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Justification: The Marine Ecology Progress Series is a leading journal in all aspects of marine ecology, publishing research fundamental for addressing ecological concerns and highlighting their implications for management such as spatial marine planning. This report provides new insight into the spatial and behavioural ecology of *Mobula alfredi* (reef manta rays), advancing a previously limited understanding that is crucial for localised conservation legislation programmes.
Variation in visitation patterns and habitat use of adult and juvenile *Mobula alfredi* (reef manta rays) at key aggregation sites in Raa Atoll, Maldives

Declaration: 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.
Abstract

*Mobula alfredi* (reef manta rays) continue to undergo rapid and persistent declines driven by pervasive anthropogenic threats. To provide effective conservation measures, a better understanding of their fine-scale habitat use is required, to identify critical aggregation sites and environmental drivers behind *M. alfredi* presence in these areas. In this study, remote underwater video systems (RUVs) were used in conjunction with abiotic environmental data and a photo-identification database to investigate small-scale habitat use and cleaning duration of adult and juvenile *M. alfredi* at three cleaning stations on western Raa Atoll, Maldives during the northeast monsoon between 2020 and 2022. Results are based on findings from 98 individuals identified from 470 sightings during daylight hours. Repeat sightings indicated high residency and site fidelity of both adult and juvenile *M. alfredi* at cleaning stations demonstrating the importance of these key aggregation areas. Coincident with previous studies, visitation by *M. alfredi* to cleaning stations was influenced by month, lunar phase, and tidal current. These environmental cues are known to affect prey availability to *M. alfredi* by providing temporally limited foraging opportunities, indicating cleaning activities may be linked to feeding in *M. alfredi*. The present study provides compelling evidence that juvenile *M. alfredi* have a stronger affinity for these cleaning stations than adults, suggesting immature individuals may be more susceptible to anthropogenic threats in this area. Juvenile *M. alfredi* cleaned for markedly shorter durations than adults, indicating larger individuals require more intense cleaning. These findings demonstrate RUVs can provide valuable insights into the temporal and behavioural visitation patterns of *M. alfredi* to cleaning stations. The results presented here provide baseline information on *M. alfredi* that can be used to inform marine spatial planning of conservation intervention programmes. This incites the need for further research to evaluate small-scale habitat use of key aggregation sites to assist conservation planning to provide protection for *M. alfredi* in other regions.
1.0. Introduction

Elasmobranchs represent a key component of marine biodiversity and are sentinel to the structural integrity and functioning of marine communities (Stevens et al. 2000, Bornatowski et al. 2014). They facilitate nutrient transport and recycling with their residency patterns and foraging movements (Peel et al. 2019). Their removal can induce cascading effects that propagate throughout the food web (Navia et al. 2010). Despite their crucial ecological role, elasmobranchs continue to face a broad range of stressors, partially driven by exponentially increasing anthropogenic activities such as overexploitation (Field et al. 2009; Humber et al. 2015) pollution (Wheeler et al. 2020, Consales and Marsili 2021), and habitat destruction (Ellis et al. 2005, Wheeler et al. 2020). The subsequent population declines and their relative effects exacerbate the risk of deterioration or change to marine ecosystem structure and function (Field et al. 2009, Halpern et al. 2015), inciting the need for effective management strategies.

Protections afforded by conservation legislation are expected to promote population recovery and amplify habitat diversity (Gilmour et al. 2022). However, it is difficult to provide the necessary coverage for wide-ranging elasmobranchs, as they are likely to move beyond stationary boundaries (Hooker and Gerber 2004). Successful protection partially relies on knowledge of species ecology including broad- and fine-scale movements, habitat use, behaviour, and spatially explicit population parameters (Ward-Paige et al. 2012). It is unlikely that adequate protection will be provided without incorporating this knowledge into conservation legislation programmes and for many elasmobranch species, this information is scarce or incomplete (Gilmour et al. 2022).

The reef manta ray *Mobula alfredi* is a conspicuous zooplanktivorous batoid species with a broad and highly fragmented geographical distribution throughout tropical and subtropical waters of the Indo-West Pacific Ocean (Couturier et al. 2014, Kashiwagi et al. 2011). Historically, coherence in mobulid taxonomy has been limited by a complex nomenclatural history and phylogenetic inferences (Marshall et al. 2009). After decades of study, the genus *Manta* was placed in synonym of *Mobula* and the
Mobulidae family was considered monogenetic, now comprising nine extant species (White et al. 2018).

*Mobula alfredi* frequent shallow coastlines or offshore oceanic islands often characterised by productive reef habitats with regular upwellings (Harris et al. 2020). Satellite and acoustic tracking and photographic identification techniques have shown long-term site fidelity and aggregation behaviours associated with feeding activities, reproductive interactions, and the presence of cleaning stations (Marshall and Bennett 2010, Anderson et al. 2011, Jaine et al. 2012, Kitchen-Wheeler et al. 2012, Weeks et al. 2015, Setyawan et al. 2022a). *Mobula alfredi* actively follow shifting patches of locally elevated zooplankton abundances triggered by environmental cues (Jaine et al. 2012, Armstrong et al. 2016, Couturier et al. 2018, Harris et al. 2020) to facilitate energetically efficient foraging activities (Luiz et al. 2009, Anderson et al. 2011). At cleaning stations, *M. alfredi* engage in associations with cleaner fish to remove bacteria, detritus and ectoparasites for physical health maintenance (Waldi et al. 2011, Stevens 2016, Dunkley et al. 2020, Armstrong et al. 2021). The ecological importance of cleaning stations and cleaner-client mutualisms to *M. alfredi* has been demonstrated in previous studies (Grutter et al. 2003, Waldie et al. 2011). For example, they are known to provide grounds for social and reproductive interactions between conspecifics (Stevens et al. 2018, Harris and Stevens 2021). They may act as a refuge site from large pelagic sharks such as *Galeocerdo cuvier* and *Carcharhinus leucas* (Marshall and Bennett 2010, Stevens 2016). Site affinity to these aggregation sites has been documented in the Maldives (Stevens 2016), Indonesia (Setyawan et al. 2018, 2020), eastern Australia (Couturier et al. 2018) and Mozambique (Venables et al. 2020), reflecting the importance of cleaning stations as a driver for movement patterns and habitat use.

