manta rays’ declines.

Figure 9: The time of day in which the sighting events occurred, split into the four categories (dawn, morning, afternoon, and dusk), with the mean number of manta ray visiting during these times. The similar pattern can be visualised in all months, with there trending to be a gradual decline as the continues, often dawn and morning exhibiting the highest abundance with lowest abundance at dusk.
### 3.2.3 | Group behaviour

Individuals arriving at the cleaning site solo was vastly more common than group behaviour, only 13.5% (84) of the sighting events contained images with more than a single *M. alfredi*. The most manta rays seen in a single event was 6 on the 29th of September 2019, however this only occurred once in three years with the most common grouping being two individuals with 60 of the 84 events being pairs. When looking at a correlation between the number of manta rays present during an event and the duration stayed, it can be concluded that is a significant correlation to one another using Pearson's correlation statistic (correlation coefficient = 0.543, p=0.0001, 95%CI [0.485,0.596]).

### 3.2.4 | Individual manta ray behaviour

From the images captured on the underwater camera system, a total of 300 images were taken allowing for identifiable photos. Some days multiple images were taken of the same individual, not all images taken are able to identify the ray due to position on the image, the side of the body shot and the distance away from the camera, also fluctuation in visibility can influence the number of examinable shots which could be taken (Figure 10). Images which are identifiable are usually taken close to the animal with the underside clearly visible.

![Figure 10](image)

*Figure 10:* Both images taken on the underwater remote camera system, however the left-hand image was unable to have the individual identified due to poor image quality, whereas the right image shows a clear belly shot with visible markings to provide an accurate identification of a specific manta ray.
The 29th of September 2019, saw the most manta rays confirmed, with 17 individuals observed. The most commonly sighted individual was MV-MA-3815, a subadult male called ‘Toon’, who was identified on 19 different days between 2019 and 2021, with an increase in sighting in 2021. Over the course of the three years, there have been 146 different *M. alfredi* identified using the Maldivian Manta Ray database, with images taken from the Eyes on the Reef project, twenty of these new identifications came in 2021. However, 102 (69.8%) individuals only appear once, meaning the remaining 30.2% appear more than once over the course of the project.

### 3.2.5 | Sex ratios

There was a total of 113 females identified compared to 187 male images. This is a split of 37.8% females contrasted to 62.2% males, indicating a strong male-dominated ratio of *M. alfredi* around the study site.

### 4 | Discussion

#### 4.1 | Environmental influence

The results of the study revealed that there was little significance on *M. alfredi* behaviour around water temperature variation, with temperature only fluctuating by one degree Celsius throughout the study period. A range of water temperatures would be required to see if variation influences abundance of reef manta rays at specific sites. Indeed, the influence of water temperature has been shown in previous studies on manta rays to be less significant than other environmental factors (Jaine et al., 2012). Findings show that manta rays are able to tolerate a range of temperatures, between 21 and 30 degrees Celsius (Dewar et al., 2008).

Seasonality appears to play a role in the abundance of manta rays around Baa atoll, with peak sightings being between July and August and a decline in the abundance during the months of October and November. This has been observed in several studies, Couturier et al (2011) identified individually recognisable manta rays using photographic identification, revealing that there was a greater abundance of *M. alfredi* during the months of June and around Lady Elliott Island. Jaine et al (2012) backed
this trend revealing there was a greater *M.alfredi* sighted between early May to mid-August, with declines in sighting numbers as you move away from August.

Site fidelity could be a major reason why manta rays return annually to Baa atoll. Several recent studies on the site fidelity behaviour of *M.alfredi* populations around the globe have shown whilst they can travel away from a site for weeks to months manta rays display strong long-term site fidelity behaviour patterns (Dewar et al., 2008; Setyawan et al., 2018; Carpentier et al., 2019; Knochel et al., 2022).

