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Project Report: Eyes on the Reef

Using remote cameras to uncover the hidden habits of reef manta rays (*Mobula alfredi*) in the Maldives

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Abstract

Reef manta rays (*Mobula alfredi*) are among the most charismatic creatures in our oceans attracting attention from tourists across the globe. These zooplanktivorous elasmobranchs are highly threatened directly and indirectly by human activities and are made further vulnerable due to their K-selective life history strategy of slow growth and low fecundity. As a result, populations have experienced global declines. In the Maldives, *M. alfredi* constitutes a major attraction for tourists, significantly contributing to the country's economy. To enforce and maintain sustainable tourism activities, research of the species' aggregation sites, behavioural patterns and the influencing environmental drivers is needed to provide robust, scientific guidance for successful conservation measurements.

In this study, three cleaning stations in the eastern Baa Atoll region of the Maldives were investigated using a novel remote underwater time-lapse camera system to allow long-term monitoring from July until November 2019. The influence of the following environmental factors on *M. alfredi* abundance was investigated: tidal phase, temperature, moon phase, time of the day and time within season (month). The moon phase was the most significant environmental predictor for *M. alfredi* with highest abundances during new moon. Higher visitation rates at cleaning stations during new moon were assumed to be a result of (1) an enhanced need for cleaning after mass feeding events and (2) more favourable cleaning conditions in the direct locations of the stations. Additionally, the times of tidal phases influenced the temporal presence of *M. alfredi* at cleaning stations with highest abundances during ebb and flood times, which might again be related to better cleaning conditions. Temperature and month showed no significant effects. However, a peak for sighting events was observed in October, possibly linked to courtship and mating behaviour.

Through photo identification, 129 different individuals could be identified showing differing sex ratios at each location and indicating a strong site affinity of observed individuals. Sighting events took up to 3.5 hours, demonstrating the essential and time-consuming role of cleaning for *M. alfredi*. Furthermore, in 25% of all sighting events manta rays were observed in groups of up to six individuals at a time. Enabling social behaviour might be an important driver for *M. alfredi* to visit cleaning stations, being partially independent of environmental factors.

Differences in manta ray abundance were also found between the three locations. As this complicates differentiating environmental drivers from variation between geographic locations, it is strongly recommended to monitor locations continuously in the future for more robust data analyses. This work presents a first approach to answer important conservational questions for *M. alfredi* with the method of remote underwater systems in the Maldives. The remote under water time-lapse camera system has proven to be a great complementary method to the standard in-water data collection, offering deeper insights into the behaviour of *M. alfredi* at cleaning stations when humans are absent.

Introduction

Reef manta rays (*Mobula alfredi*) are charismatic, very large zooplanktivorous elasmobranchs (Couturier *et al*., 2012) that belong to the taxa Mobulidae, which currently includes eight species (White *et al.*, 2017). *M. alfredi* is distributed circumglobally in tropical and subtropical waters (Couturier *et al.*, 2012). Due to their conservative life history traits including slow growth, late maturity and low fecundity Mobulidae are highly vulnerable to overexploitation (White *et al.*, 2006; Dulvy *et al.*, 2014; Stewart *et al.*, 2018).

In the last two decades, the threat of both targeted and bycatch fisheries has resulted in *M. alfredi* population declines (Croll *et al.*, 2016; Lawson *et al.*, 2017). The main reason to target Mobulidae is the demand for their gill plates, which are used in traditional Asian medicine and are believed to cure a variety of aliments (Croll *et al.*, 2016; O'Malley *et al.*, 2016). Additionally, unregulated tourism and habitat destruction (Murray at al., 2019), as well as climate change and pollution can have a dramatic impact on the animals, their habitat and food supply (Richardson, 2008; Manta Trust, 2019a). Consequently, they are currently listed as "Vulnerable to Extinction" on the IUCN Red List of Threatened Species (Marshall *et al.*, 2019).

Since the 1980s, non-consumptive uses of marine resources have become increasingly popular due to their far greater long-term value in comparison to the short-term benefits of consumptive uses (O'Malley *et al.*, 2013). This value is not only of ecological and social nature, but also the economic benefits can increase significantly (Bäuer, 2003; Gallengher & Hammerschlag 2011; O'Malley *et al.*, 2013). One option of non-consumptive use is ecotourism; in the marine realm ecotourism mainly focusses on observing megafauna (O'Malley *et al.*, 2013).

The Republic of Maldives, having the largest known population of *M. alfredi* in the world, is estimated to generate ~US$8.1 million annually directly from manta ray focused diving and snorkelling activities (Anderson *et al.* 2011). Since 2007 the Manta Trust has identified over 4.900 different individuals of reef manta rays from more than 70.000 photo identification (photo-ID) sightings, which makes it one of the most intensively studied populations in the world (Manta Trust, 2020). As a result of recognising the value of living reef manta rays, a number of measures have led to their protection on a governmental level such as banning exports of all ray species and products (Anderson *et al.*, 2011), creating marine protected areas (Anderson *et al.*, 2011) and the implementation of management plans to enforce sustainable tourism practices and strict regulations within marine protected areas, such as Hanifaru Bay (EPA, 2011; Manta Trust, 2020).

Hanifaru Bay is located at the eastern edge of Baa Atoll, where mass aggregations of feeding mantas can be observed from May to December each year (Stevens, 2016). During the Southwest Monsoon, deep-water upwelling occurs outside the atoll walls and transports nutrient rich water to the surface, resulting in a high abundance of zooplankton (Doty & Oguri, 1956; Stevens, 2016). Due to the tides and currents, the nutrient rich water is sucked inside the atoll channels, and restrained by the atolls and reefs within them (Stevens, 2016; Manta Trust, 2019a). Because of its unique outer reef structure, Hanifaru Bay particularly traps vast amounts of zooplankton, which makes it a key aggregation site for *M. alfredi* (Stevens, 2016). However, Maldivian reef manta rays not only favour these channels and lagoons to feed. Additionally, they visit cleaning stations close to the channels where they can be frequently accessed by tourists (Kitchen-Wheeler, 2010; Stevens, 2016).

At cleaning stations, usually a reef outcrop or a coral bommie, megafauna such as *M. alfredi* rid themselves of parasites, bacteria and detritus build-up with the help of cleaner fish (Clark, 2010; Stevens, 2016). In addition to cleaning, manta rays may aggregate at cleaning stations for several reasons: Firstly, metabolic and physiological functions such as digestion and gestation are thought to be enhanced due to the elevated temperature in the shallow waters of the cleaning stations (Hight & Lowe, 2007; Jirik & Lowe, 2012). Secondly, the stations possibly act as a refuge from pelagic sharks (Marshall & Benett, 2010) and cetaceans (Anderson, 2005). Moreover, the majority of courtship and mating behaviour of manta rays has been reported at cleaning stations (Stevens, 2016). Therefore, cleaning stations are important gathering points where social behaviour of *M. alfredi* can be observed (Stevens, 2016). Studying these central aggregation sites, the behavioural patterns and the influencing environmental drivers, contributes to filling knowledge gaps essential for *M. alfredi* conservation.