*Mobula alfredi* are highly mobile and capable of covering wide expanses by undertaking long-range migrations over hundreds of kilometres (Luiz et al. 2009, Graham et al. 2012, Braun et al. 2015). Consequently, they are more likely to encounter anthropogenic threats in both protected and unprotected areas (Jaine et al. 2012). Targeted and bycatch fisheries partially driven by the demand
for Mobulid gill plates have prompted rapid and persistent population declines in *M. alfredi* (Lawson et al. 2017). Unmanaged tourism industries, habitat degradation and the climate crisis are examples of the many anthropogenic threats that exacerbate population declines (Harris and Stevens 2021). For example, the destruction of shallow reef habitats for boat access can disrupt visitation patterns to habitats (Barr and Abelson 2019). As ectothermic planktivores, *M. alfredi* respond rapidly to environmental drivers and are particularly sensitive to the potential effects of climate change (Jaine et al. 2012). *Mobula alfredi* are inherently vulnerable to extinction as they exhibit conservative life history traits such as slow growth rates, low fecundity and late maturity that hinder the recovery of depleted populations (Marshall et al. 2022). The accumulation of such anthropogenic pressures can push *M. alfredi* populations to critical thresholds, inducing shifts in population structure and stability, and marine food chain dynamics (Rohner et al. 2013, Lawson et al. 2017). Despite existing conservation measures, ample evidence of population declines co-occurring with ongoing fishing pressure continues to materialize (Lewis et al. 2015, Stevens and Froman 2018). Therefore, it is important effective protection is provided by identifying priority areas and environmental drivers that influence movement patterns to these areas.

The knowledge required to identify areas of conservation concern such as the broad spatiotemporal distribution of *M. alfredi* and factors that influence visitation and site fidelity is well documented (Harris et al. 2020, Harris and Stevens 2021). At present, there is a severe paucity of studies addressing the ecological reliance of *M. alfredi* on discrete habitats such as cleaning stations, and the factors that influence their use. Juvenile movements and residency patterns to key aggregation sites remain understudied (Setyawan et al. 2022a) with investigations analysing sexually mature individuals and juveniles opportunistically (Jaine et al. 2014, Couturier et al. 2018, Peel et al. 2019). Given that *M. alfredi* are in a global state of decline (Marshall et al. 2022), the need to conserve juvenile life stages by identifying critical habitats has become increasingly apparent. To assess the potential implications of anthropogenic stressors and provide more effective ecosystem-based management (EBM), a more detailed understanding of the individual temporal visitation patterns and small-scale habitat use by
adult and juvenile *M. alfredi* to ecologically important aggregation sites such as cleaning stations is required.

The Maldivian archipelago comprises 26 geographical atolls and supports the largest known subpopulation of *M. alfredi* (Kitchen-Wheeler et al. 2012). Their distribution patterns are strongly influenced by seasonal variance and weather patterns dictated by the South Asian Monsoon (Armstrong et al. 2016). Biannual reversing monsoon currents and localized upwellings deliver nutrients into the euphotic zone, generating shifting phytoplankton blooms and zooplankton hotspots (Radice et al. 2019). *Mobula alfredi* follow and exploit these rich feeding grounds that are often concentrated in productive channels and lagoons (Anderson et al. 2011, Armstrong et al. 2016, Stevens 2016, Stevens et al. 2018). Migration patterns along the monsoonal down-current edge of atolls vary seasonally with shifting distributions of chlorophyll-a (Chl-a; Armstrong et al. 2016). During the northeast monsoon (Iruvai) between December and March, *M. alfredi* use shallow reef habitats along the western edges (Harris et al. 2020). Conversely, between May and October during the southwest monsoon (Hulhangu), aggregations primarily occur on the eastern edges. These predictable distribution patterns have enabled the identification of key aggregation sites that are a focal point for ecotourism activities such as snorkelling and SCUBA diving (Stevens 2016).

Local and global threats continue to diminish the Maldives’ *M. alfredi* subpopulation despite their economic significance and protection from target fisheries (Harris et al. 2020). In total, 32 marine protected areas (MPAs) cover 0.5% of the surrounding waters of the 26 atolls in the Republic of Maldives (Stevens and Froman 2018, Harris et al. 2020). It is doubtful this limited area accommodates the movement of *M. alfredi* or provides adequate protection. Moreover, it is unlikely that protection will be afforded on a broad scale that could meet the range of such a mobile species (O’Leary et al. 2018). Anthropogenic threats continue to act as a driving force for *M. alfredi* population structure and function (Field et al. 2009, Rohner et al. 2013). Therefore, it is important to identify key aggregation sites so conservation measures can be focused and dedicated effectively (Harris and Stevens 2021).
Current knowledge of spatiotemporal variation in the distribution of *M. alfredi* has derived from an amalgamation of research using underwater visual census (UVC; Jaine et al. 2012), photo identification (Harris et al. 2020), and acoustic and satellite telemetry (McCauley et al. 2014, Couturier et al. 2018, Peel et al. 2019, Harris and Stevens 2021). Technological innovations in underwater video cameras have provided a plethora of accessible and affordable methods for non-extractive sampling of marine taxa (Bennett et al. 2016, Shortis and Abdo 2016). In particular, remote underwater video systems (RUVs) have garnered considerable praise in recent years for monitoring diurnal and seasonal patterns of behaviour, activity, and abundance (Lowry et al. 2012, Mallet and Pelletier 2014). Coupled with their ability to generate permanent data-rich records of qualitative information on habitats and empirical evidence of species, there have been extensive applications globally (Goetze et al. 2019). Despite this, studies using RUVs to study the small-scale habitat use by *M. alfredi* are scarce.