Knochel et al (2022) studied a population of *M.alfredi* in Sudan using acoustic tracking, discovering that whilst some individuals travelled distances of over 125km away from the bay they subsequently were later detected in the bay, it was then concluded this population have a strong degree of site fidelity. Setyawan et al (2018) carried out a similar study on thirty-nine reef manta rays tagged in Raja Ampat, Indonesia, to investigate the residential behaviour and found that 87% of the tagged mantas displayed re-visitation events.

Theories behind why site fidelity may occur are due to the important oceanographic structure of a site, allowing for the concentration of valuable marine resources, such as the congregation of food. In addition, the changing monsoons throughout the Maldivian archipelago influence the Maldivian *M.alfredi* population (Anderson, Adam & Goes, 2011). During the Hulhangu (Southwest monsoon) mass aggregations of foraging manta rays develop on the eastern edge of the nation’s atolls, which include Baa (Armstrong et al., 2021). This mass event is due to the structure of the atolls, steep walls and strong monsoon winds that drive up deep cold water, containing vast concentrations of plankton (Stevens, 2021). Hence, some of the reef mantas within this study, 30.2% which are re-captured, display site fidelity behaviour due to the abundance of vital marine resources in the area throughout the study season.

The celestial cycle has shown to have a dramatic influence over the congregation of manta rays, not only in the Maldives but studies globally have shown the impact the moon phase has on behaviour (Lavender et al., 2021). The marine environment is influenced daily via hydrodynamic processes caused by the moon, tidal strength can be based on the moon phase, with the strongest tides evident during a new or full
moon (Barlow et al., 1986; Rohner et al., 2013). Furthermore, the illumination via the moon phase influences the availability of light (Hernandez-Leon et al., 2001). Both of these fore-mentioned factors can directly influence the abundance of plankton, thus impacting marine predators’ behaviour (Hernandez-Leon et al., 2001; Benoit-Bird et al., 2009).

The light levels produced by the moon influence the circumlunar rhythms of planktonic species. The depth of plankton is determined by the levels of illumination, a greater degree of brightness provided during full moons leads plankton to be located at deeper depths, compared to new moons (Calbet, 2020). Furthermore, illumination influences the density that plankton congregate at, with low-light conditions making planktonic species more sporadic (Barr and Abelson, 2019). Concentrations of plankton are low throughout low-light conditions due to a lack of environmental cues to trigger coordinated movements (Barr and Abelson, 2019).

Thus, foraging behaviour of *M.alfredi* is influenced by light levels, with high levels of moonlight more likely inducing deeper night-time foraging by *M.alfredi* (Lassauce et al., 2020), with reef manta rays hunting the downward vertical migrations of prey throughout these illuminated periods (Gliwicz, 1986). Alongside this low-light conditions make foraging less effective due to the inconsistent densities of prey (Barr and Abelson, 2019). Hence, under low-light conditions such as new moon it would be more favourable for *M.alfredi* to visit cleaning stations, as both foraging and cleaning behaviours do not often take place at the same site (Stevens, 2016; Lassauce et al., 2020). In contrast, an absence of *M.alfredi* from cleaning stations would be expected when light levels are higher, forcing congregations of prey together triggering foraging and allowing for the most energy-efficient behaviour, crucial to survival (Meekan et al., 2015).

However, there is a high abundance of manta rays recorded during full moon phases, when light levels are at a peak, consequently inducing elevated foraging behaviour. With this rise in foraging behaviour the build-up of detritus increases, resulting in more frequent visitation to cleaning sites, hence explaining the greater number of sightings around full moon lunar phases. When local foraging sites provide the greatest prey catch per unit of effort for the manta rays, consequential
M.alfredi require a greater level of cleaning to rid themselves of increased waste build-up (Dewar et al., 2008; Anderson et al., 2011). Looking at specific years' abundance of M.alfredi around moon cycles (Figure 6), results show that 2020 and 2021 both have the highest abundances during full moons when illumination levels would be greatest.