Various environmental drivers have been shown to influence patterns of movement in elasmobranchs (Freedman & Roy, 2012; Rohner *et al.*, 2013; Schlaff *et al.*, 2014; Harris *et al.* 2020). In this study, the focus has been put on the following factors: tidal phase, temperature, moon phase, time of the day and time within season (month). All of which have been demonstrated to influence the occurrence of planktivorous elasmobranchs at cleaning stations (O'shea *et al.*, 2010; Rohner *et al.*, 2013; Barr & Abelson 2019).

Most published field research on the behaviour of *M. alfredi* has been conducted predominantly at cleaning stations (Couturier *et al.*, 2012; Flowers *et al.*, 2016; Stevens, 2016). However, long-term studies monitoring cleaning stations are scarce (O'Shea *et al.*, 2010; Barr & Abelson, 2019). SCUBA and free diving only offer a short glimpse into manta ray activity at these sites each day, greatly limiting data collection and the ability to monitor cleaning stations in the absence of human presence.

The project "Eyes on the Reef" by the Manta Trust aims to improve this knowledge by using a novel remote underwater time-lapse camera system to allow long-term monitoring of three cleaning stations in the Maldives. The objective of this study is to assist conservation planning by (1) determining the influence of environmental factors on reef manta ray abundance at cleaning stations and (2) investigating the behavioural patterns of this species at cleaning stations in the absence of human presence.

Material and Methods

Study area

Three cleaning stations located in the key manta aggregation sites within eastern Baa Atoll in the Maldives were sampled from 4th of July until 24th of November 2019 (Fig. 1). Exact locations are not disclosed due to previous thefts of research equipment and for conservation purposes. Therefore, locations depicted in Figure 1 are an approximation of the study site localities. The first cleaning station Vinaneiy Faru (VF) had a depth of seven metres. The second, Thiladhoo Faru (TF), showed a depth of around eight metres and the third, Dhandhoo Diner (DD), was the shallowest station with five metres. At all stations the direction of currents constantly changes, depending on the rising and falling of the tide. The predominant cleaner fish species observed at these stations are the blue-streaked cleaner wrasse (*Labroides dimidiatus*), the bicolour cleaner wrasse (*Labroides bicolour*), the moon wrasse (*Thallasoma lunare*) and the two-tone wrasse (*Thallasoma amblycephalum*) (Stevens, 2016).

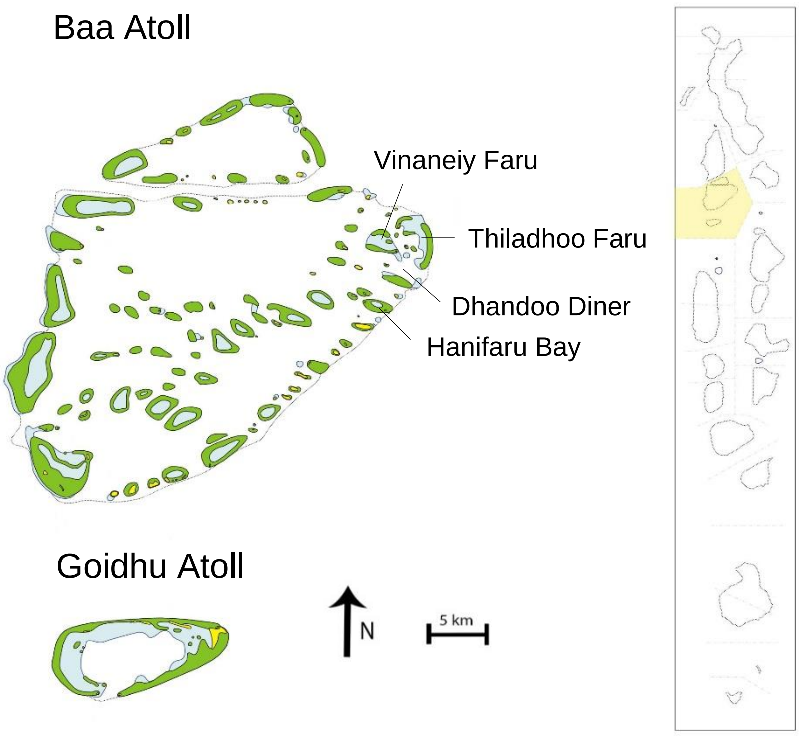

Fig. 1: Map of Baa Atoll region showing approximate locations of the studied cleaning stations and the feeding aggregation site Hanifaru Bay. The region in relation to the rest of the Maldives Archipelago (shaded in orange) is shown on the right (modified after Manta Trust, 2019a).

Experimental design

The three cleaning stations chosen for the project were based on short term remote underwater video surveys (up to 4 hours) conducted during 2017-2019 field seasons. In 2017, during routine site surveys, positions of cameras on particular coral bommies were selected based on frequent manta ray encounters. For the "Eyes on the Reef" project the time-lapse camera system was deployed randomly between the different cleaning stations. The majority of time it was stationed at Vinaneiy Faru (52 days), followed by Thiladhoo Faru (23 days) and lastly Dhandhoo Diner (17 days).

Data collection

For data collection, a GoPro Hero 4 Camera (San Mateo, USA; resolution 1080p; fps 30; mode wide angle) was secured in a self-made camera housing (Fig. 2 C, D dimensions: 31.5x20x16.5 cm). The camera setup was placed consistently two metres away from the edge of the cleaning station, measured with a one metre long section of PVC pipe. The back end of the housing was lowered further into the sand to ensure the camera was in a slightly upward facing position (Fig. 2 A, B). The GPS location and direction of lens angle were collected at the beginning of deployments.

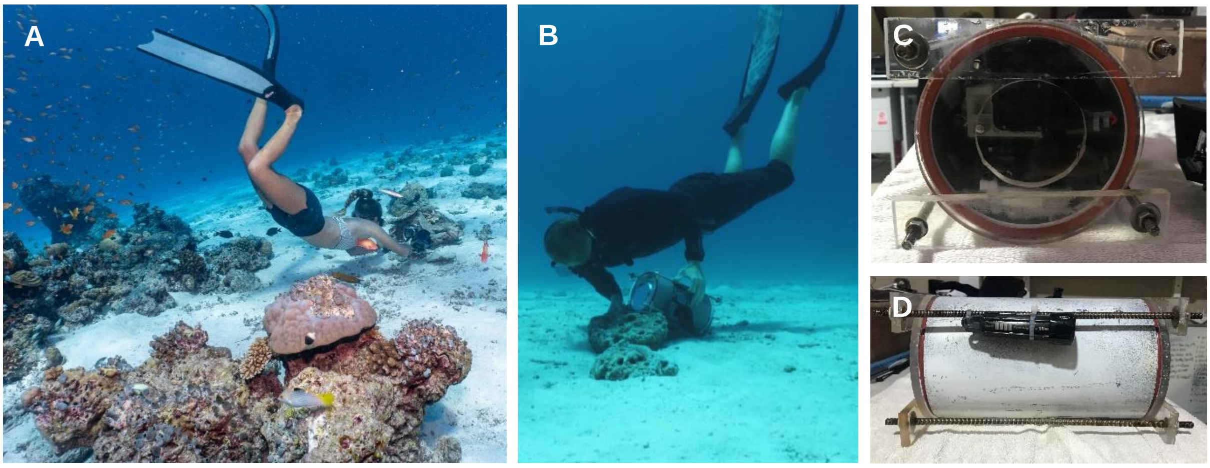

Fig. 2: Photographs from the field: (A, B) Placements of the camera system in an upwards facing position 2m away from the edge of the cleaning station. (C) Self-made camera housing from the front and (D) from the side with the attached HOBO water temperature logger.