This study explores the temporal variation in occupancy and small-scale habitat use of adult and juvenile *M. alfredi* at major aggregation sites on the western side of Raa Atoll, Maldives during the northeast monsoon. Using RUVs, photo identification records, and abiotic environmental data, the study will specifically investigate: 1) If sex and/or maturity status affects the temporal visitation patterns of *M. alfredi* to cleaning stations, 2) whether sex and/or maturity status affects the duration of time spent cleaning by individuals and 3) the importance of month, cleaning station, lunar phase, tidal current and temperature in driving visitation patterns of juvenile and adult *M. alfredi*.

2.0. Materials and Methods

2.1. Study Location

This study was conducted at three *M. alfredi* cleaning stations located in Maamunagau Finolhu, situated on the west side of Raa Atoll, Maldives (5.6006° N, 72.9461° E; Figure 1). Data were obtained during the northeast monsoon when *M. alfredi* are known to exhibit predictable aggregation behaviours. Remote underwater video systems (RUVs) were deployed for consecutive years from 2020-2022, for the duration of January and April.
2.2. Remote underwater video system design and deployment

Data were collected using RUVs with the same set-up deployed at each site. This comprised a single high-definition GoPro Hero 4 Camera (resolution 1080p; fps 30; mode wide angle) secured in a GoPro camera housing (Figure 2). To ensure stability, the camera was affixed to a 1-kilogram (kg) dive weight using cable ties. At each site, RUVs were placed at a distance unique to each cleaning station, measured using a 3-metre tape measure. At site A, RUVs were deployed at the base of the cleaning station at 8 metres (m) depth approximately 15 centimetres (cm) above the seafloor. The RUVs were positioned north of the cleaning station. At site B and C, RUVs were deployed at 12 m depth approximately 3 m distance from the cleaning station. At site B, RUVs were placed 50 cm above the

Figure 1. Locations of the three manta ray cleaning stations studied at Maamunagau Finolhu, western Raa Atoll, Maldives.
seafloor, north of the cleaning station. At site C, RUVs were 30cm above the seafloor, positioned west of the cleaning station. A total of 317 RUVs were deployed over the three sites between 2020 and 2022. Trained staff from the Manta Trust (www.mantatrust.org) deployed and collected RUVs each day via snorkelling and boat. Depending on the battery charge, the RUVs ran for periods of between 25-240 minutes between the hours of 7:00 and 18:00.

Figure 2. Still image of remote underwater video system set up (photographed by Jess Haines).

2.3. Environmental variables

Abiotic environmental variables that may have influenced temporal and behavioural visitation of *M. alfredi* were measured, estimated, or taken from online data sources for every survey regardless of the presence or absence of *M. alfredi*. Values for average water temperature were obtained from a U22-001 HOBO Water Temperature Pro v2 Data Logger, set to record the average temperature
(degrees Celsius) at Site B every hour throughout a surveyed day. The tidal current was defined by the individual responsible for RUVs deployment as either ebb (outgoing), slack, or flood (incoming; Barr and Abelson 2019). Raw data for the moon phase were taken from online moon phase calendars (https://www.timeanddate.com/moon/phases/maldives/male).

2.4. Remote underwater video footage analysis

Spatial and temporal variation in visitation patterns was observed by analysing RUVs footage on QuickTime Media Player version 10.5. Each of the 317 replicates comprised between 25-240 minutes of video footage and was visually analysed by two observers. Video analysis commenced immediately after the cameras were positioned and continued until the recording finished due to low battery power or retrieving of RUVs.

2.4.1 Photo identification

*Mobula alfredi* display unique ventral body pigmentation patterns that remain unchanged throughout their life (Stevens 2016) and can be used to differentiate one individual from another using photo identification (Figure 3). The Maldivian Manta Ray photo-identification database is maintained by the Manta Trust and contains verified images of individual manta rays collected by trained Manta Trust staff, citizen scientists, or dedicated research trips. In this study, still images of the ventral surface were taken from each encounter in the RUVs footage and compared to the Maldivian Manta Ray photo-identification database. Trained manta staff completed manual photo-identification assessments of individual *M. alfredi* by using a two-step verification process as described by Stevens (2016). A manta was catalogued as a new individual if the ventral patterns did not match with those of any animal present in the database.
Figure 3. The unique ventral patterns of *Mobula alfredi* used to differentiate between individuals where (a) shows the two areas used to identify individuals using the Manta Trust’s photo-identification database. The primary identification area (yellow) includes markings on the ventral surface between the gill slits and on the lower abdomen. The secondary identification area (red) comprises patterns on the pectoral fins (Stevens 2016), (b) an example of a still photograph showing ventral colouration of *M. alfredi* obtained from remote underwater video footage at Site A cleaning station.
2.4.2. Sex, maturity status, and primary behavioural activity

As described in methods by Nicholson-Jack et al. (2021), a sighting event was defined as the confirmed identification of an individual reef manta ray on a specific date at a particular location. Here, a total of 470 sighting events of at least one manta were identified from 31,715 minutes of footage. During each sighting event, sex, maturity status (adult, subadult or juvenile) and primary behavioural activity (cleaning, cruising, or feeding) were recorded for all individuals identified in this study (Stevens 2016; Stevens et al. 2018). Sex was determined by the presence of claspers in male individuals (Marshall and Bennett 2010). Male *M. alfredi* were considered immature (juvenile) by the presence of small, uncalcified or partially calcified claspers (Stevens 2016). Adult female *M. alfredi* were identified by the presence of reproductive scars, visible pregnancy or an estimated disc width exceeding 320 cm (Marshall and Bennett 2010; Stevens 2016). Cleaning behaviour was defined as an individual maintaining a stationary position or displaying slow movements while being cleaned by cleaner fishes. Following methodology by Murie et al. (2020), cleaning interactions were recorded and timed from when a manta entered the field of view and ceased following departure, providing the individual did not return for ≥ 5 minutes. Individuals swimming with their mouths open and cephalic lobes unfurled during a given sighting event were considered as displaying feeding behaviour. Cruising behaviour was recorded when individuals were observed swimming with their mouths closed and cephalic lobes completely rolled.