This study’s findings correspond with similar behaviour patterns exhibited in previous studies (Stevens, 2016; Krüger, 2020), with the highest abundances of M.alfredi at the cleaning station across all three years occurring during new moon and full moon periods (Figure 6). Research in the surrounding area revealed that ideal conditions for reef manta ray feeding in the Hanifaru Bay occur around both new and full moons (Armstrong et al., 2021). The conditions of Hanifaru Bay for feeding during these moon phases may be more optimal than other foraging sites exposed to greater environmental stressors or altering structure influencing prey behaviour. Combined with the unique oceanography of the bay generating mass congregations of trapped plankton, caught in eddied water providing prolific foraging grounds for planktivores (Neves, 2009).

Expanding further research in the Baa Atoll region, via studying more sites would provide more detail on the theory of M.alfredi abundance at cleaning stations being higher during these lunar periods. Provides evidence on light levels and abundance of manta rays such as increased sightings during new moon periods due to unfavourable foraging conditions, supporting the notion low light conditions influence prey aggregations.

The wind is a key environmental influence on many aspects of the ocean. Wind drastically impacts the mixing of water layers through various natural processes upwelling, downwelling and waves of both size and speed (Rinke et al., 2007; Kim et al., 2014). These processes alter the movement of plankton, upwellings can be greatly intensified with an increase in wind speeds, and when upwellings occur there is a bloom in plankton (Yoder et al., 1983). In relation to wave influence over plankton, when there is an increase in wave frequency and magnitude it provides a higher concentration of plankton assemblage (Lennert-Cody and Franks, 1999).
Both of these environmental processes strengthen with higher wind speeds (Figure 7). As a result, the power of each process intensifies, forming more dense aggregations of plankton, previous studies have shown how foraging behaviour can be impacted by surface currents and wind speeds (Yoda et al., 2014). Such variables would provide prime foraging conditions for manta rays, hence when wind speeds are greater results would be expected to indicate a decline in the number of ray sightings at cleaning sites. This is because energy consumption for large marine planktivores is higher, requiring vast quantities of food to survive (Barr and Abelson, 2019). Consequently, there is probably a trade-off between cleaning and foraging behaviour suggest that when conditions are optimal for feeding, such as higher wind speeds, there will be less opportunity to clean (Schofield et al., 2017).

The data shows a similar behaviour pattern, with there being fewer sightings of *M. alfredi* at higher wind speeds (Figure 7). Harris and Stevens (2021) found a similar pattern pertaining to lower wind speeds increasing the frequency of cleaning visitation. However, whilst on average there are a greater number of sightings at lower wind speeds, speeds occurring at less than 10 knots per hour there are still some sightings are higher speeds. These high wind speed sightings could be due to prolific feeding conditions, which then require the manta to visit a cleaning station, triggered by parasite load (Treasurer, 2002) and their physical health (Waldie et al., 2011) post forage.

Wind direction has been shown previously to influence the abundance of manta rays (Anderson et al., 2011; Harris et al., 2020), with winds driving productivity and supporting populations of *M. alfredi* throughout the Maldives (Harris et al., 2020), as well as the wind direction influencing the distribution of prey (Harris et al., 2020), which thus alters the movement of predatory species such as *M. alfredi*.

The study sites are located on the eastern edge of Baa, and this means that West winds are the direction which blow into the site. As previously suggested *M. alfredi* follow the distribution of plankton, which is influenced by wind direction an expected pattern arises. The highest abundance of prey blown in and accumulate in the shallow coastal feeding grounds would be in westward winds, and the sighting of manta rays hence would be higher. The data indicates this pattern, demonstrates the
highest abundances occurring during Westerly winds and the lowest during Easterly winds.