For each day the moon phase, average water temperature and the high tide time (GMT+5) was recorded. The water temperature was measured using the HOBO Water Temperature Pro v2 Data Logger (Bourne, USA) that recorded hourly. Moon phase was recorded via online moon phase calendars (https://www.timeanddate.com/moon/phases/maldives/male). Tide charts were provided by Moto and Moosa, operators of a diving company in the Maldives. If both high tides occurred within the survey time, the highest high tide time was recorded. If only one high tide occurred within the survey period that one was recorded, regardless if it was the highest or not. For further analyses, low tide times were recorded in the same way. To define an intermediate state between high and low tide, the ebb or flood tide times (depending on chosen low tide) were determined by the midpoint between high and low tide.

The time-lapse recorder was setup to turn on before sunrise and off again after sunset. Accordingly, the recorded time differed from day to day, but always lay within the time frame of 5:45 to 18:45 hours. Recordings were made for periods of up to 13 hours a day. The time-lapse recorder was configured to take one photo each minute. Overall, 92 days were recorded between July and November 2019 with a total of 57.376 photos taken.

Analysis

Firstly, the number of manta rays captured in each photo was counted. Additionally, so-called ‘sighting events’ were recorded for each day, consisting of event time, duration and maximum number of manta rays in one photograph (MaxN). As photos were only taken every 60 seconds with a limited field of view, it was likely that a manta ray present at the site would not be recorded in every single photo for the entire duration it spent on the station. Therefore, a sighting event was continuously counted even if manta rays were absent in a few photos. If 10 or more minutes passed without a manta ray being captured in a photo, it was assumed that the individual(s) left the cleaning station for a period of time and the next appearance was counted as a new sighting event (Peel, 2019). From the maximum number of rays present in the sighting events, the number of manta rays present per day was estimated.

Secondly, photos of the ventral spot pattern of mantas rays were pulled out for photo identification. The ventral skin markings among manta rays are a unique “fingerprint” that remains unchanged throughout the animal’s life and thus, can be used to identify each individual (Marshall & Pierce, 2012; Stevens, 2016). This study used the black spots between the gill slits and upon its lower abdomen (Fig. 3). All photo-ID’s from this study were manually matched to the individuals of the Maldivian Manta Ray Database from the Manta Trust.

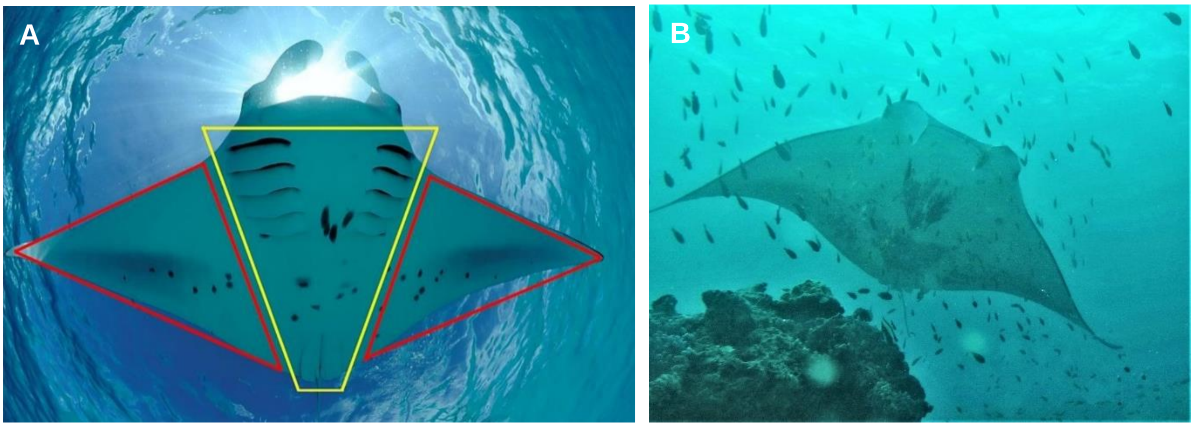

Fig. 3: Photographs of the unique spot patterns on the ventral surface of each *Mobula alfredi*. (A) shows the primary (yellow) and secondary (red) areas used to identify individuals (Stevens, 2016). (B) Photograph taken by the GoPro on the 01/11/2019 in the Vinaneiy Faru cleaning station.

Data Analysis

A Generalized linear model (GLM) with ranks was performed to identify the significant environmental variables influencing manta ray's abundance. Manta ray abundance was measured as MaxN, which is generally acknowledged to provide a conservative estimate of abundance (Cappo *et al.*, 2004; Campbell *et al.*, 2015; Sherman *et al.*, 2018). Significant variables were determined by a stepwise selection procedure and the model was reduced accordingly. When variables showed significant differences, a Tukey test was conducted to determine which states within each variable were different. To test the homogeneity of variance, a one-way ANOVA with squared residuals (equals Levene test) was performed. The influence of the tides was further investigated. High, low and ebb/flow tide times were not only correlated with MaxN, but also with the sighting event times. Pearson's correlation coefficient r was calculated. Correlations were also performed to show the relationship between different abundance indices used.

All statistical tests were conducted with the software SAS Studio at a significance level of $\alpha = 0.05$. Only for the test for homogeneity of variances α was set to 0.1.

Additionally, descriptive statistics were used to summarise further research aspects. All data was recorded and assessed using Microsoft Excel 2019.

Results

Assessing different indices to represent manta ray abundance

Three different indices have been determined to represent manta ray abundance: MaxN, estimated number of manta rays and the abundance score (number of photos with manta rays present per day/total number of photos per day). Main statistical analysis was performed with MaxN. For graphical depiction however, different indices have been chosen, depending on the availability of corresponding data and suitability to present different aspects. In Table 1 it is shown that all indices are highly correlated with each other. Accordingly, they have been used to represent manta ray abundance.

Tab. 1: Pearson's correlation coefficient r and p-value for the three different indices representing manta ray abundance.

		MaxN	**Estimated No.**	**Abundance Score**
MaxN	*r*	1.00000	0.82203	0.78604
	p		<0.0001	<0.0001
Estimated No.	*r*	0.82203	1.00000	0.63133
	p	<0.0001		<0.0001
Abundance Score	*r*	0.78604	0.63133	1.00000
	p	<0.0001	<0.0001	

Influence of environmental factors

The GLM explained 18% of the total deviance, using 5 degrees of freedom. The analysis revealed that only two of the five investigated variables (month, high tide time, moon phase, temperature and location) showed a significant influence on the manta ray abundance (MaxN). The final model (p = 0.0035) includes the variables moon phase (p = 0.0259) and location (p = 0.0112). The one-way ANOVA with squared residuals confirmed the parametric assumption of homogeneity of variances (p = 0.4142). Although the variables themselves were statistically significant, not all states within both variables were significant. For moon phase, the Tukey test revealed a significantly higher abundance at new moon compared to the third quarter. Manta ray abundance does not differ significantly between new moon, first quarter and full moon, as well as between full moon, first quarter and third quarter. However, abundances of first quarter and full moon are very close to the abundance during the third quarter (Fig. 4). For location, the Tukey test showed that manta ray abundance is significantly higher in Thiladhoo Faru compared to Vinaneiy Faru and Dhandhoo Diner. In contrast, there is no significant statistical difference between Vinaneiy Faru and Dhandhoo Diner (Fig. 5).