2.5. Statistical analysis

All statistical tests were conducted with SPSS Statistics version 24.0 statistical software to analyse patterns. The Chi-squared test was used to determine whether there was a significant difference between observed and expected frequencies of *M. alfredi* to cleaning stations. This ensured the frequency of *M. alfredi* sightings was not attributable to sampling intensity and allowed diurnal patterns of visitation to cleaning stations to be observed. The frequency of sightings per survey was
converted into the mean sighting rate per minute of sampling for each survey to account for potential
effort-mediated bias associated with variation in sampling duration.

2.5.1 Environmental variables

Tests of normality found samples did not conform to normality and could not be transformed to fit
the assumptions of parametric tests. Therefore, the Kruskal-Wallis one-way analysis of variance
(ANOVA) by ranks test was used to assess differences in mean sighting rate per minute of sampling
across years, months, sites, tidal currents, and lunar phases. After identifying significant
environmental variables that influenced the mean sighting rate of *M. alfredi*, the Mann-Whitney test
was used. The influence of the continuous predictor temperature was investigated by calculating
Spearman’s Rank Correlation Coefficient.

2.5.2. Duration of time spent cleaning

The mean duration of time spent cleaning was calculated for each individual identified to account for
pseudoreplication. To determine if there was a significant difference between the mean cleaning time
in adults and juveniles the Mann-Whitney U test was conducted. Tests for comparison between
subadults were excluded from this analysis due to lack of replicates, reducing the extent they could
be deduced as significantly different to larger sample sizes.

The Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks test was conducted to determine
the significance of the intraspecific difference in mean time spent cleaning between maturity statuses
and sex (adult males, juvenile males and juvenile females). Tests for comparison in cleaning time were
not conducted for adult females or subadults due to the lack of replicates. The Mann-Whitney U test
was used to test for significant differences in mean cleaning time between remaining maturity
statuses and sex.
3.0. Results

Overall, a total of 98 individual reef manta rays from 470 sighting events were identified using the Manta Trust’s identification database over 116 days of the study months between 2020 and 2022. Cleaning behaviour accounted for 99.15% (n = 466) of the primary behaviour exhibited in sightings, with cruising behaviours recorded for the remaining 0.75% (n = 4%).

Of the 98 individuals identified, 4% were adult females (n = 4), 24.5% adult males (n = 24), 29.6% juvenile males (n = 29), 36.7% juvenile females (n = 36), 4% subadult males (n = 4) and 1% subadult females (n = 1; Figure 4a). This suggests a slight male bias as 58.1% (n = 57) of the individuals identified were male and 41.8% (n = 41) were female. Of the sightings, 83.6% (n = 393) were juveniles, 14.5% were adults (n = 68) and 1.9% (n = 9) were subadults (Figure 4b). A total of 56% (n = 55) of the individuals identified in this study were resighted (Figure 4c), with 9.2% (n = 9) of these individuals observed every year (2020, 2021 and 2022), 4.08% (n = 4) sighted in both 2020 and 2021, and 22.4% (n = 22) sighted in both 2021 and 2022. Of the 55 resighted individuals, 69% (n = 38) were juveniles, 27.3% (n = 15) adults, and 3.6% (n = 2) subadults (Figure 4c) indicating juveniles have a stronger affinity for cleaning stations than adults and subadults.
Figure 4. Individuals identified using the Manta Trust’s photo-identification database displaying percentage composition of (a) maturity status and sex of the total individuals identified (n = 98) from sighting events, (b) total sightings (n = 470) in relation to maturity status, and (c) total individuals (n = 98) resighted or sighted once, with percentage composition representing maturity status and sex of resighted individuals.
3.1. Diurnal visitation patterns of *Mobula alfredi* to cleaning stations

There was a significant difference between the observed and expected frequency of *M. alfredi* (Chi-square test, $X^2_{11} = 111.69$, $P < 0.05$; Figure 5), indicating a random pattern of sightings throughout the day.

![Figure 5. Comparison of total sampling duration in minutes (dark grey) and total frequency of *Mobula alfredi* (light grey) sighted at a given time interval throughout the day (Chi-square test, $X^2_{11} = 111.69$, $P < 0.05$).](image)

The mean sighting rate of *M. alfredi* per minute of sampling gradually increased to peak site use between 13:00-13:59 and rapidly declined thereafter (Figure 6), indicating a preference for cleaning during this time interval.
Figure 6. The mean sighting rate of *Mobula alfredi* per minute of sampling at a given time interval throughout the day, indicating peak site use between 13:00-13:59.

3.2. Influence of environmental variables on mean sighting rate of *Mobula alfredi*

There was a weak negative relationship between the mean sighting rate of *M. alfredi* per minute of sampling and temperature (Spearman’s Rank Correlation Coefficient, $r_s = 0.121, P < 0.05$). However, regression analysis could not confirm a significant relationship ($P > 0.05$) indicating the effect of temperature on visitation of *M. alfredi* to cleaning stations was limited. The year of sampling did not influence mean sighting rates (Kruskal Wallis H, $X^2 = 0.736, P > 0.05$).
3.2.1. Month, Site, Tidal Current, and Lunar Phase

Throughout the months of the northeast monsoon, the sighting rates of *M. alfredi* differed significantly (Kruskal Wallis H, $X^2_3 = 16.78, P < 0.005$), indicating a clear decline from January into subsequent months (Figure 7).