These easterly winds may force manta rays to move site, this could be into deeper water to forage or to the opposite side of the atoll, where winds blow plankton. Further research could be performed to track the horizontal and vertical movement of Baa’s manta rays throughout various wind directions. Cloud cover is influenced by wind, many studies found a correlation between higher wind speeds and lower cloud cover (Abbood et al., 2021). Whilst not studied as a factor in this report, cloud cover percentage has been shown to impact the presence of manta rays (Barr and Abelson, 2019). The decreased percentage of cloud cover allows for greater light intensities, resulting in the formation of plankton aggregations triggering foraging behaviour (Armstrong et al., 2016). Hence, high wind speeds mean a decrease in cloud cover and thus the absence of manta rays from cleaning stations, which is a pattern witnessed in this study (Figure 7).

4.2 | Behavioural patterns
Whilst there were no night-time observations in this study, during the recording daylight hours the time of day at which M.alfredi were observed shows a peak number of sighting events over all three years within dawn and morning periods, which are from record start time to 12:00. As the day progresses the number of sightings declines, this pattern is seen throughout all months over the entire study period. Similar patterns have been found in previous studies (Setyawan et al., 2018; Andrzejaczek et al., 2021). These results suggest that coastal vertical migrations occur daily, whereby manta ray movements are motivated by the distribution of plankton which follows a diurnal migration (Stewart et al., 2016; Andrzejaczek et al., 2021), forcing M.alfredi offshore to forage at night and return to surface waters to rewarm between dives. These deep later dives could be forced due to mesopelagic sources of plankton (Burgess, 2017), alternatively to forage on benthic species of plankton emerging at night in shallow coastal areas (Alldredge and King, 1980).

When manta rays appear during these daytime periods it may be to rewarm their body which can aid in digestion and remove parasites from previous foraging events.
(Murie et al., 2020), as well as partake in social interactions with one another, such as courtship alongside cleaning behaviour (Burgess, 2017). This could also be due to plankton remaining at greater depths or buried within benthic substrate subsequently making them unobtainable. It is important to note that the monitoring of movement and internal body regulations for Maldivian reef mantas outside of the cleaning station was not observed in this study. This does not necessarily equate to night-time foraging; further tracking would be required to confirm information. Other factors could be responsible for movement patterns such as intraspecific interactions or low oxygen waters.

The time that *M. alfredi* spent at each cleaning station varied, the longest recorded time 271 minutes in November 2020, with the average time at the cleaning station over the three years being 16.17 minutes. This is similar to other studies, indeed O’Shea et al. (2010) observed cleaning events lasting up to 5 hours with an average of 31 minutes, and Venables et al. (2020) saw cleaning events lasting up to 8.2 hours with a mean visit duration of 25.41 minutes. Cleaning for a *M. alfredi* is clearly an essential and time-consuming part of well-being, with the longest event lasting being 4.52 hours, however other studies have similar long patterns. Harris and Stevens (2021) found some individuals were staying at cleaning sites for up to 8.5 hours per day, with the mean duration varying between sites.

The activities that occur at cleaning stations are not limited to the metabolic benefits (Jirik & Lowe, 2012), cleaning (Barr and Abelson, 2019) and predator avoidance (Marshall & Benett, 2010). These aggregations allow courtship behaviour and social relationships (Stevens, 2016). In this study, there were groups of manta rays up to six visiting the cleaning station in a single event, with individuals often returning to the site with the same groupings which indicates a possible social structure. Identifying social behaviour within populations has only been done in limited sub-populations of manta rays, such as in Indonesia (Perryman et al., 2019), hence this type of non-solitary behaviour is of interest. A better understanding of natural social behaviour can advise conservation efforts and the implementation of strategies to protect marine fauna (Stewart et al., 2018).
Nevertheless, the gathering of multiple manta rays could be due to social or courtship-related behaviour. The predominantly male-dominated sex ratio of the identified manta rays across all three years could be due to the social preferences of those individuals present, being a social animal, *M. alfredi* have exhibited behaviours which require cooperation with one another to be effective such as cyclone feeding as well as generic interactions with one another (Perryman, 2020). However, reproductive behaviour could be a reason for the sex differences (Deakos, 2010).

