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Influences of reef manta ray (*M. alfredi*) visitation
patterns to a remote cleaning station and the
implications for conservation.

By

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Impact Summary Preface

Reef manta rays are one of the largest of all ray species, with wing spans reaching up to four meters. Unfortunately, the reef manta ray is threatened by both bycatch and targeted fisheries. The gill plates of reef manta rays are harvested for Chinese medicine, despite there being no scientific evidence the gills contain any medicinal properties. This threat, paired with the slow maturity rate and low offspring yield (K-selected), has led the reef manta ray to be listed as Vulnerable to Extinction by the ICUN Red List of Threatened Species – and the Manta Trust endeavours to improve this.

The Manta Trust is dedicated to the protection and conservation of mobulid species across the globe. Much of the work done by the Manta Trust varies from educational outreach, campaigning for better mitigating actions and conducting crucial research. All of which are critical in working towards the implementation of new laws, policies and legislations to help legally protect reef manta rays. The key to implementing successful conservation strategies that protect the reef manta ray is to develop an understanding of the species spatial ecology and how various factors may influence this behaviour. However, this can be challenging for highly mobile species. Therefore, if patterns in movements and aggregations can be established, this can inform conservation efforts. An example of science informed mitigation actions includes the implementation of marine protected areas (MPA), such as the Chagos Archipelago, British Indian Ocean Territory (hereafter Chagos). Previous studies have investigated the drivers of visitations to feeding sites, but little research has focused exclusively on cleaning stations. In this study, the Manta Trust conducted data collection to identify the environmental factors that influence reef manta ray visitation patterns to cleaning stations. This research is important to the Manta Trust as the results will help to build a clear picture of the behavioural and spatial ecology of the reef manta ray population that utilise the North Ile des Rats (NIIdR) Cleaning Station in Chagos. Results can be applied to the wider populations of reef manta ray that reside throughout Chagos (and across the globe) and help to formulate successful and scientifically supported conservation strategies. The data analysis in this study used generalised additive models (GAMs) to statistically analyse the detections data against environmental influences such as; wind speed, wind direction, rainfall, sea surface temperature, time of day and tidal phase.

Of the environmental variables analysed, time of day, tidal phase and wind direction were found to carry some significance in influencing reef manta ray visitations to the NIIdR cleaning station. Furthermore, the individuals with the most detections and longest durations of visits to the cleaning station were females, yet those with the least detections to the location were males. Interestingly, the likelihood of observing visitations increased between 13:00 o'clock and 23:00 o'clock, with peak likelihood at 18:00 o'clock. This is unexpected, as cleaner wrasse are inactive at night. These results suggest that the NIIdR cleaning station is a multipurpose reef, not just for cleaning activities. This indicates that individuals (expectedly, juveniles) utilise the reef during the night as a predator avoidance strategy and due to being inexperienced in foraging offshore. Furthermore, the results indicate that the NIIdR reef is utilised by the reef manta ray throughout multiple life stages, further supporting the importance of this habitat. Moving forward, the Manta Trust will use these results to complete further investigations and build upon current scientific findings. Moreover, as Chagos is already an established MPA, the findings from this study will help inform the maintenance and improvement of protective measures for the reef manta rays that live within the archipelago. This will support future conservation campaigns conducted by the Manta Trust within areas of high aggregations and utilisation of the species. The results may be used to build upon current knowledge and consequently, conservation, of the reef manta ray throughout Chagos and wider locations.

Acknowledgements

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Executive Summary

Reef manta rays (*Mobula alfredi*) face multiple threats, including overexploitation from targeted and bycatch fisheries and pressure from unregulated eco-tourism. A combination of these risks with slow maturation rate and low reproductive output has led to *M. alfredi* being listed as Vulnerable to Extinction on the ICUN Red List of Threatened Species. Highly mobile species, such as *M. alfredi*, can be challenging to protect. Therefore, it is imperative to not only identify key aggregation sites of *M. alfredi*, but to develop an understanding of patterns of these visitations as well as factors that may influence them. Previous studies have investigated the fine-scale oceanic drivers of visitations to aggregation sites, but few have focused primarily on cleaning stations. The Chagos Archipelago (hereafter; Chagos) was established as a no-take Marine Protected Area (MPA) in 2010, with a coverage of 600,000km². All of the islands are uninhabited (except for Diego Garcia, southernmost atoll), as a result, the marine environment is relatively untouched and in excellent ecological condition. Thus, this makes Chagos an excellent location for observing undisturbed behaviours of the *M. alfredi* sub-population which reside there. The study location focuses primarily on a known aggregation hotspot used as a cleaning station, namely North Ile de Rats cleaning station (NIIdR). The Manta Trust tagged 20 *M. alfredi* Individuals with acoustic transmitter tags whilst within the Egmont atoll and deployed an omnidirectional acoustic receiver at the NIIdR cleaning station (13.6 meter depth and 2 meters above the seabed). Generalised Additive Models (GAMs) were used in R[®] to analyse the relationship between the presence and absence of detections and environmental influences, these influences were; time of day, windspeed (km/h), wind direction (°), rainfall (presence and absence), tidal phase and sea surface temperature (°C). A total of 17 *M. alfredi* individuals were detected between the 3rd of December 2019 and the 13th of March 2020, collating a total of 3,601 detections throughout the data collection period. The individuals with the top three total detections were all females (407, 395 and 359 detections) and the three individuals with the least total detections (43, 61 and 70 detections) were all male. Suggesting a species demographic segregation may occur at the site. The greatest total time spent at the NIIdR cleaning station was by individual CG-MA-0035 (juvenile female) with a total duration of 1,390 minutes across 407 detections. GAMs highlighted three key predictors in *M. alfredi* visitations to the NIIdR cleaning station, including time of day, tidal phase and wind direction. The variables were tested in separate GAMs, which found that likelihood of visitations increased; between ~13:00 and 23:00 o'clock, with peak likelihood at 18:00 o'clock for time of day, three to four hours before flood tide and with the most significant wind directions of 50° (north easterly). Results for time of day suggest the NIIdR cleaning station is a multipurpose aggregation site, as visitations peak at and after sunset, when cleaner wrasse are no longer active. These results can be used to improve visitation predictability to the NIIdR cleaning station, inform the improving of protective measures within Chagos and wider regions for *M. alfredi* (such as informing the implementation of new MPAs at unprotected aggregation sites).

1.0 Introduction

Reef manta rays (*Mobula alfredi*), of the family Mobulidae, are among the largest of all ray species, reaching wingspans of up to four meters (Nicholson-Jack *et al.*, 2021). These elasmobranchs can be found throughout tropical and sub-tropical waters of the Indo-West Pacific Oceans in highly fragmented populations (Braun *et al.*, 2015; Couturier *et al.*, 2012; Harris *et al.*, 2021; Jaine *et al.*, 2012). Unfortunately, the species face overexploitation from targeted and bycatch fisheries (Harris *et al.*, 2021; Nicholson-Jack *et al.*, 2021). This species is cartilaginous and, much like sharks are targeted for their fins, the gill plates of the species are harvested for Chinese medicine (Braun *et al.*, 2015; Jaine *et al.*, 2012) despite there being no scientific evidence of the gills having medicinal properties. In addition, *M. alfredi* are also at risk from boat strike, pressure from unregulated eco-tourism operations and are easily disturbed by anthropogenic activity (Braun *et al.*, 2015; Harris *et al.*, 2021; Murray, 2019). A combination of overexploitation and following typical K-selected life traits (slow maturation rate and low reproductive output) has contributed to the sharp decline in *M. alfredi* populations (Braun *et al.*, 2015; Couturier *et al.*, 2012; Germanov *et al.*, 2019; Ward-Paige *et al.*, 2013). Consequently, they are listed as Vulnerable to extinction on the IUCN Red List of Threatened Species (Germanov *et al.*, 2019; Harris *et al.*, 2020.).