![Figure 7](image)

**Figure 7.** Mean sighting rate of *Mobula alfredi* per minute of sampling (± standard error) between January, February, March, and April with a decline in cleaning station use over the months from January (Kruskal Wallis H, $X^2_3 = 16.78, P < 0.005$).

The sighting rates of *M. alfredi* over the study period all differed significantly (Table 1), with the exception of sighting rates between January and February, denoting the presence of similar visitation patterns to cleaning stations during these months.
Table 1. Results of Mann-Whitney test to compare mean sighting rate of *Mobula alfredi* per minute of sampling across sampling months. Significant results (*P* < 0.05, *P* < 0.005) indicate a marked difference between the mean monthly sighting rates. No significance (*ns*) indicates there was no significant difference in sighting rates between the months (*P* > 0.05).

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Mean sighting rate per minute of sampling differed significantly between sites (Kruskal Wallis H, \(X^2_2 = 10.023, P < 0.05\)) with sightings rates at Site B markedly higher than Site A (Mann Whitney test, \(U_2 = 10.023, P < 0.005\)), indicating *M. alfredi* prefer cleaning at site B over site A (Figure 8). There was no significant difference in the mean sighting rates at site C compared to site A (Mann Whitney test, \(U_2 = 1581, P > 0.05\)) or site B (Mann Whitney test, \(U_2 = 1136, P > 0.05\)), suggesting this preference did not apply to site C.
Figure 8. Mean sighting rate of *Mobula alfredi* per minute of sampling (± standard error) across studied sites (Kruskal Wallis H, $X^2 = 10.023, P < 0.05$).

Visitation patterns to cleaning stations differed significantly with tidal current (Kruskal Wallis H, $X^2 = 15.139, P < 0.005$; Figure 9). Mean sighting rates during an ebb or slack current did not differ (Mann Whitney test, $U = 7892, P > 0.05$). Conversely, mean sighting rates were significantly lower at a flood current compared to an ebb (Mann Whitney test, $U = 2817, P < 0.001$) and slack current (Mann Whitney test, $U = 2107, P < 0.001$), suggesting the temporal presence of *M. alfredi* to cleaning stations is influenced by tidal current.
The mean sighting rates of _Mobula alfredi_ differed significantly under the influence of the lunar phase (Kruskal Wallis H, $X^2_3 = 17.746$, $P < 0.005$; Figure 10). Sightings of _M. alfredi_ were significantly higher during a full moon cycle compared to a third quarter (Mann Whitney test, $U_1 = 123.500$, $P < 0.005$), new moon (Mann Whitney test, $U_1 = 156$, $P < 0.005$) and first quarter phase (Mann Whitney test, $U_1 = 144$, $P < 0.005$). There were no significant differences between all remaining lunar phases ($P > 0.05$), suggesting the full moon phase may influence the use of cleaning stations by _M. alfredi_.

*Figure 9.* Mean sighting rate of _Mobula alfredi_ per minute of sampling (± standard error) with tidal current (Kruskal Wallis H, $X^2_2 = 15.139$, $P < 0.005$).
Figure 10. Mean sighting rate of *Mobula alfredi* per minute of sampling (± standard error) with lunar phase (Kruskal Wallis $H, \chi^2_3 = 17.746, P < 0.005$), demonstrating a clear peak in cleaning station use during a full moon.

### 3.3. Duration of time spent cleaning by *Mobula alfredi*

On average, the duration of time spent cleaning ranged from 1 – 47 minutes per individual and differed between maturity statuses (Figure 11). The cleaning duration was significantly lower in juveniles compared to adults (Mann Whitney test, $U_1 = 508, P < 0.001$) denoting the presence of ontogenetic variability in cleaning duration, which may be attributable to differences in size class.
Figure 11. Average duration of time spent cleaning by adult and juvenile *Mobula alfredi* (± standard error), with significantly less time spent cleaning by juveniles compared to adults (Mann Whitney test, $U_1 = 508, P < 0.005$).

Adult female *M. alfredi* were observed to clean for the longest duration (36 minutes ± 12.67), while juvenile males cleaned for the shortest duration (7 minutes ± 6.45). The duration of time spent cleaning by adult males, juvenile males and juvenile females differed significantly (Kruskal Wallis H, $X^2_2 = 6.526, P < 0.05$; Figure 12).
Figure 12. Average duration of time spent cleaning (± standard error) by adult male (AM), juvenile male (JM) and juvenile female (JF) *Mobula alfredi* (Kruskal Wallis H, $X^2 = 6.526, P < 0.05$).

Further analysis revealed that adult males cleaned for significantly longer than juvenile males (Mann Whitney test, $U = 218, P < 0.05$) and juvenile females (Mann Whitney test, $U = 287, P < 0.05$). However, cleaning duration between juvenile males and juvenile females did not differ (Mann Whitney test, $U = 766, P > 0.05$), reflecting the difference in cleaning duration between adult and juvenile *M. alfredi*. 
4.0. Discussion

To date, little information exists regarding small-scale habitat use by either adult or juvenile *M. alfredi*. In this study, integrating results from RUVs and environmental data with information from a photo-identification database has enabled patterns in the behaviour and temporal visitation to cleaning stations of *M. alfredi* to be detected, providing crucial insight into their small-scale habitat use. These results provide compelling evidence of a juvenile aggregation site using an innovative data collection technique. Trends reflect the role of environmental drivers during the northeast monsoon and suggest that *M. alfredi* have a strong affinity for cleaning stations on the west side of Raa Atoll, Maldives.