An explanation for the reduced number of female manta rays is due to the level of parental investment that is required into producing offspring. As a result, females gain more benefits from remaining at a single site, whereas males benefit from having a more migratory pattern between congregation sites to seek a suitable partner (Germanov et al., 2019). Nevertheless, the project still revealed there were females visiting the cleaning station, hence further research into other study sites would allow for conclusions about the female’s movement to be drawn, with studies already showing that multiple sites can vary sex ratios and are not often equal (Kruger, 2020).
4.3 | Future work

Factors which may influence the behaviour of *M. alfredi* around Baa atoll may not have been included in this study. Future work adding other unaccounted-for environmental factors could show alternate influencers which are not currently linked. Cloud cover has previously been shown to be a significant influence on manta ray behaviour (Barr & Abelson, 2019; Farmer et al., 2022). With a higher density of cloud cover occurring the light intensity is decreased, this reduces sunlight penetration into the water column thus reducing the rate at which plankton can photosynthesize, reducing aggregation densities and therefore making prey more sporadic (Evans and Parslow, 1985). Resulting in more energy expenditure for *M. alfredi* to hunt prey, hence during times of decreased cloud cover, an expected result would be reduced sighting events on cleaning stations as we would expect foraging behaviour to be exhibited.

The sea state should also be a variable considered when monitoring the behaviour of manta rays. Densities of plankton can be influenced by the turbidity and currents in the water (Hieronymi and Macke, 2010). These large-scale hydrodynamic processes such as turbulence and water motion can thus influence the high or low densities of plankton (Yen and Bundock, 1997). As a result, this would impact the congregative behaviour of *M. alfredi*, whose feeding effusiveness is dependent on environmental conditions for the aggregation of prey. With poorer sea conditions, (greater wave action and more turbulence) an increasing trend in the number of sightings at cleaning stations could be expected, with reduced foraging conditions elsewhere.

Finally, a biological factor for further investigation is the quality of cleaning and its effectiveness. These sites studied have a predominant cleaning species, the diurnal blue streak cleaner wrasse (*Labroides dimidiatus*). Cleaner wrasse primary activity hours are from sunrise to sunset (Slobodkin and Fishelson, 1974), and are also affected by hydrodynamic processes such as current speeds and sea state (Oliver et al., 2011) such factors may impair their ability to clean, resulting in clients avoiding the service and moving on (Bshary and Schaffer, 2002).
5 | Conclusion

Using research into the behavioural patterns of reef manta rays around the world has allowed for the designation of effective conservation and management strategies (Sutherland, 1998). Nevertheless, sub-populations of manta rays in differing geographical locations may vary in behavioural displays hence studies, such as those performed by the Manta Trust in specific locations such as Baa atoll, allow insight into *M. alfredi* behaviour. This work has provided insight into the environmental factors which may influence the behaviour of sub-populations of reef mantas surrounding Baa Atoll in the Maldives. Using this data has assisted in the designation of marine protected areas and other management strategies such as closures to public interference, regulating fishing rules locally and integrating governmental policy all may increase the protection of Maldivian reef mantas, a reduction in the rate of decline (Graham et al., 2012; Stewart et al., 2018).

The use of time-lapse underwater recording systems deployed by the Manta Trust research team has shown to be a useful aid in monitoring manta rays when there is a lack of human presence. The ability to monitor a site continuously for months over the course of three years has revealed important information on *M. alfredi* behaviour. The results of the study evidentially show the environmental conditions influence the rate of abundance at cleaning stations, when the conditions for foraging are unfavourable the density of *M. alfredi* at monitored cleaning stations is higher. Nevertheless, during periods of prime foraging conditions such as full moons, when prey aggregations are higher leading to increasing feeding activity there is a greater need for body maintenance for *M. alfredi*, which can lead to uncreased visitation rate to cleaning stations.

Studies such as these provide answers to key behavioural questions, relating to *M. alfredi* specifically in the Maldives. However, continual studying gathering more data is necessary to successfully guide future conservation planning and scientific guidance for the ongoing protection of Maldivian Reef Mantas.
Citations


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