Over the last decade, there has been a considerable focus of research to help gain a better understanding of the spatial, temporal and behavioural ecology of mobulid species to help inform conservation efforts (Axworthy *et al.*, 2019; Harris *et al.*, 2020; Harris *et al.*, 2021; O'Shea *et al.*, 2010; Peel *et al.*, 2020; Setyawan *et al.*, 2018). Successful conservation of *M. alfredi* requires knowledge of the species behavioural ecology (Axworthy *et al.*, 2019; Germanov *et al.*, 2019; Jaine *et al.*, 2012). However, implementing successful conservation strategies can prove challenging for highly mobile species as they are able to transit out of protected areas and are capable of long-range movements (Armstrong *et al.*, 2016; McCauley *et al.*, 2013). Therefore, when implementing successful mitigation actions for *M. alfredi*, it is important to identify key aggregation sites. Moreover, it is imperative to identify any

visitation patterns to these aggregation sites and factors that may influence them (Harris *et al.*, 2021; McCauley *et al.*, 2013).

1.1 Aggregation Behaviour

A typical behavioural characteristic of this species is aggregation behaviour and site fidelity (Harris *et al.*, 2021). *M. alfredi* will often aggregate in specific locations which are utilised by the species as cleaning stations, feeding or breeding grounds (Couturier *et al.*, 2018; Setyawan *et al.*, 2018; Harris *et al.*, 2020). These aggregation sites most often occur in discrete habitats, such as coastal reefs, therefore making the spatial conservation of these sites extremely important (Harris *et al.*, 2020; McCauley *et al.*, 2013). Aggregation sites utilised by *M. alfredi* for feeding are usually separate to sites visited for cleaning activities (O'Shea *et al.*, 2010) - nonetheless, multipurpose reef locations have been previously identified (Germanov *et al.*, 2019; Jaine *et al.*, 2012; Setyawan *et al.*, 2018). *M. alfredi* utilise particular discrete reef habitats whereby they interact with other *M. alfredi*, mate and are cleaned by cleaner wrasse for parasite removal (Côte, 2000; Harris *et al.*, 2021). The locations whereby *M. alfredi* participate in symbiotic interactions with cleaner wrasse are known as cleaning stations (O'Shea *et al.*, 2010; Harris *et al.*, 2021).

Previous investigations have identified that a particular population of *M. alfredi* displays increased visitations to a reef utilised as a foraging site amongst the Great Barrier Reef (Southwest Australia) prior to low tide (Armstrong *et al.*, 2016). This was explained by the observation of higher zooplankton biomass during a low tide (Armstrong *et al.*, 2016), but this cannot be applied to cleaning station visitations as the reason for visiting these locations is not food driven. Previous findings have also illustrated that species demographics (such as sex and sexual maturity) can also play a role in where *M. alfredi* aggregate and the time spent at these hotspots (Germanov *et al.*, 2019; Harris *et al.*, 2021). For instance, juveniles have been recorded to display longer visitations to these aggregation sites, which has been

attributed to inexperience of feeding behaviours as well as prey avoidance due to being more vulnerable to predators (Harris *et al.*, 2021). Juveniles exhibiting greater visitations to these locations can also indicate a nursery location (Germanov *et al.*, 2019). While recent work has investigated the fine-scale oceanographic drivers of feeding aggregation behaviour (Harris *et al.*, 2021), there has been little recent investigation into environmental influences - such as tidal phase, on visitation patterns of these elasmobranchs to cleaning stations (Barr and Abelson, 2019; O'Shea *et al.*, 2010).

Previous studies have investigated the tide-related periodicity of *M. alfredi* and sharks to a reef utilised as a cleaning station in the coral sea, on the outer Great Barrier Reef (O'Shea *et al.*, 2010). The visitations recorded within this study were collected through direct observation and remote video capture, which identified strong patterns between tide and reef visitations by the elasmobranchs (O'Shea *et al.*, 2010). It was found that 59% of *M. alfredi* that were observed engaging in cleaning activities during an ebbing tide and *M. alfredi* sighting numbers did not vary across time of day (O'Shea *et al.*, 2010). This highlights a relationship between *M. alfredi* visitations to cleaning stations and tidal phase, but not time of day. Yet, this contrasts findings from another investigation in Raja Ampat, Indonesia, which found that detections of individuals peaked at around midday for three of the cleaning and feeding stations (Setyawan *et al.*, 2018). This particular study did not investigate tidal influence on *M. alfredi* visitation patterns to aggregation sites (Setyawan *et al.*, 2018). Increased visitations to cleaning stations by *M. alfredi* during the day has been attributed to the cleaner wrasse being inactive at night (Harris *et al.*, 2021). Further studies have also demonstrated a relationship between visitations to feeding and cleaning sites and lunar phase (Jaine *et al.*, 2012). Gaining an understanding of behavioural and environmental influences on aggregation patterns will help predict prominent levels of visitations to aggregation sites and thus assist the planning of long-term protection for mobulids (Armstrong *et al.*, 2016; Barr and Abelson, 2019).

Time spent by *M. alfredi* at cleaning stations has been shown to be variable, between 1 and 300 minutes with a mean visitation length of 31 minutes (O'Shea *et al.*, 2010). Furthermore, all *M. alfredi* observed at the oceanic reef (Great Barrier Reef, Coral Sea) utilised the reef for cleaning activities and none displayed feeding behaviours (O'Shea *et al.*, 2010). The distinguishing of a relationship between tidal phase and cleaning station visitations by *M. alfredi* is a crucial identification of behavioural ecology. Despite this, the use of direct observation and remote video capture could mean that some observations were missed due to individuals not being captured within the video capture range.

1.2 Movement Ecology and Tracking Methods

There are several different methods used to monitor *M. alfredi* movements. The data from these methods help to gain an understanding of how, why and when *M. alfredi* aggregate at different sites and display different behaviours (Couturier *et al.*, 2018; Harris *et al.*, 2021; Setyawan *et al.*, 2018). Photo-Identification (hereafter photo-ID) is a cost effective and straightforward way to monitor site fidelity of *M. alfredi*, as each specimen has ventral markings that are unique in the same way that a thumb print is unique to an individual human (Peel *et al.*, 2020). However, the use of photo-ID alone can produce seasonally biased datasets that do not represent annual movement patterns, due to the difficulty of reaching aggregation sites (Peel *et al.*, 2020). The data collected can also be biased to areas that are already previously known as aggregation sites to *M. alfredi* (Harris *et al.*, 2020; Peel *et al.*, 2020). Nonetheless, when supplemented with other data methods, the use of photo-ID can allow for a larger tracking range of individuals to be monitored (Couturier *et al.*, 2018; Harris *et al.*, 2021).

Passive acoustic telemetry can complement and expand the value of photo-ID as a tracking method. The data collection can be continuous and over extended periods of time and when

tracking fine-scale movement (Couturier *et al.*, 2018; Harris *et al.*, 2021). For example, the use of passive acoustic telemetry has previously aided investigations of *M. alfredi* movements over 682 days and the findings demonstrated strong site fidelity as well as seasonal movements (Setyawan *et al.*, 2018). When a tagged individual swims within the detectable range (160 m; Harris *et al.*, 2021) the acoustic tag emits a code to the receiver – this detectable range therefore limits the tracking of individuals to this range only (Peel *et al.*, 2020). In comparison to satellite tags, acoustic tags are lower in cost, which means more individuals can be tagged (Peel *et al.*, 2020).