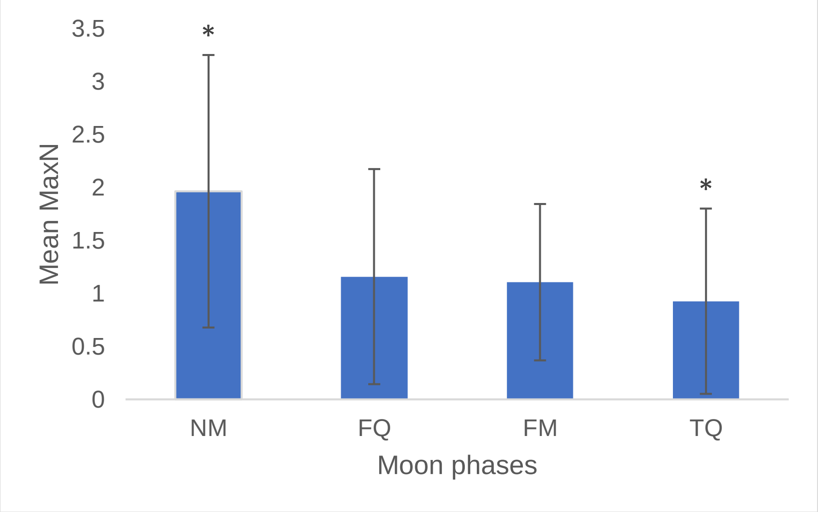

Fig. 4: Maximum of observed reef manta rays in one photo per day (MaxN) for the three moon phases (NM = New Moon, FQ = First Quarter, FM = Full Moon, TQ = Third Quarter). *'s indicate states that differ significantly from each other. Values indicate mean ± SE.

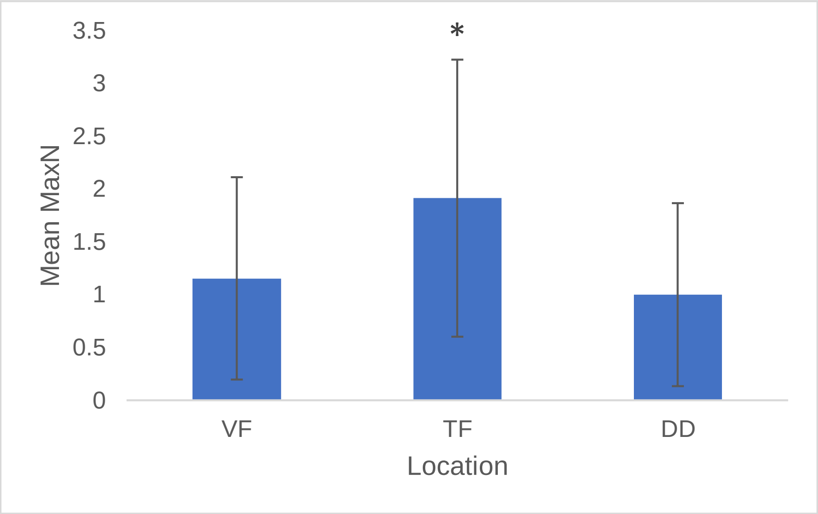

Fig. 5: Maximum of observed reef manta rays in one photo per day (MaxN) for the three different locations (VF = Vinaneiy Faru, TF = Thiladhoo Faru, DD = Dhandhoo Diner). * indicates significantly different state. Values indicate mean ± SE.

The variables high tide time, temperature and month did not show a significant influence on manta ray abundance. When looking at the manta ray abundance plotted against the single days, it becomes obvious that peaks within the month mostly appear during or around new moon phases (shaded in light blue, Fig. 6).

Even though no significant trend could be found for manta ray abundance between months, the average time manta rays spent at cleaning stations has a clear peak in July and a minimum in September. August, October and November display similar values for average time spent at the cleaning stations (Fig. 7). For the sighting events per day, showing how often the manta rays visited the cleaning stations, July shows a high abundance again. Surprisingly, the most sighting events per day occurred in October (Fig. 7).

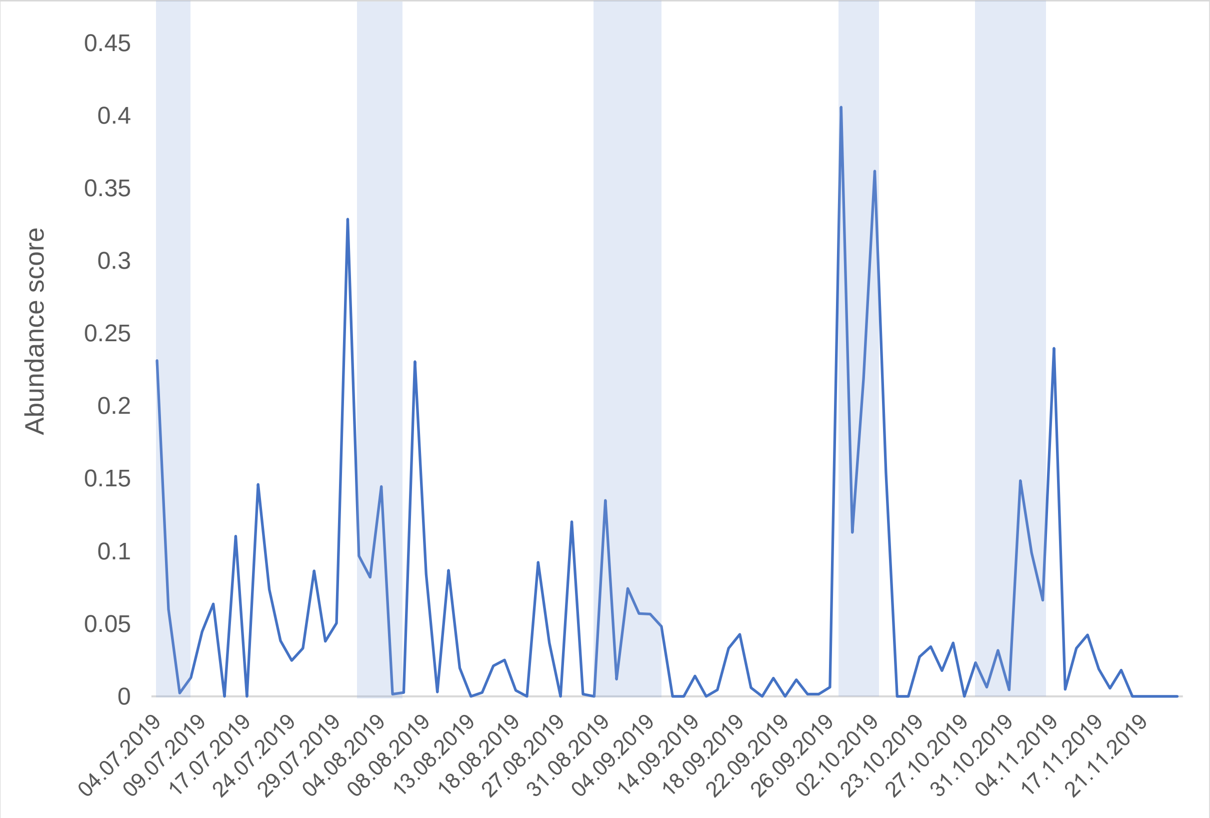

Fig. 6: Reef manta ray abundance score for selected days in regular intervals throughout all sampled months. Light blue rectangles indicate new moon phases.