4.1. Residency and site fidelity

The strong site fidelity and residency displayed by both adult and juvenile *M. alfredi* at the studied cleaning stations highlight the importance of these areas to their spatial and behavioural ecology. Sighting events primarily comprised cleaning behaviours, suggesting *M. alfredi* visit these sites to solicit cleaning services. Consistent with previous studies, juvenile *M. alfredi* displayed a higher affinity for these cleaning stations than adults (Cerutti-Pereyra et al. 2014, Peel et al. 2019, Harris et al. 2021), reflecting their significance to immature individuals. Cleaning stations provide grounds for cleaning interactions between cleaner fish and client species. Typically, the key advantages associated with this cleaner-client symbiosis include the removal of ectoparasites or diseased tissue to facilitate client maturation and growth of client species (O’Shea et al. 2010, de Souza et al. 2014). However, this does not explain why juveniles were observed in more prominent frequencies than adults; hence, it is important to highlight additional causalities that may have influenced the visitation patterns of *M. alfredi* to these cleaning stations such as courtship and mating (Stevens 2016, Stevens et al. 2018), metabolic demands (Hight and Lowe 2007, Jirik and Lowe 2012), and predator avoidance (Marshall and Bennett 2010, Peel et al. 2019). Given that juveniles are yet to reach sexual maturity, it is unlikely that courtship and mating influenced the affinity of juveniles to cleaning stations (Stevens et al. 2018). Hypotheses to explain juvenile affinity to cleaning stations include energy efficiency, which reportedly
limits smaller-body individuals from travelling offshore (Peel et al. 2019, Harris et al. 2021), and predator avoidance (Stevens 2016, Harris et al. 2021). Juvenile *M. alfredi* may use cleaning stations as a refuge site to avoid predation (Nicholson-Jack et al. 2021) by pelagic sharks (Marshall and Bennett 2010). Predator avoidance has been highlighted as a possible driver for a more pronounced abundance of small juveniles than expected (Stevens 2016). This may elucidate why juveniles were more prevalent than larger adults as they are smaller and more susceptible to predation (Stevens 2016). Either or both hypotheses could explain the prevalence of juvenile *M. alfredi* observed here. However, as morphometric dimensions were not recorded in this study, further research is required to confirm whether the size class of *M. alfredi* influences the strength of affinity to cleaning stations.

Previous studies have reported a significantly higher chance of observing female *M. alfredi* rather than males at cleaning stations (Couturier et al. 2014, Stevens 2016, Germanov et al. 2019), contradicting the results presented here. Sex-based differences in observations of adult *M. alfredi* could be attributable to social preferences (Germanov et al. 2019) or reproductive behaviour (Deakos 2010, 2012). During the peak mating season, cleaning stations can act as lekking sites for sexually receptive females to choose their sexual partner (Young et al. 2009) prompting a shift from female to male bias (Marshall and Bennett 2010, Stevens et al. 2018). As only 4 females but 24 males were observed, it is likely there was a strong male bias in adults at the studied cleaning stations, denoting the presence of a functioning lek (Stevens 2016). Adult female *M. alfredi* are highly philopatric, often showing strong site fidelity to specific locations during peak mating season (Germanov et al. 2019). Given the low frequency and abundance of adult females observed in this study, it is likely seasonal reproductive strategies did not align with the benefits offered by this location and adult females migrated to an aggregation site elsewhere. This could explain the sex-based differences in habitat use by adult *M. alfredi*. 


4.2. Influence of environmental variables on *Mobula alfredi* visitation patterns

Biological, environmental, and temporal variables are known to influence *M. alfredi* sightings at key aggregation sites (Jaine et al. 2012, Rohner et al. 2013, Armstrong et al. 2016, Harris et al. 2020, Harris and Stevens 2021). As the intensity of these factors varies between locations and manta subpopulations (Harris et al. 2021), it is important to identify locally specific drivers of visitation patterns. In this study, *M. alfredi* presence at cleaning stations gradually increased throughout the morning, reaching a peak between 13:00 and 13:59, thereafter declining into the evening. Similar diurnal visitation trends were observed at Lady Elliot Island, Australia where cleaning behaviour peaked between 12:00 and 14:00 (Jaine et al. 2012). It is likely these diurnal patterns in site occupancy are associated with feeding activities (Harris and Stevens 2021). During the night, *M. alfredi* move offshore into deeper waters to forage on diel vertical migrating zooplankton (Couturier et al. 2013, Braun et al. 2014, Peel et al. 2019). Following foraging activity, *M. alfredi* are known to attend cleaning stations for ectoparasite removal or to facilitate digestion (Clark 2010, Harris and Stevens 2021). Hence, their preference for being cleaned in the morning to early afternoon could explain other diurnal visitation trends to cleaning stations observed here. However, diurnal visitation patterns to cleaning stations vary across literature. Acoustic telemetry along the Kona Coast of Hawaiʻi revealed movement away from cleaning stations to an offshore afternoon/evening and nocturnal foraging area (Clark 2010). In Indonesia, a reduced temporal appearance of *M. alfredi* following the hours of 09:00 and 10:00 was observed using passive acoustic telemetry (Setyawan et al. 2018) and a combination of satellite and acoustic telemetry (Setyawan et al. 2022a). By contrast, some studies observed no diurnal trend in the hourly use of cleaning stations by *M. alfredi* (O’Shea et al. 2010). This suggests temporal peaks in cleaning behaviour are highly variable and likely to be influenced by location, seasonality, or sampling method.