A study in the Maldives used both photo-ID data and acoustic telemetry to investigate the long-term visitations and small-scale movements of *M. alfredi* around known aggregation sites (Harris *et al.*, 2020). The study found that all tagged individuals showed high affinity to the aggregation sites and visitations to a cleaning station increased during lower wind speeds (<5ms⁻¹) and three hours after high tide (Harris *et al.*, 2020). The study suggested that *M. alfredi* may visit cleaning stations at times of the day when environmental conditions are not suitable for feeding (Barr and Abelson, 2019; Harris *et al.*, 2020). Furthermore, results from studies indicated that visitation patterns may be associated with difference in habitat use based on species demographics – such as sex and maturity status (Harris *et al.*, 2020; Harris *et al.*, 2021).

1.3 Chagos Archipelago, British Indian Ocean Territory

A population of *M. alfredi* inhabit the Chagos Archipelago (hereafter Chagos), which was established as a no-take Marine Protected Area (MPA) in 2010 and hence became the largest MPA in the world (at the time), at 600,000 km² (Sheppard *et al.*, 2011). Thus, Chagos provides a key habitat to *M. alfredi* (Andrzejaczek *et al.*, 2020; Harris *et al.*, 2021). All the islands are uninhabited and have been so for at over 50 years, with the exception for the

southernmost atoll, Diego Garcia (Sheppard *et al.*, 2011). As a result, the marine environment is seemingly untouched and in excellent condition when compared to other areas, such as the Maldives (Sheppard *et al.*, 2011). Therefore, the region is of particular importance for conservation efforts to ensure the environment does not deteriorate in the same way as surrounding areas. Moreover, because of the excellent environmental conditions, the marine habitats within Chagos make ideal areas for scientific investigation (Sheppard *et al.*, 2011). There is a known hotspot within Chagos (specifically, Egmont Atoll) where *M. alfredi* frequently engage in cleaning activities, named North Ile de Rats Cleaning Station (hereafter NIdR). A recent on *M. alfredi* spatial ecology to a feeding site within the Egmont Atoll investigated the fine-scale oceanic drivers of these visitations (Harris *et al.*, 2021). This study demonstrated that visitations increased with the intrusion of cold-water bores that transported zooplankton through the narrow topography of the marine environment, offering preferable conditions for *M. alfredi* foraging (Hosegood *et al.*, 2019; Harris *et al.*, 2021). This occurred during a flood tide; however this investigation did not focus on visitations to cleaning stations. Thus, the current study focuses primarily on the visitation patterns of *M. alfredi* to the NIdR cleaning station. With the use of photo-ID, acoustic telemetry and modelling techniques, this study aims to examine detection data from tagged individuals, analyse the detection data against environmental influences (such as tidal phase), highlight patterns that will improve future predictability of *M. alfredi* movements and make recommendations for future conservation efforts based on the results found.

2.0 Methodology

2.1 Study Location

The Chagos Archipelago is located 450 km south of the Maldives at the southernmost end of the Lakshadweep-Maldives-Chagos ridge (Harris *et al.*, 2021; Sheppard *et al.*, 2012). The entire archipelago is made up of seven atolls and more than 60 islands (Harris *et al.*, 2021). The exceptional environmental conditions and lack of anthropogenic activity within Chagos makes the location excellent for observing undisturbed behaviours of *M. alfredi*. The NIdR

cleaning station (-6.64, 71.32) is a remote reef within the Egmont atoll, southwest Chagos, in which *M. alfredi* have been seen to frequent (Harris *et al.*, 2021; figure 1).

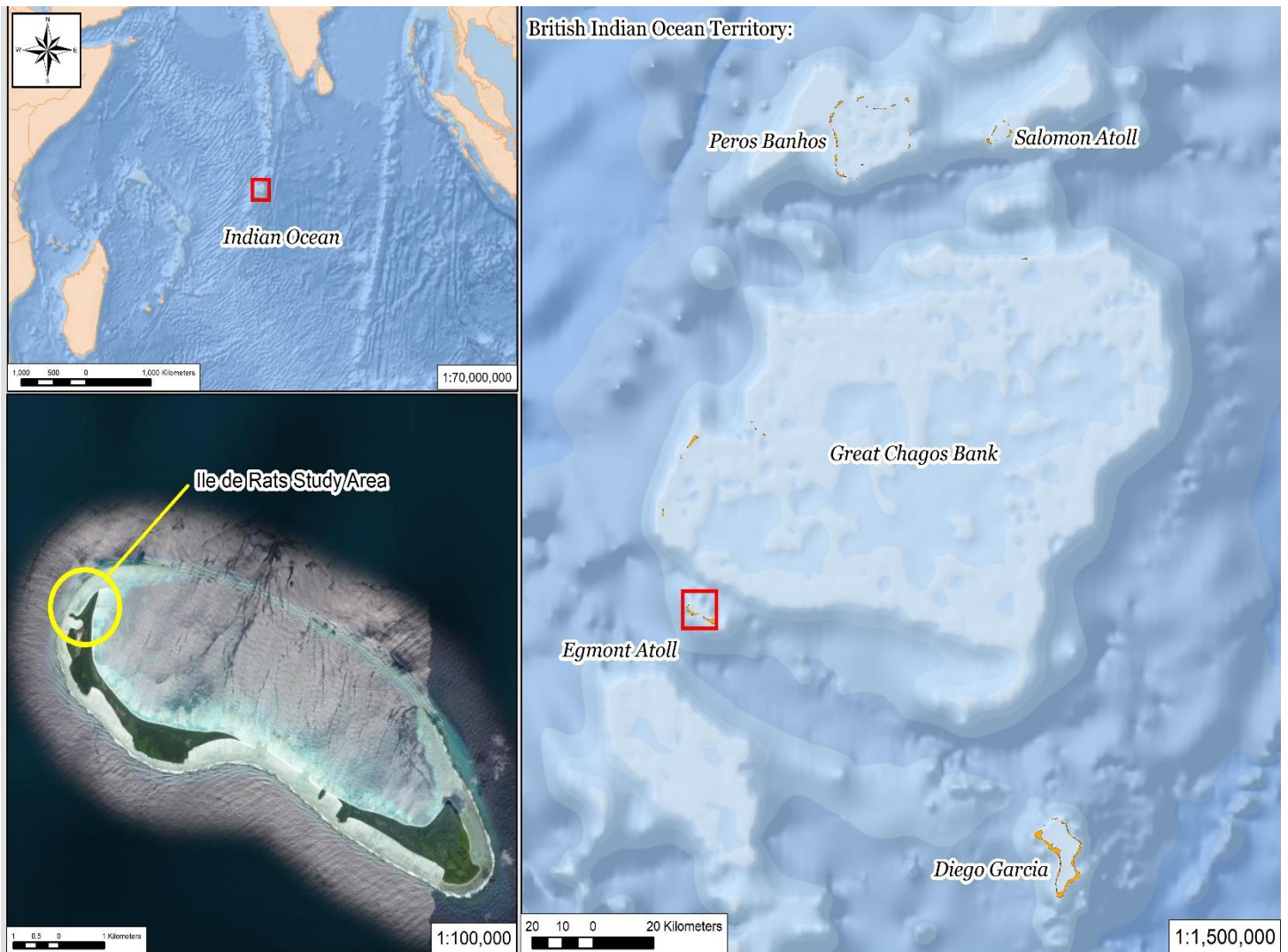


Figure 1: The central Indian Ocean with Chagos Archipelago: British Indian Ocean Territory indicated within the red box (left inset). The Chagos Archipelago with Egmont Atoll indicated with the red box (right). Egmont Atoll and the location of Ile de Rats indicated by the yellow circle and line (left).