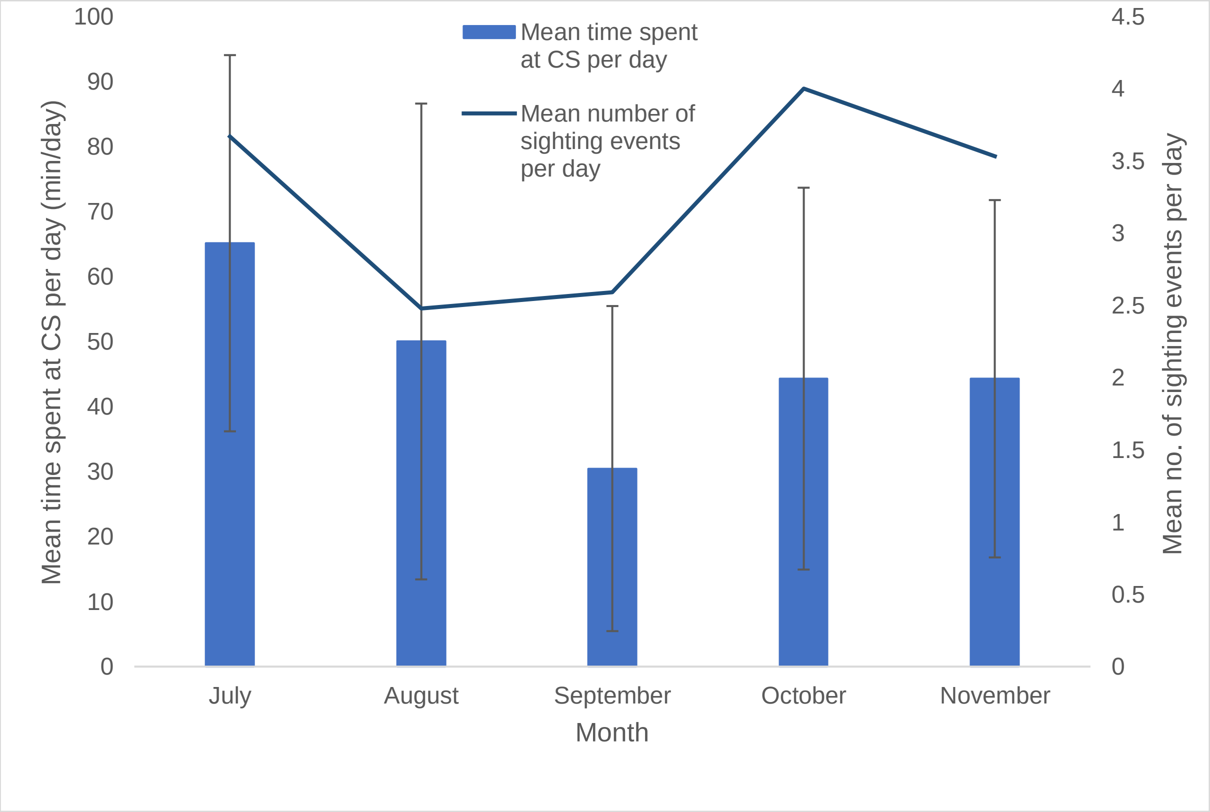

Fig. 7: Time reef manta rays spent at cleaning stations (mean ± SE) and mean number of sighting events per day for the five investigated months.

The high tide time had no significant effect on MaxN. An additional correlation between MaxN and the low tide times and flood/ebb tide times revealed no significant trend either ($p = 0.4549$, $p = 0.8756$, respectively).

As MaxN does not contain information on when the manta rays were present, further correlations between the times of the single sighting events and the high, low and ebb/flood tide times were performed. The high tide time and sighting event times were highly significantly correlated ($p < 0.0001$), but the correlation coefficient was rather small ($r = 0.13923$) indicating that only a small percentage of variability in manta ray abundance can be explained by the high tide. The correlation coefficient for the low tide time ($p < 0.0001$) was even smaller, only half of the high tide times' coefficient ($r = 0.07445$). The correlation for the ebb/flood times ($p < 0.0001$) revealed the highest correlation ($r = 0.16573$).

The abundance pattern at various times of day can be observed in Figure 8. Abundance gradually increases, reaching a peak between 9:00-10:00 in the morning after which, abundance gradually decreases again. Generally, 41% of all manta rays could be observed within only three hours of the day, between 9:00-12:00 (Fig. 8).

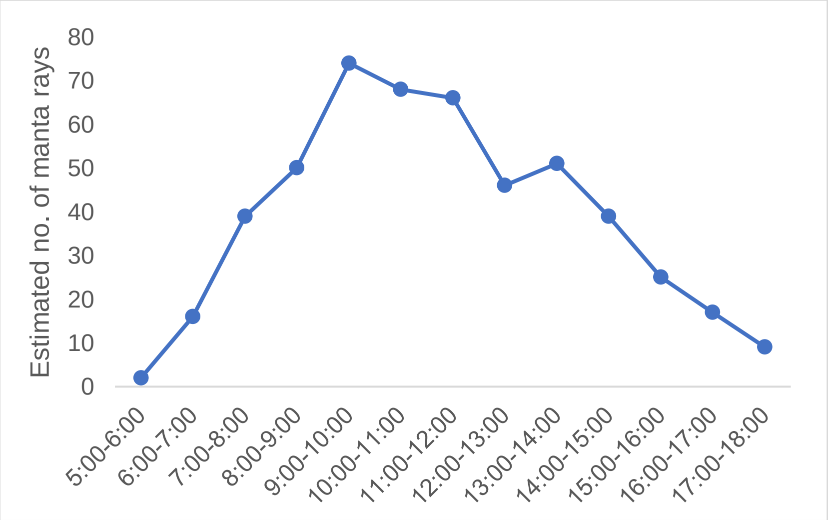

Fig. 8: Estimated number of reef manta rays per time interval over all months.

Behavioural patterns and identity of manta rays

In order to assess the duration of time that manta rays spent cleaning, the number of sighting events were plotted against the sighting event durations (Fig 9). More than a third (35.64%) stayed for ≤ one minute; it is assumed that these mantas were just passing by and not stopping to clean. Cleaning behaviour took place between two to 217 minutes (mean = 23 minutes). Almost 50% of the individuals recorded stayed between two and 20 minutes (Fig. 9).