Predictable utilization of key aggregation sites is often linked to foraging opportunities in planktivorous elasmobranchs, including *M. alfredi* (Anderson et al. 2011, Harris et al. 2020, Harris and
The South Asian Monsoon (SAM) is a known driver for shifting productivity hotspots that strongly influence the spatiotemporal distribution patterns of *M. alfredi* (Anderson et al. 2011, Harris et al. 2020). Biannual migrations of *M. alfredi* with seasonally reversing monsoon currents allow individuals of this subpopulation to exploit the most readily available source of planktonic food, inducing seasonal and predictable peak abundances (Kitchen-Wheeler et al. 2012, Armstrong et al. 2016, Harris et al. 2020). This study was conducted during the northeast monsoon, where island circulation patterns and nutrient enrichment of surface waters induce shifting distributions of chlorophyll-a (Chl-a) enhancement and localised abundances of zooplankton along the western sides of atolls (Stevens and Froman 2018). Sampling month was identified as a significant driver of *M. alfredi* visitation to cleaning stations, with a clear decrease in sighting rates from January and April. Similar results reported during the northeast monsoon on the western sides of Maldivian atolls found manta presence was influenced by shifting Chl-a concentrations and zooplankton blooms induced by the SAM (Anderson et al. 2011). It is likely that the SAM influenced patterns observed here. However, Chl-a concentrations and zooplankton biomass were not analysed, hence the prospective relative effects of the SAM cannot be determined with certainty.

Previous studies have shown changes in water temperature can influence *M. alfredi* fine- and broad-scale movement patterns (Dewar et al. 2008, Jaine et al. 2012, Couturier et al. 2018, Peel et al. 2019). This is often attributable to the link between primary production as increased temperatures elevate ocean stratification, reducing the accessibility of zooplankton to *M. alfredi* (Xiu et al. 2018). In this study, the average temperature had a limited effect on *M. alfredi* visitation to cleaning stations, suggesting temperature does not affect cleaning station use at Raa Atoll, Maldives.

The role of tidal-driven distribution and behavioural patterns in association with the foraging activity of *M. alfredi* are well documented (Schlaff et al. 2014, Harris et al. 2020). The present study provides evidence that supports the theory of tidal current as a driver for visitation patterns to cleaning stations in *M. alfredi*. Similar to previous studies (O'Shea et al. 2010, Anderson et al. 2011), the sighting rates
observed here were highest during an ebb or slack current, and lowest at a flood current. During a flood current, fine-scale oceanographic processes deliver dense assemblages of zooplankton by the upward propagation of cold-water bores more frequently than during an ebb current (Leichter et al. 1996, Woodson 2018). Under these optimal foraging conditions for energy-efficient feeding (O’Shea et al. 2010, Armstrong et al. 2016, Barr and Abelson 2019), it is more beneficial for *M. alfredi* to exploit these rich feeding grounds rather than visit cleaning stations. This demonstrates the link between *M. alfredi* feeding ecology and visitation to cleaning stations, inciting the presence of a trade-off between energetically efficient feeding and cleaning (Barr and Abelson 2019).

Moon phase is a known environmental cue that affects *M. alfredi* cleaning and feeding activities (Dewar et al. 2008, Jaine et al. 2012, Rohner et al. 2013, Barr and Abelson 2019, Harris and Stevens 2021, Knochel et al. 2022). During new and full moon phases, a combination of both monsoon and strong lunar currents induces hydrodynamic processes that concentrate patches of zooplankton (Jaine et al. 2012, Rohner et al. 2013, Harris and Stevens 2021, Knochel et al. 2022). Consequently, *M. alfredi* are presented with favourable feeding conditions. Foraging activities involve nocturnal movements into deeper and cooler waters facilitated by physiological adaptations, such as counter-current heat exchange that allow *M. alfredi* to exploit feeding grounds at temperatures below their physiological tolerances, but at a high metabolic cost (Lassauce et al. 2020). *Mobula alfredi* may associate with cleaning stations to thermoregulate and meet metabolic demands (Hight and Lowe 2007, Jirik and Lowe 2012, Stevens et al. 2018). In this study, *M. alfredi* were more frequently sighted during a full moon phase than any other lunar phase. Given the full moon typically increases manta feeding activities (Knochel et al. 2022), it is likely *M. alfredi* preferentially visited the cleaning stations in this study to physiologically prepare for or recover from energetically inefficient feeding activities offshore (Nicholson-Jack et al. 2021). Peak foraging activities during the full and new moon may be linked to cleaning stations use; however, the sighting rates observed here during a new moon do not support this theory. The present study provides further evidence of the moon phase as a driver for behavioural...
and temporal trends in visitation to cleaning stations, highlighting the potential influence of feeding ecology on cleaning.

It is difficult to ascertain the explanation behind site as a driver for mean sighting rate as either environmental parameters or site characteristics could be responsible for differences. At site A, there was a significantly lower mean sighting rate than at site B. While it is likely *M. alfredi* feeding ecology is a key driver of cleaning station use, it is important to recognise the role of the cleaning performance of cleaner wrasse in driving the presence of *M. alfredi* at cleaning stations. Studies have reported high water flow impairs the ability of cleaner wrasse to provide adequate services to client animals (Bellwood and Wainwright 2001, Oliver et al. 2011). Cleaning effectiveness relies on the performance of cleaner wrasse, suggesting that under high flow speeds, client attendance will be reduced (Barr and Abelson 2019). As site B was slightly deeper than site A, the conditions may have been more favourable for cleaner wrasse under high flow conditions (Armstrong et al. 2021). However, as neither flow speeds nor cleaner fish presence were monitored during this study, the causality behind the preference for site B over site A remains speculation.

The present study provides evidence of environmental drivers influencing small-scale habitat use by *M. alfredi*. Determining the direct causality of *M. alfredi* presence at cleaning stations is problematic, as it is likely to rely on an amalgamation of both biotic and abiotic factors. Previous studies suggest *M. alfredi* use cleaning stations under conditions that are unfavourable to foraging efficiency (Barr and Abelson 2019, Harris and Stevens 2021). Evidence presented here suggests foraging ecology is likely associated with the use of cleaning stations.