2.2 Acoustic Tag Deployment

The Manta Trust deployed 20 VEMCO V16-4x acoustic transmitter tags (Vemco Inc., Nova Scotia, Canada) onto 20 *M. alfredi* individuals between 19th of November and 3rd of December 2019 whilst freediving. All individuals were tagged whilst within the Egmont atoll. Each of the tags were deployed on the right dorsal musculature using modified

Hawaiian hand slings whilst swimming behind the *M. alfredi* (Harris *et al.*, 2021). Prior to tagging, the ventral side of each individual was photographed for photo-ID and sex and size were recorded (Table 1, a tag was believed to have failed, thus there is only the details for 19 individuals provided within this table). All activities were approved by the University of Plymouth Animals in Science Ethics Committee under permit ETHICS-24-2019 (Harris *et al.*, 2021).

Table 1: The identification of the tagged *M. alfredi* individuals (Manta ID), the tag deployment date, location, and demographics of each individual

Manta ID	Sex	Maturity status	Deployment Date	Deployment Location
CG-MA-0035	F	Juvenile	20/11/2019	Ile Sipaille
CG-MA-0046	F	Adult	25/11/2019	Ile Tattamuca
CG-MA-0070	F	Adult	01/12/2019	Ile Lubine
CG-MA-0088	M	Juvenile	28/11/2019	Ile Lubine
CG-MA-0094	F	Adult	30/11/2019	Ile Sipaille
CG-MA-0112	M	Juvenile	01/12/2019	Ile Lubine/Sipaille
CG-MA-0117	F	Juvenile	20/11/2019	Ile Lubine
CG-MA-0118	M	Juvenile	19/11/2019	Ile Sipalle
CG-MA-0119	F	Juvenile	19/11/2019	Ile Sipalle
CG-MA-0120	F	Adult	19/11/2019	Ile Sipalle
CG-MA-0121	M	Juvenile	19/11/2019	Ile Sipalle
CG-MA-0124	M	Adult	20/11/2019	Ile Lubine
CG-MA-0125	M	Juvenile	20/11/2019	North IDR Cleaning St
CG-MA-0139	F	Adult	25/11/2019	Ile Tattamuca
CG-MA-0140	M	Juvenile	30/11/2019	North IDR Cleaning St
CG-MA-0141	F	Adult	28/11/2019	Ile Lubine
CG-MA-0142	F	Juvenile	02/12/2019	Ile Tattamuca
CG-MA-0151	M	Adult	01/12/2019	Ile Lubine
CG-MA-0161	M	Adult	02/12/2019	Ile Carre Pate

The omnidirectional acoustic receiver (VR2W-69 kHz, Vemco Inc., Nova Scotia, Canada) was deployed at a depth of 13.6 meters (m) with the receiver approximately 2 m above the seabed at NIdR cleaning station (-6.64, 71.32). The receiver was equipped with a HOBO® water temperature Pro v2 data logger. The detection range of the acoustic receiver was approximately 160 m, as determined by range testing (Harris *et al.*, 2021).

2.3 Data Analysis

All tag detection data were imported into VUE software (version 2.6.2). False detections were filtered out of the data set using the False Detection Analyser. By doing this, all detections suspected to be false are removed, however, very few detections from VEMCO systems for acoustic telemetry are false (Simpfendorfer *et al.*, 2015). Detection data were filtered into presence and absence data sets (presence or absence of detections) in hourly bins. The environmental influences analysed were; wind speed (km/h), wind direction (°), sea surface temperature (°C), time of day (hour), the presence of rainfall and tidal phase (which was expressed as hours before and after a flood tide). Furthermore, the presence or absence of patterns found will be discussed relating to how these results could aid future conservation efforts of the *M. alfredi* species. Sea surface temperature, wind speed, wind direction and rainfall data were sourced from Meteoblue Data. These datasets were recorded in hour intervals. Microsoft EXCEL was also used to filter the data into hourly bins, presence and absence of rainfall and detections, as well as separating male and female detections. The environmental variables analysed within this study were chosen based on influences analysed in previous investigations as well as to fill previous knowledge gaps concerning influences of visitations to cleaning stations.

Generalised additive models (GAMs) were used to establish the relationship between the *M. alfredi* detections and the environmental influences. This study used R® version 4.1.0 with the

MGCV library (Embling *et al.*, 2010; Wood, 2006) to produce the GAMs (R Core Team, 2021). Smooth and circular smooth GAMs were implemented for each environmental influence in relation to the presence and absence detection data. GAMs can be used with non-parametric data (Hastie *et al.*, 2005) to analyse variable and non-linear relationships between responses and predictors (Guisan *et al.*, 2002). Binominal GAMs were used for all models created - all of which were created as separate variables. Variables were included in the models and decided as significant if the GAMs showed a result above 0 (figures 3a, b and c) and with a P – value of $P < 0.001$.