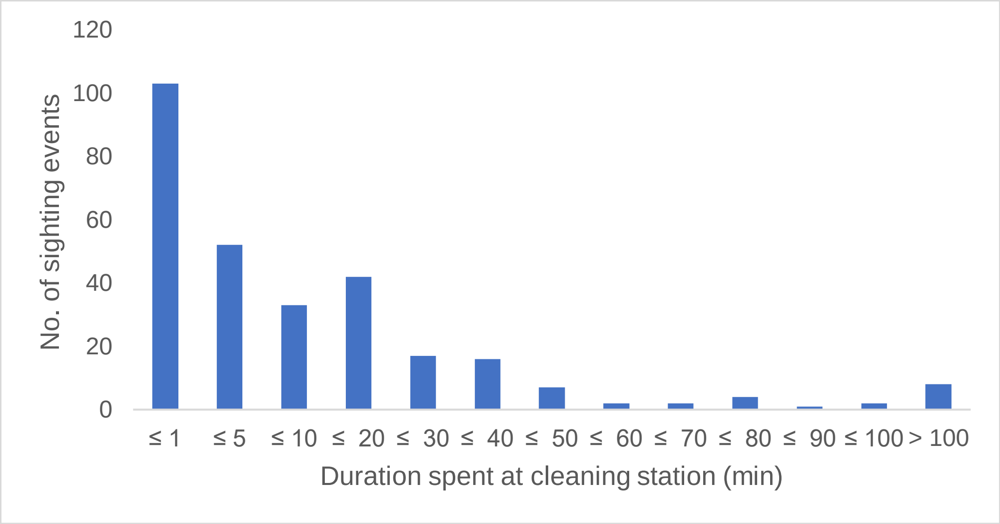

Fig. 9: Number of sighting events plotted against the durations of sighting events.

To estimate how often manta rays clean together, sighting events of ≤ one minute were excluded, assuming these individuals were just passing by and not cleaning. Figure 10 shows that around 75% of all mantas observed in this study have been cleaning alone. Therefore, almost 25% of the manta rays recorded were cleaning in company, mainly in groups of two to three, but reaching up to six individuals at a time.

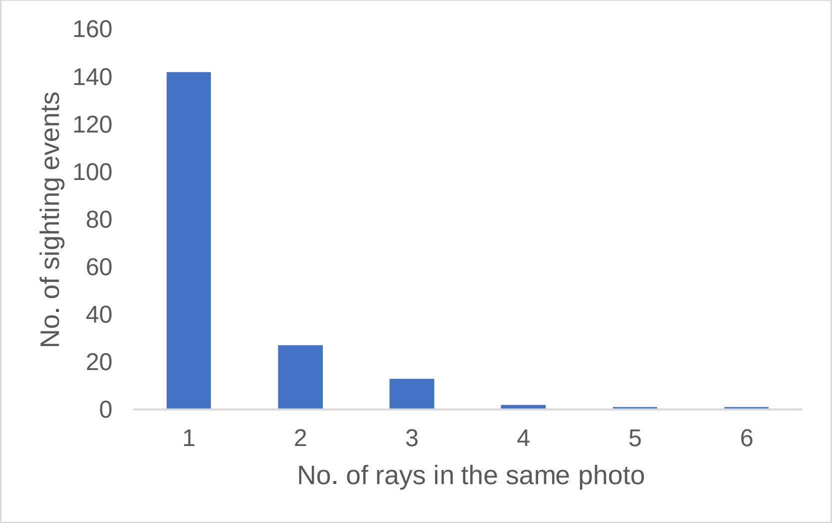

Fig. 10: Amount of sighting events shown for the number of reef manta rays observed at the same time in one photo.

The number of manta rays confirmed through photo identification was 194, which is about half of the number of estimated rays (361). This is not surprising, considering that manta rays needed to be quite close and swim at a specific angle over the camera so that the ventral spot pattern could be photographed by the stationed camera. Often manta rays were only observed at a distance, would turn their back to the camera or the distinct pattern was obscured by other animals or part of the coral reef.

In total, 129 different individuals from the Maldivian Manta Ray Database were identified. The most sighted individual was Dimples (MV-MA-1407) (Fig. 11). Dimples, an adult male, has been sighted on seven different days in four of the five investigated months (not in August). The most manta rays were confirmed on the 29th of September, where in total 17 different individuals were observed at the cleaning station Thiladhoo Faru.

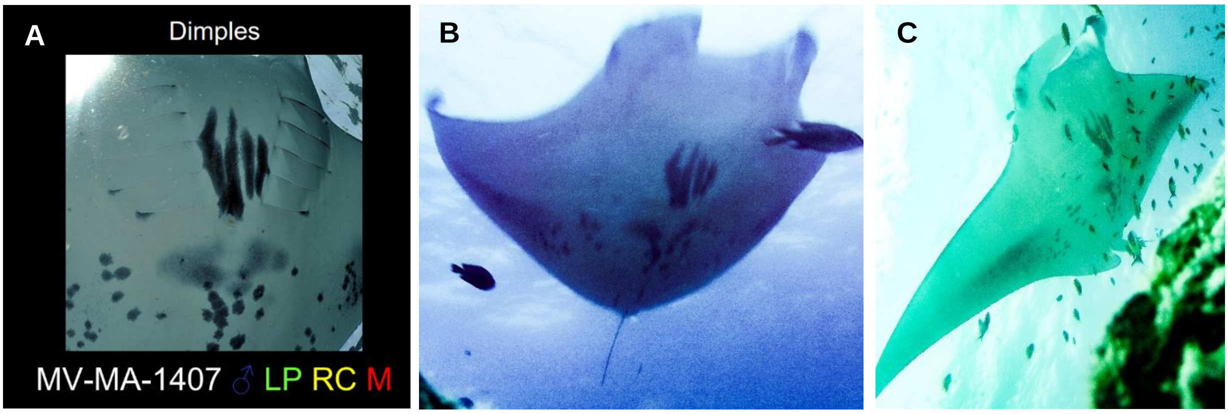

Fig. 11: Photographs of the unique spot pattern on Dimple's ventral surface. (A) Slide from the Maldivian Manta Ray Database containing Dimples' official code, gender and information of damages (coloured letters). (B, C) Photos of Dimples taken by the GoPro on two different days.

The overall sex ratio was 52% female and 48% male. In Figure 12 the sex ratio is compared between each of the three different cleaning stations.

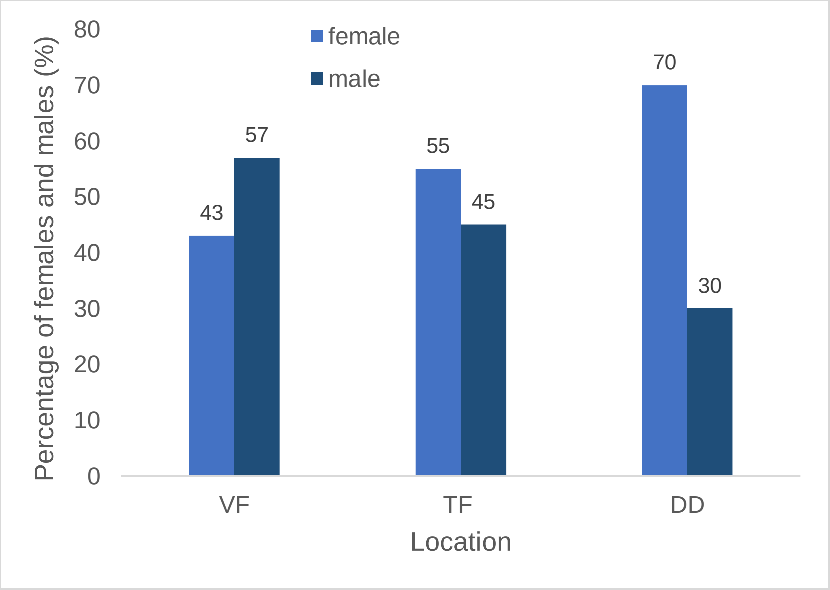

Fig. 12: Sex ratio for the three different locations (VF = Vinaneiy Faru, TF = Thiladhoo Faru, DD = Dhandhoo Diner).