### 4.3. Cleaning duration

This study marks the first report to quantify *in-situ* cleaning durations of adult and juvenile *M. alfredi* at three major aggregation sites at Raa Atoll, Maldives. Adults cleaned for considerably longer than juveniles, suggesting cleaning duration may increase with maturity. It is likely that size class influenced cleaning duration as juveniles are generally smaller than adult *M. alfredi* (Stevens 2016) and cleaner
fish have shown a preference for larger clients over small-bodied individuals (Grutter 1995, de Souza et al. 2014, Sun et al. 2016, Coward 2017). This preference typically manifests itself in the form of prolonged encounters between larger clients and their symbionts (de Souza et al. 2014, Sun et al. 2016). Here, the duration of cleaning events in juvenile *M. alfredi* was notably less than in adults, supporting the hypothesis that morphometric dimensions could influence cleaning duration. However, the extent to which this theory can be applied to differences in cleaning duration between adult male and female *M. alfredi* is unclear. The maximum average cleaning duration of 36 minutes was observed in adult female *M. alfredi*. By contrast, adult males cleaned for an average of 12 minutes. Strong evidence of sexual dimorphism in the size of *M. alfredi* continues to materialize (Marshall and Bennett 2010, Deakos 2012, Stevens 2016, Setyawan et al. 2022b) with adult females growing larger than males by as much as 16% (Marshall and Bennett 2010). While it is possible that size class influences cleaning duration, the present study did not incorporate morphometric measurements of *M. alfredi*. Moreover, replicates of adult female cleaning durations were limited, reducing the robustness of data and the extent their average cleaning duration can be compared to adult males and juveniles. Therefore, further study with a sufficient sample size is required to ascertain whether morphometric dimensions influence the cleaning duration of adult and juvenile *M. alfredi*.

Sampling constrained to diurnal hours may have reduced the scope for detecting patterns associated with environmental parameters that influenced small-scale habitat use by *M. alfredi*. It is unlikely that the observations here were affected by limited sampling hours as observations of *M. alfredi* at cleaning stations predominantly occur during the day when cleaner fish are active (Couturier et al. 2018, Peel et al. 2019, Harris and Stevens 2021). However, it is important to acknowledge that sampling duration during the day was not consistent throughout the three years of data collection for this study. Although converting observations into a mean sighting rate may have rectified this, it is possible there was some effort-mediated sampling bias. It is advised that future studies sample consistently throughout the year to avoid bias associated with sampling intensity and ensure key factors that influence visitation patterns to cleaning stations can be identified.
4.4. Implications for conservation

*Mobula alfredi* are in a global state of decline and are vulnerable to extinction (Marshall et al. 2022). The majority of nearshore surrounding waters of the Maldives are unprotected from anthropogenic threats such as marine resource exploitation by fisheries. Active protection has been provided for Hanifaru MPA in Baa Atoll, Maldives (Harris and Stevens 2021); however, this alone is not sufficient to accommodate small- and broad-scale movement patterns of juvenile and adult *M. alfredi*.

Knowledge of the environmental variables that influence fine-scale habitat use by *M. alfredi* can be applied to conservation legislation to reduce exposure to anthropogenic threats and minimize the relative effects (Harris et al. 2020). Here, short-term fluctuations in environmental parameters influenced the presence of *M. alfredi* at key aggregation sites. Therefore, it is important that future studies incorporate robust datasets of sighting trends and environmental fluctuations over a broad spatiotemporal scale to ensure population parameters are genuine.

Strong site fidelity and residency patterns by juvenile *M. alfredi* at these cleaning stations indicate that immature individuals are particularly vulnerable to the direct and indirect effects of anthropogenic threats at key aggregation sites. Typically, nursery habitats offer advantageous conditions such as ideal foraging opportunities, a refuge from predation, and opportunities for socialising with conspecifics (Setyawan et al. 2022a). It can be suggested that the sites studied here offer such conditions that facilitate the survivorship of juvenile *M. alfredi*. While there is evidence that these cleaning stations on the western side of Raa Atoll could function as a nursery area for juvenile *M. alfredi*, it cannot be confirmed without further research that directly addresses the three proposed criteria that define an elasmobranch nursery habitat (Heupel et al. 2007).

This study provides baseline information that can be used to assist conservation legislation planning by providing ecological insight into small-scale habitat use by both adult and juvenile *M. alfredi* so conservation strategies can be focused and implemented effectively at these key aggregation sites. It is important to highlight these observations refer to one of the hundred unprotected aggregation sites.
in the Maldivian archipelago subject to anthropogenic threats. As *M. alfredi* exhibit high connectivity between aggregation sites (Harris and Stevens 2021), it is crucial further study is undertaken to research adjacent habitats important to *M. alfredi* and confirm the inferences of patterns reported here.

### 5.0. Conclusions

The present study provides quantitative evidence that demonstrates the ecological importance of cleaning stations to both adult and juvenile *M. alfredi*. Strong site fidelity and residency patterns displayed by juveniles reflect the crucial role of these key aggregation sites for the survivorship of early life stages, indicating these cleaning stations may act as nursery grounds. While this cannot be confirmed by the results presented here, it can be recognised as a strong possibility that requires further research. This study identified environmental drivers of visitation of *M. alfredi* to cleaning stations known to affect small-scale habitat use and their prey availability, indicating a possible link between *M. alfredi* feeding activity and frequency at cleaning stations. However, further research that incorporates zooplankton and *M. alfredi* abundances is necessary to address this over a broad spatiotemporal scale. These findings suggest RUVs in conjunction with abiotic environmental data can be used to detect temporal and behavioural visitation patterns in *M. alfredi* at key aggregation sites and to address knowledge gaps in the use of cleaning stations in other localities. This study extends the current knowledge of fine-scale habitat use by *M. alfredi* in the Maldives and provides information that can facilitate localised spatial marine planning to ensure the long-term conservation of *M. alfredi*. 
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