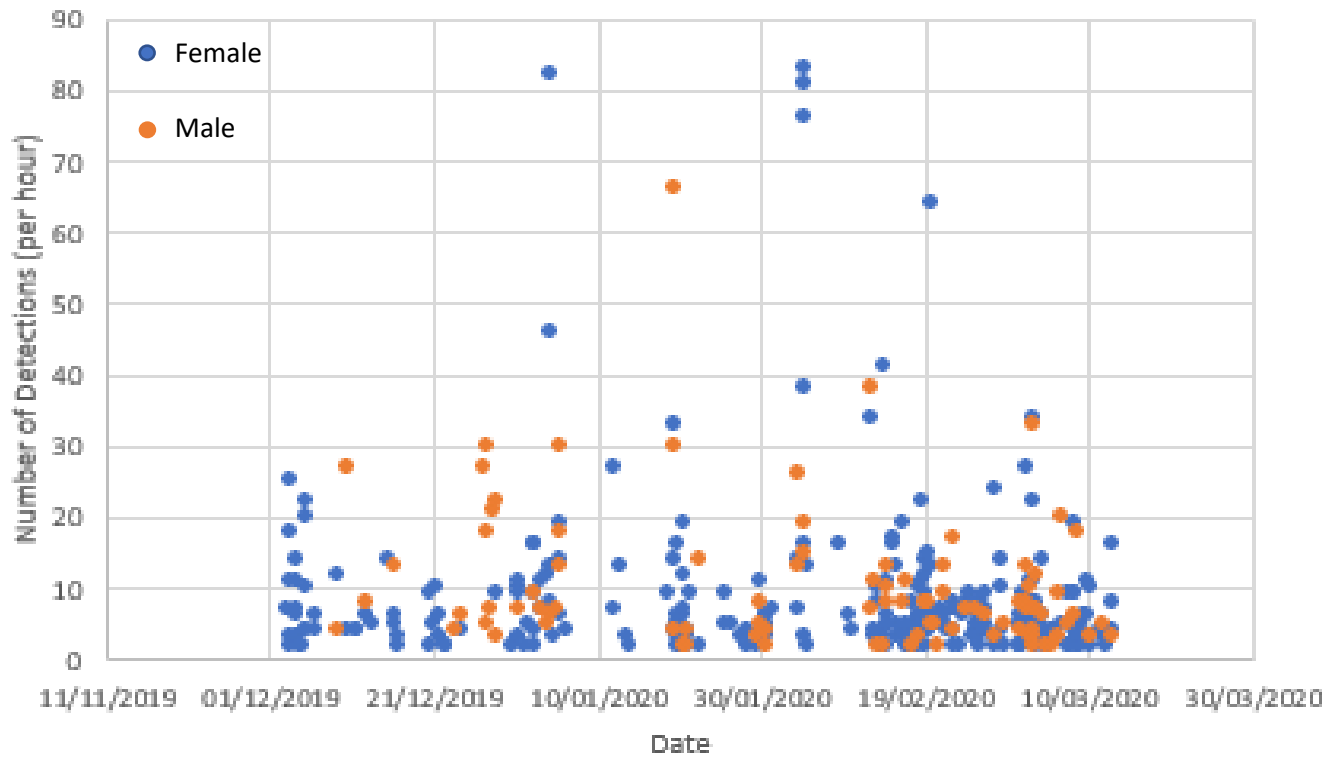
3.0 Results

After tagging, two of the tags were not detected and a third was not detected until May 2020 (two months after the end of the data collection period). Thus, giving a total of 17 individuals providing data for analysis. There were 3,601 detections recorded between the 3rd of December 2019 and the 13th of March 2020. Detections were almost continuous throughout the study period, with clear peaks in January and February (Figure 2a). The individual with the longest resident event was CG-MA-0142 (juvenile female) with a duration of 293 minutes, commencing at 09:50am, the 4th of January 2020 (figure 2b). The days with the most total detections recorded were the; 5th of December, 4th of January, 4th of February and the 3rd of March, with 193, 173, 347 and 273 detections being recorded (respectively).

The individual that spent the least time at the site was CG-MA-0118 (juvenile male), spending a total of 51 minutes at the site throughout the data collection period across 43 detections. The top three individuals with the highest detection numbers were CG-MA-0035 (juvenile female), CG-MA-0094 (adult female) and CG-MA-0046 (adult female) with a total of 407, 395 and 359 detections (respectively) across the data collection period. The lowest 3 individuals were CG-MA-0118, CG-MA-0140 (juvenile male) and CG-MA-0124 (adult

male), these individuals were detected 43, 61 and 70 times, respectively. Individual CG-MA-0035 spent the greatest total duration at the cleaning station across the data collection period. A total duration of 1,390 minutes across 407 detections.

(a) Male and Female - Number of Detections Per Hour



(b) Male and Female - Visitation Duration

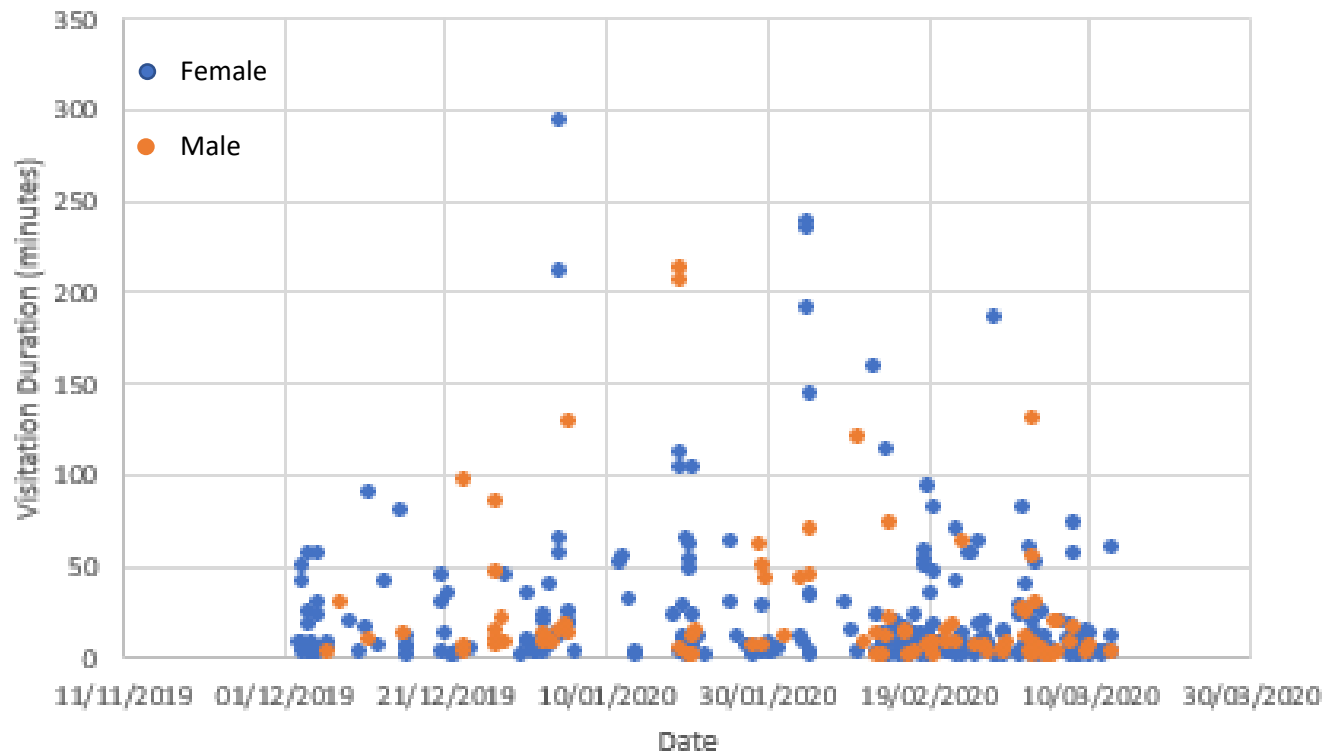


Figure 2: Scatter plots formulated with EXCEL visualising a) the number of male and female detections per hour and b) the visitation durations of males and females at the NIdR cleaning station throughout the data collection period

The month with the highest number of detections recorded was February, with 1,492 detections. January saw the second highest number of detections within a month, with 837 detections. Number of detections per hour (figure 2a) illustrated the dates and time with the highest detections in one hour occurring on the 4th of February, with a total of 86 detections between 15:00 and 16:00 o'clock. A notable quiet period of no detections occurred between the 7th and 11th of January (figure 2a, b).

3.1.1 Generalised Additive Models (GAM)

Of the variables analysed, tidal phase, wind direction and time of day were found to be significant in explaining patterns in visitations to the NIdR cleaning station (figure 3). The peak likelihood of observing a detection is between three and four hours before a flood tide at the study location (figure 3a). The wind direction GAM (figure 3c) indicated the likelihood of detections at the NIdR cleaning station increased at a direction of 50° (North easterly winds). All GAMs were plotted as separate variables, and thus results are significant separately.