Regarding site affinity, 35 manta rays were observed repeatedly. 66% of those manta rays were always found at the same cleaning station, even throughout the different months. The remaining 34% visited other cleaning stations at least once.

Discussion

Influence of environmental factors

Among all environmental factors assessed, the moon phase was found to be a potential predictor of *M. alfredi* abundance at cleaning stations. The moon phase influences the marine environment by hydrodynamic processes triggered by the tides, creating the strongest tidal intensity during new and full moons (Neumann, 1981; Rohner *et al.*, 2013). Additionally, the moon phase has an influence on light availability (McFarland, 1999; Cohen & Forward, 2005). Both of the above-mentioned effects can directly influence the zooplankton abundance (Kingsford *et al.*, 1991; Benoit-Bird *et al.*, 2009; Webster *et al.*, 2015).

Barr & Abelson (2019) suggest that under low light conditions plankton are scattered, resulting in less efficient foraging for *M. alfredi*. As cleaning and feeding activities usually do not occur at the same time and place (O’shea *et al.*, 2010; Barr & Abelson, 2019; Pers. Recomm. Kaitlyn Zerr, 2020), visiting cleaning stations under low light conditions would be more beneficial for *M. alfredi*. In contrast, higher light intensity results in the formation of plankton aggregations, triggering the foraging behaviour and hence, the absence of manta rays at cleaning stations. Furthermore, high levels of moonlight induce downward vertical migrations of macrozooplankton (Webster *et al.*, 2015), resulting in deeper night-time diving of *M. alfredi* likely to be linked to foraging (Lassauce *et al.*, 2020).

These findings correspond with highest abundance of *M. alfredi* at cleaning stations during new moon phases found in this study (Fig. 4). However, both full moon and new moon present the most favourable conditions for feeding in Hanifaru Bay (Pers. Recomm. Kaitlyn Zerr & Tam Sawers, 2020). Therefore, the new moon phase might show different effects on the different sites within the Baa Atoll region. Hanifaru Bay could display favourable feeding condition during new moon, while conditions might be less favourable at cleaning stations due to their more exposed location and the surrounding reef structure. On the other hand, the increased cleaning activity during new moon might be in-line with increased feeding activity in Hanifaru Bay. After mass feeding events, the need to rid themselves of detritus build-up might increase and accordingly, manta rays would spend more time at near-by cleaning stations. Additionally, the elevated temperature in shallow waters of cleaning stations could aid the digestion (Hight & Lowe, 2007). However, higher abundances at cleaning stations were not observed during full moon, objecting this assumption. A lot more data will have to be collected over several seasons to identify more concrete trends. It is assumed that a combination of more favourable cleaning conditions in the direct locations of the cleaning stations and the enhanced need for cleaning after mass feeding events in Hanifaru Bay result in a higher abundance of *M. alfredi* at cleaning stations during new moon phases.

The other significant variable affecting the abundance of *M. alfredi* was the location itself (Fig. 5). Reasons for that might be various, as not all cleaning stations have been sampled evenly throughout the months, making it difficult to determine if the studied environmental variables or any other trait of the location itself was the driving factor. The variation between and within geographic locations must be considered and must be presented with a sufficient sample in order to be able to uncover species behaviour (Schlaff *et al.*, 2010). Nevertheless, due to the close proximity and similar characteristics of the observed cleaning stations all data has been pooled over the different locations to raise sample size. Still, it is strongly recommended for future sampling to either continuously monitor all three stations or, if only one camera system is available, only monitor one station in order to make convincing statements on the influence of environmental factors.

Temperature did not have a major impact on *M. alfredi* abundance in this study, as the sampled cleaning stations were all located in the same area with similar characteristics, resulting in mostly constant temperatures throughout the season.
There were no significant differences between the months within the season regarding *M. alfredi* abundance. However, Figure 6 shows that the abundance peaks always match the new moon phases, or at least the days close to a new moon phase. This corresponds with the preceding findings of higher abundances during new moon, as a result of favourable cleaning conditions and the enhanced need for cleaning after mass feeding events. The average time *M. alfredi* spent at cleaning stations was highest in July and lowest in September (Fig. 7). When comparing this to the Manta Trust's Annual Report for the Baa atoll region 2019 there is no such trend visible. July and September had almost the same number of sightings for the whole Baa Atoll region and Hanifaru Bay, the main feeding site. Interestingly though, the peak for sighting events at the cleaning stations was found in October (Fig. 7), whereas in Hanifaru Bay the lowest number of sightings (40% less compared to other months) occurred in October (Manta Trust, 2019a). This might be another indication for changing temporal and spatial movement patterns of *M. alfredi* depending on the conducted activity (cleaning or feeding). Furthermore, courtship behaviour and mating generally take place at cleaning stations and are much more frequently observed during October and November in the Maldives, when the two monsoons transition from one to the other (Stevens, 2016; Manta Trust, 2019b).

It has been investigated in several publications that tidal-driven movement and behavioural patterns of elasmobranchs can be associated with foraging tactics, energy conservation strategies and predator avoidance (see review Schlaff *et al.*, 2014). This study shows that times of tidal phases have an influence on the temporal presence of *M. alfredi* at cleaning stations. It has to be mentioned that all investigated times of tidal phases correlated significantly, but only showed a slightly positive effect on the times of the sighting events. Therefore, it is assumed that tides have an influence on *M. alfredi* abundance, but it is likely a

small contributor to a much more complex system. The intermediate state between high and low tide, the ebb and flood times, had a slightly higher influence on the abundance than high tide times and more than double the effect of low tide times. Feeding activity in Hanifaru Bay is known to be highest at full and new moon particularly around the high tide, displaying optimal conditions for feeding (Pers. Recomm. Tam Sawers, 2020). When the tide falls (ebb tide) manta rays probably move to the cleaning stations to get rid or detritus build-up after feeding and to aid digestion. For rising tide (flood tide) the manta rays might be more active on cleaning stations near to the feeding aggregation sites, waiting for optimal feeding conditions during high tide. The low tide probably shows the lowest abundance of *M. alfredi* as it is furthest away from high tide with optimal feeding conditions.
These results correspond with previous findings suggesting strong tidal patterns of *M. alfredi* at cleaning stations and a higher level of cleaning interaction during ebb times (O'shea *et al.*, 2010). Another study found increasing sightings of *M. alfredi* around high tide, peaking within the first hours of ebb tide (Jaine *et al.*, 2012). In the study from O'Shea *et al.* (2010) plankton availability at the observed location was low during ebb tides, suggesting better conditions for cleaning and thus, a higher abundance at the stations. Determining zooplankton concentrations during different tidal phases at the monitored locations in Baa atoll would offer a greater understanding of the relation between abundance of *M. alfredi* and tidal phase.