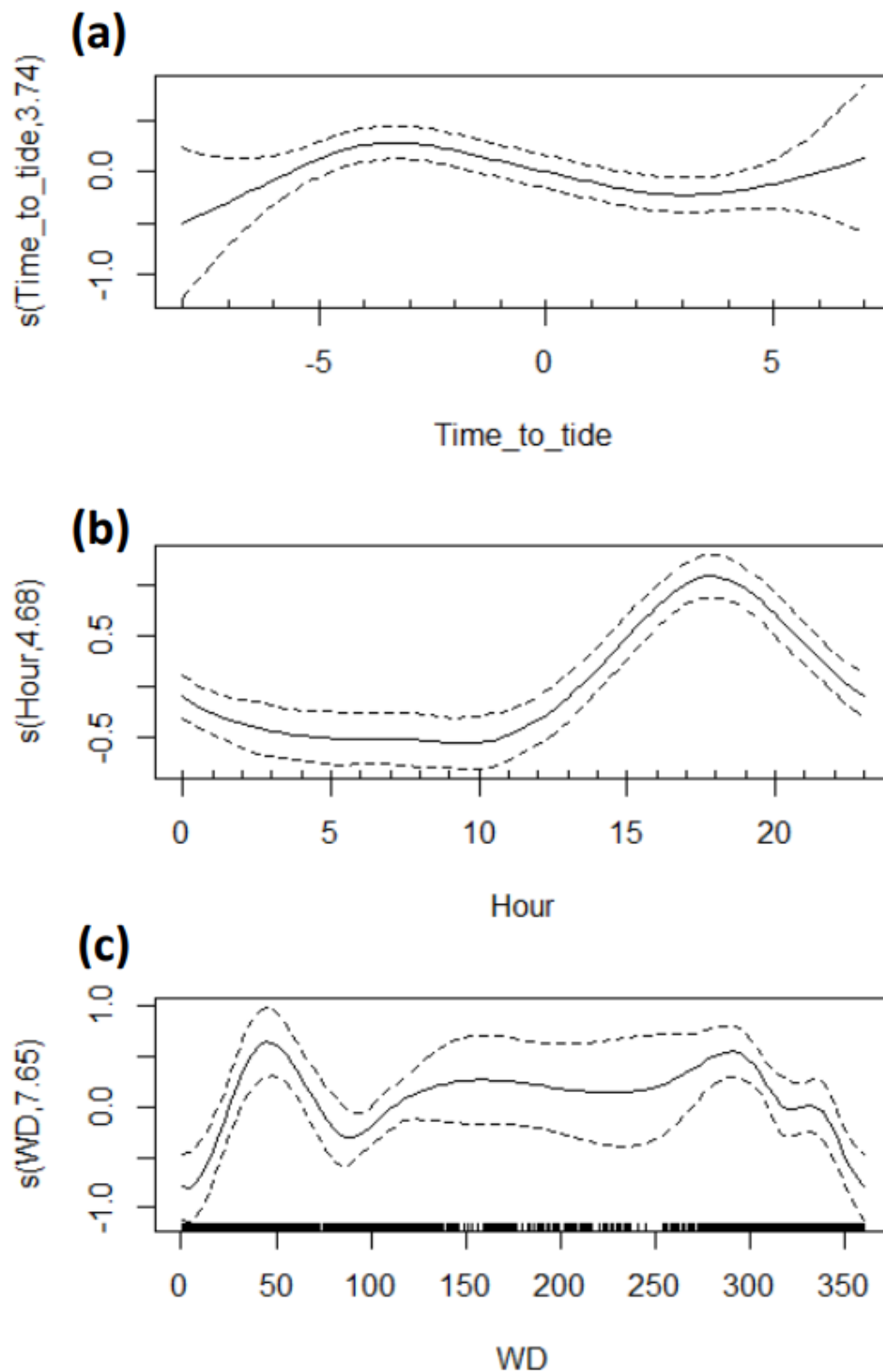


Figure 3: Relationships between the presence and absence of the detections of *M. alfredi* at the NIIdR cleaning station and (a) tidal phase (whereby 0 is high tide, -6 is 6 hours before and 6 is 6 hours after high tide), (b) time of day (in analogue hours) and (c) wind direction (WD, °) whereby 0° is north and 180° is south

Hour of day was the most important predictor of *M. alfredi* visitations to the NIdR cleaning station in the GAMs, with a deviance explained of 5.70% (table 2). The likelihood of detecting *M. alfredi* at the NIdR cleaning station increased for time of day between ~13:00 and 23:00 o'clock, with peak likelihood at 18:00 o'clock, or sunset (figure 3b). The wind direction GAM exhibited a deviance explained of 2.00% (table 2), with likelihood of detections increasing just below 50 ° winds, which then dropped at ~ 75 °. The likelihood of detections then increased slightly ~ 120 ° - 200 ° and peaked again at ~ 290 ° (or between southeast and southwest winds and north westerly winds, respectively). The peak likelihood of detections increasing with 50° winds (north easterly; figure 3c). The final significant variable was tidal phase (time to tide), whereby the GAM model exhibited a P – value of P<0.01, yet a deviance explained of 0.70% (table 2). The GAM results of detections against tidal phase illustrated the likelihood of detections at the cleaning station rose around four hours before a flood tide (figure 3a).

Table 2: Results of binominal GAMs indicating relationships between the presence and absence of detections at the NIdR cleaning station and the environmental variables; tidal phase (time to tide), wind direction (WD) and time of day (Hour).

Time to Tide (Time_to_Tide)		Wind Direction (WD)		Time of Day (Hour)	
Calculation	Result	Calculation	Result	Calculation	Result
Edf	3.80	Edf	7.70	Edf	4.70
P – value	P<0.01	P – value	P<0.001	P – value	P<0.001
Deviance explained	0.70%	Deviance explained	2.00%	Deviance explained	5.70%
UBRE	-0.063	UBRE	-0.071	UBRE	-0.109

4.0 Discussion

The results from this study support previous findings of high site fidelity to identified aggregation sites and cleaning stations by *M. alfredi* (Axworthy *et al.*, 2019; Braun *et al.*,

2015; McCauley *et al.*, 2013). Of the 20 tagged individuals, 17 were detected at the NIdR cleaning station, accumulating 3,601 detections between the 3rd of December 2019 and the 13th of March 2020. These results reaffirm the importance of cleaning stations for *M. alfredi* as key aggregation sites. The individuals with the greatest visitation durations (293 minutes at a single event and 1,390 minutes overall) were juvenile females, whereas the individual with the least detections (43) was a juvenile male. Furthermore, the three individuals with the most detections (407, 395, 359) were also all females and those with the least (43, 61, 70) were males. This therefore indicates that demographics within the species may influence where individuals aggregate, how often and the duration of the visitation (Germanov *et al.*, 2019). This supports previous findings that juvenile *M. alfredi* will spend less time away from the safety of cleaning hotspots due to inexperience and increased risk to prey (Harris *et al.*, 2021; Setyawan *et al.*, 2018). In addition, the results also indicate that an inter species demographic segregation occurs at the NIdR location, due to males typically spending less time at the NIdR cleaning station in comparison to the females.

4.1 Environmental Variables

Time of day was statistically the most influential of the variables analysed within this investigation. The GAMs presented the likelihood of visitations increased between ~13:00 and 23:00 o'clock, peaking at 18:00 o'clock – or sunset. Furthermore, time of day presented a P-value of $P < 0.001$ and a deviance explained of 5.70%. The Edf (Estimated degrees of freedom) and UBRE (Unbiased Risk Estimator) values (of 4.70 and -0.109, respectively) for time of day were also the greatest of the significant variable results. These findings are supported by previous studies which found diel phase to be influential to visitations by *M. alfredi* to aggregation sites (Harris *et al.*, 2021; Jaine *et al.*, 2012; Setyawan *et al.*, 2018), but contrasts findings from another investigation that found no relationship between visitations and time of day (O'Shea *et al.*, 2010). However, to find an increase of visitation likelihood to the NIdR cleaning station beyond sunset and into the night, is unexpected. *M. alfredi* utilise cleaning stations for a symbiotic interaction whereby cleaner wrasse perform parasitic

removal from the *M. alfredi*, yet cleaner wrasse are only active throughout the day (Côté, 2000; Harris *et al.*, 2021). This may be explained by previous findings that highlight juveniles being detected at aggregation sites more frequently at night than adults (Harris *et al.*, 2021). Due to the smaller size and inexperience of juvenile *M. alfredi*, travelling offshore for foraging is less energetically efficient and carries an increased risk from offshore predators to the vulnerable juveniles (Harris *et al.*, 2021). Considering this alongside the results of the greatest total visitation and duration being a juvenile *M. alfredi*, explains why the results demonstrated increased visitations at the NIdR cleaning station beyond the time of day of cleaner wrasse activity.

Previous investigations have found that *M. alfredi* travel further offshore during the night to forage upon diel vertically migrating zooplankton (Couturier *et al.*, 2018; Harris *et al.*, 2021; Jaine *et al.*, 2012). This therefore demonstrates that the NIdR cleaning station may be utilised as a multipurpose aggregation site, whereby the *M. alfredi* will partake in social behaviours. Juvenile females were amongst those with the greatest total detections and time spent at the NIdR location, therefore this may suggest that the NIdR cleaning station is an important habitat for *M. alfredi* during all life stages. This is similar to recent findings of *M. alfredi* habitat utilisation within the Egmont Atoll (Harris *et al.*, 2021).

Wind direction has been found to influence *M. alfredi* visitations to aggregation sites (Armstrong *et al.*, 2016; Couturier *et al.*, 2018; Harris *et al.*, 2020), which has been affiliated to wind influencing plankton availability (Armstrong *et al.*, 2016; Couturier *et al.*, 2018; Harris *et al.*, 2020; Harris *et al.*, 2021). Results from the GAMs exhibited P – values, deviance explained, Edf and UBRE of $P < 0.001$, 2.00%, 7.70 and -0.071, respectively. These results support previous findings of wind direction as a predictor variable for *M. alfredi* visitations to aggregation sites (Armstrong *et al.*, 2016; Couturier *et al.*, 2018; Harris *et al.*, 2020; Harris *et al.*, 2021). However, previous investigation on visitations to aggregation sites by *M. alfredi* in relation to wind direction have found different wind directions to be significant. Different wind directions influencing visitations to cleaning stations at various

locations is expected, as there are several environmental factors unique to each location that affect this. It has been suggested that *M. alfredi* go to cleaning stations when conditions are not favourable for feeding activities, as feeding activities must occur in optimal conditions to allow for minimum energy expenditure (Harris *et al.*, 2020; Harris *et al.*, 2021). The GAMs utilised to analyse wind direction against the presence and absence of detections illustrated the likelihood of *M. alfredi* visitations to the cleaning station increased with peak likelihood around 50°, or north easterly winds. Similarly, a study in the Maldives found that primary productivity decreased with north easterly monsoons, which suggests foraging activities are lessened during these conditions and thus cleaning behaviours may increase (Harris *et al.*, 2020), which thus supports these findings. When considering these results alongside previous findings, this may therefore demonstrate that north easterly wind directions do not provide optimum foraging conditions within other locations and thus the *M. alfredi* aggregate at the NIdR cleaning station during these states.

Due to the Chagos archipelago's global geographical location, the local tidal range there is low. Despite this, GAMs demonstrated the likelihood of visitations to the NIdR cleaning station increased between three and four hours before a flood tide. The statistical analysis conducted to determine the relationship between visitations and tidal phase also presented a P-Value of $P < 0.01$ (as well as a Edf and UBRE of 3.80 and -0.63 respectively), yet a deviance explained of only 0.70%. This result contrasts findings of the majority of *M. alfredi* cleaning behaviours being observed during an ebbing tide to a cleaning station at the outer Great Barrier Reef (O'Shea *et al.*, 2010). Yet, Previous studies have illustrated that cold-water bores exhibit an upward propagation which varies with tidal phase which can provide a pulsed delivery of plankton for *M. alfredi* consumption within the Chagos archipelago (Harris *et al.*, 2021; Hosegood *et al.*, 2019). Recent studies of fine-scale oceanographic drivers of *M. alfredi* to feeding aggregation sites within the Egmont Atoll indicated that preferable feeding conditions occur during the early stages of a flood tide (Harris *et al.*, 2021). Considering this in relation to the GAM results for tidal phase, this may suggest that the *M. alfredi* would partake in cleaning behaviours at the NIdR cleaning station whilst waiting for suitable foraging conditions before a flood tide (Barr and Abelson, 2019).

This investigation did have limitations and could be expanded through further research. For instance, the 4 days with the highest numbers of detections (5th of December, 4th of January, 4th of February and the 3rd of March) were 30, 31 and 27 days apart (respectively). Previous investigations have found a relationship between increased visitations to foraging sites and moon phase, particularly around a new and before a full moon (Jaine *et al.*, 2012). The same investigation demonstrated decreased observations in cleaning behaviours around new and full moons (Jaine *et al.*, 2012). These findings could therefore demonstrate a relationship between *M. alfredi* visitations to the NIdR cleaning station and moon phase. To confirm this, further investigations with fraction of moon illuminated, or lunar phase would be required. In addition, all environmental variables analysed within this study were done so as separate variables. Future investigations could build upon these findings by combining multiple variables to give greater precision when improving predictability of *M. alfredi* movement patterns. Moving forward, the results from this study should be utilised to maintain the protection of *M. alfredi* populations within the Chagos archipelago, as well as improving protective measures for the future. Moreover, the results demonstrate the multipurpose utilisation of the NIdR cleaning station, highlighting the location as crucial reef habitat for the *M. alfredi* that were detected there. Building upon the results from this investigation will further develop knowledge of the spatial and behavioural ecology of the Chagos *M. alfredi* population, which can then be used to scientifically inform future conservation and mitigation actions (Axworthy *et al.*, 2019; Braun *et al.*, 2015). The site fidelity and utilisation of the NIdR reef by the *M. alfredi* further instils the importance of maintaining the Chagos MPA (Andrzejczek *et al.*, 2020; Sheppard *et al.*, 2012).

5.0 Conclusion

Studying *M. alfredi* visitations to the NIdR cleaning station in relation to environmental variables offers a valuable insight into the species behavioural and spatial ecology. The results from the GAMs presented that time of day, tidal phase and wind direction are influential in visitation likelihood to the NIdR cleaning station. Thus supporting future predictability of these visitations. The study provides evidence that the likelihood of

visitations to the NIIdR cleaning station increased with north easterly winds, four hours before a flood tide or between 13:00 and 23:00 o'clock in the afternoon. This information demonstrates when this discrete reef habitat is most likely to be utilised by the *M. alfredi* subpopulation of the NIIdR area. Additionally, the results provide evidence that not only do the *M. alfredi* appear to utilise the NIIdR cleaning station in observable patterns, but that the location is used as a multipurpose location – not just for cleaning activities, by *M. alfredi* through various life stages. Ensuring the maintenance current protection (Chagos MPA), as well as establishing new protective measures in other aggregation sites is made evidently imperative by the results of this investigation. In addition, the results provide a comprehensive representation of *M. alfredi* spatial ecology surrounding the NIIdR cleaning station in relation to several environmental influences. Therefore, the results will improve the predictability of visitations to the NIIdR cleaning station, which may be used to collect more efficient and comprehensive data sets to expand upon the findings within the current investigation. Finally, the results will inform the design of appropriate mitigation actions and conservation protective measures for *M. alfredi* throughout Chagos and wider tropical and subtropical regions.