M. alfredi presence at cleaning stations was found to increase gradually, reaching a peak at 9:00-10:00 in the morning and after that, gradually decreased again (Fig. 8). As described before, only a small portion of their temporal appearance at cleaning stations can be explained by the tidal phase. Setyawan *et al.* (2018) monitored *M. alfredi* at different feeding and cleaning sites in Raja Ampat, Indonesia with passive acoustic telemetry. They detected the exact same patterns of the temporal appearance of *M. alfredi* throughout the day*,* suggesting they move deeper and further away at night, probably as foraging tactic. Because of the change in location of plankton during night, there are less manta rays towards the evening and it simply takes them more time to return in the mornings. Accordingly, the observed pattern might be typical for *M. alfredi* abundance during the day in coastal areas. The preference for cleaning in the morning is probably a response to feeding during the night in colder waters. In the following mornings they can rid themselves of parasites and aid digestion in the warmer and shallow water of the cleaning stations (Hight & Lowe, 2007; Clark, 2010). Other studies found no hourly trend (O'shea *et al.*, 2010) or slightly different patterns, peaking early in the morning and between 12:00-14:00 (Jaine *et al.*, 2012).

Behavioural patterns and identity of manta rays

Cleaning behaviour was recorded up to 3.5 hours with a mean of 23 min per sighting events. This corresponds with findings from O'shea *et al*. (2010), who observed cleaning up to 5 hours with a mean of 31 min. Furthermore, O'shea *et al.* (2010) confirmed through photo identification that single individuals stay around 60 min at a cleaning station. As not all individuals could be confirmed during the sighting events of this study, it is likely that multiple manta rays visited the cleaning stations adding up to these long event durations. However, for the longest sighting event (217 minutes), one individual was confirmed to be present for almost the whole time (215 minutes), showing the importance of cleaning for *M. alfredi*, as it presents an essential and obviously time-consuming activity for the manta rays well-being (Grutter, 1999; Ros *et al.*, 2010).

As mentioned before the reasons for *M. alfredi* to aggregate at cleaning stations include, but are not limited to, metabolic benefits (Hight & Lowe, 2007; Jirik & Lowe, 2012), predator avoidance (Anderson, 2005; Marshall & Benett, 2010) and the search for a mating partner (Stevens, 2016). Enabling such behaviour and structuring social relationships might be one of the main causes to visit cleaning stations (Stevens, 2016; Perryman *et al.*, 2019).

In this study almost 25% of all sighting events did not observe solitary individuals, but groups of up to six manta rays. In other studies, manta rays have been observed in groups at cleaning stations as well (Perryman *et al.*, 2019), taking up to 16% of all observed encounters (O'shea *et al.*, 2010). O'shea *et al.* (2010) have also frequently observed social interactions among individuals before, during or after cleaning.

In addition to investigating how often multiple manta rays clean together, there is particular interest in investigating how often the same individuals clean together indicating a form of social structure. The same individuals were observed repeatedly together at the cleaning stations only twice on differing days across the whole data set. Quantifying structured social relationships in manta rays has only been done for an Indonesian population so far (Perryman *et al.*, 2019). This topic forms an interesting research focus, that will help to understand manta ray's natural behaviour and consequently help maximising conservation efforts. However, more specific data relating to the consistent identification of manta rays on cleaning stations is needed to address this issue.

Almost the same portion of males (48%) and females (52%) were identified at the three cleaning stations. This is consistent with the overall sex ratio for the Maldivian reef manta ray population (Stevens, 2016). However, the sex ratio varied considerably between the different cleaning stations (Fig. 12). Vinaneiy Faru had a slightly male-biased sex ratio. In contrast, the individuals identified at Thiladhoo Faru showed no big difference between sexes while Dhandhoo Diner demonstrated a strongly female-biased sex ratio. Differing sex ratios at cleaning stations across the same area have been observed in other studies as well (Germanov *et al.*, 2019; Perryman *et al.*, 2019).

Sex-based differences in habitat use for *M. alfredi* might be linked to social preferences. Perryman *et al.* (2019) have suggested that *M. alfredi* forms communities with different social structures and sex ratios, that are associated with site fidelity. Another explanation for sex-based differences is the reproductive behaviour (Deakos, 2010). Female manta rays, having the greater parental investment, gain a greater choice of mates by remaining in one aggregation site, while males benefit from moving around sites in the search of a female (Germanov *et al.*, 2019). This might explain the much higher abundance of females in Dhandhoo Diner. However, sample size at Dhandhoo Diner was considerably smaller compared to the other two sites, possibly resulting in a biased sex ratio.

The term 'site affinity' is used, when a difference between site fidelity and residency cannot be demonstrated clearly (Couturier *et al.*, 2011). Therefore, photo identification studies can generally describe site affinity as one of the broadest forms of philopatry (Flowers *et al.*, 2016). Multiple studies have given evidence for site affinity of *M. alfredi* (see review Flowers *et al.*, 2016). In this study, the majority (66%) of repeatedly observed *M. alfredi* were found at the same cleaning station, even throughout the different months, indicating a strong site affinity. O'shea *et al.* (2010) have documented much fewer repeated sightings (n = 3) at cleaning stations, suggesting relatively high exchange rates and the probability of more unknown cleaning stations close by.

As described before, site affinity might also be related to social structures in *M. alfredi*: Perryman *et al.* (2019) even suggest that food availability and habitat quality might not be as important as individual environmental or social preferences for *M. alfredi's* site affinity. The findings of this study only present a first approach on the site affinity of reef manta rays to the three investigated cleaning stations. More data collection and a more comprehensive analysis is needed for concluding statements.

In conclusion, remote underwater time-lapse camera systems are a useful tool to collect versatile long-term data without the need of humans to be present. The deployments of cameras can complement the SCUBA and free diving data collection of the Manta Trust. For example, nine individuals were sighted only on camera deployments and not by human observation, three of which were newly identified in 2019. While human observers may increase the number of confirmed photo-IDs and provide a detailed report on cleaning station activity, the addition of remote underwater cameras allows for continuous monitoring at the locations.

The results strongly indicate higher abundances of *M. alfredi* at cleaning stations, when the environmental conditions are more favourable for cleaning compared to feeding. It was also assumed, that an enhanced need for cleaning after mass feeding events had higher abundances of *M. alfredi* at cleaning stations as a consequence. However, presence at cleaning stations might be to a certain extent independent of environmental factors, as the social component might play a large role in influencing aggregations of *M. alfredi* at particular sites. In correspondence, the most sighting events were observed in October, one of the predominant months for courtship and mating behaviour of *M. alfredi*.

This work provides us with a first approach on answering important questions of behavioural patterns and influencing environmental drivers of *M. alfredi* at cleaning stations in the Maldives. More data collection and further research is necessary to provide robust, scientific guidance for successful conservation measurements and sustainable tourism of *M. alfredi*.

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Statutory Declaration

I hereby declare that I have authored this report independently and used only the sources and resources cited.

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig verfasst habe und keine anderen Quellen und Hilfsmittel als die angegebenen verwendet habe.

Lotte Krüger