6.0 References

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Appendix

GAM codes R®:

```
library(mgcv)
```

```
pairs(presabs[9:19])
```

```
gam1 <- gam(Presence_Absence~s(Hour), family=binomial, data=Darcy_Data_for_R)
```

```
summary(gam1)
```

```
plot.gam(gam1)
```

```
gam2 <- gam(Presence_Absence~s(Time_to_tide), family=binomial,  
data=Darcy_Data_for_R)
```

```
summary(gam2)
```

```
plot.gam(gam2)
```

```
gam3 <- gam(Presence_Absence~s(SST), family=binomial, data=Darcy_Data_for_R)
```

```
summary(gam3)
```

```
plot.gam(gam3)
```

```
gam4 <- gam(Presence_Absence~s(rainfall_MM), family=binomial,  
data=Darcy_Data_for_R)
```

```
summary(gam4)
```

```
plot.gam(gam4)
```

```
gam5<- gam(Presence_Absence~s(WD,bs="cc"), family=binomial, data=Data_for_R)
```

```
summary(gam5)
```

```
plot.gam(gam5)
```

```
gam6 <- gam(Presence_Absence~s(WS), family=binomial, data=Darcy_Data_for_R)
```

```
summary(gam6)
```

```
plot.gam(gam6)
```

```
Gam9<-gam(Presence_Absence~s(WD,bs="cc")+s(Hour,bs="cc")+s(Time_to_tide,bs="cc"),  
family=binomial, data=Darcy_Data_for_R) #as test
```

```
Summary(gam9)
```

```
plot.gam(gam9)
```

```
Gam10<- gam(Presence_Absence~s(Hour,bs="cc"), family=binomial,  
data=Darcy_Data_for_R)
```

```
summary(gam10)
```

plot.gam(gam10)

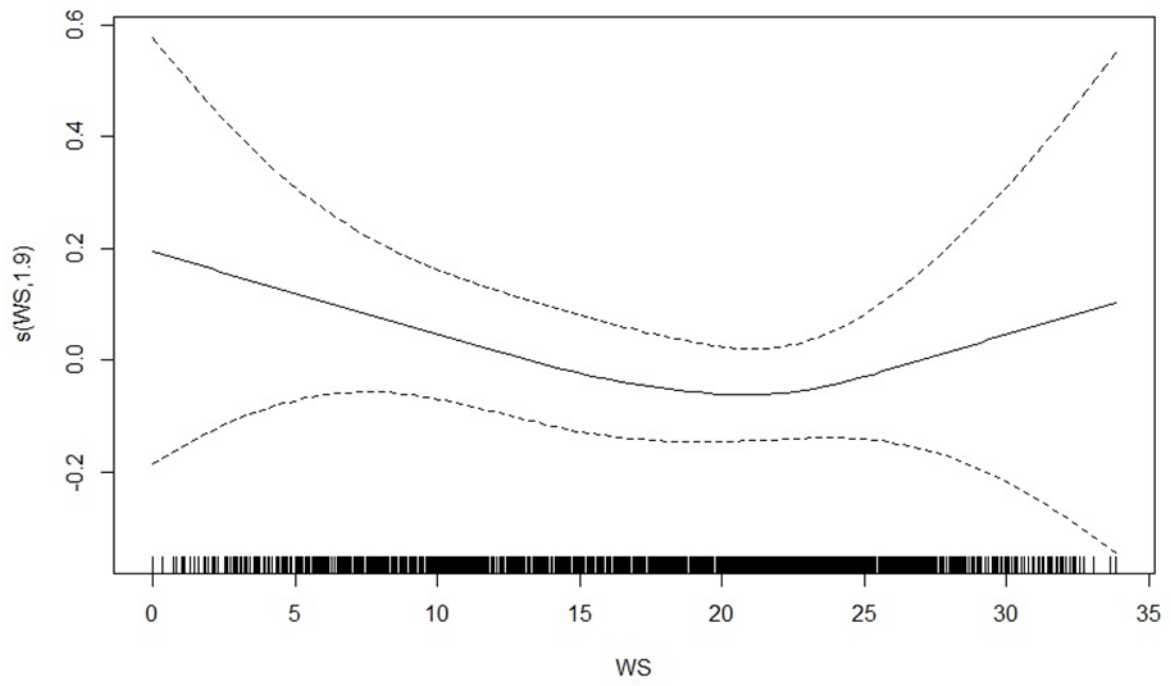


Figure A.1: GAM of wind speed (km/h) in relation to presence and absence of detections of *M. alfredi*

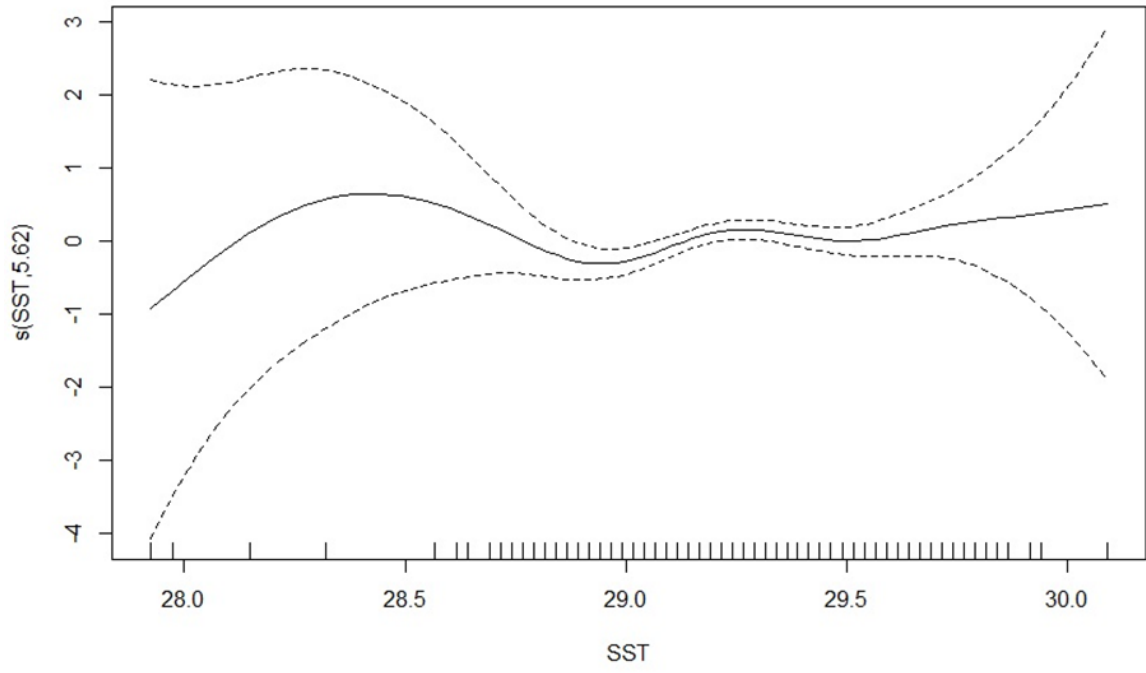


Figure A.2: GAM of sea surface temperature ($^{\circ}\text{C}$) in relation to the presence and absence of detections of *M. alfredi*

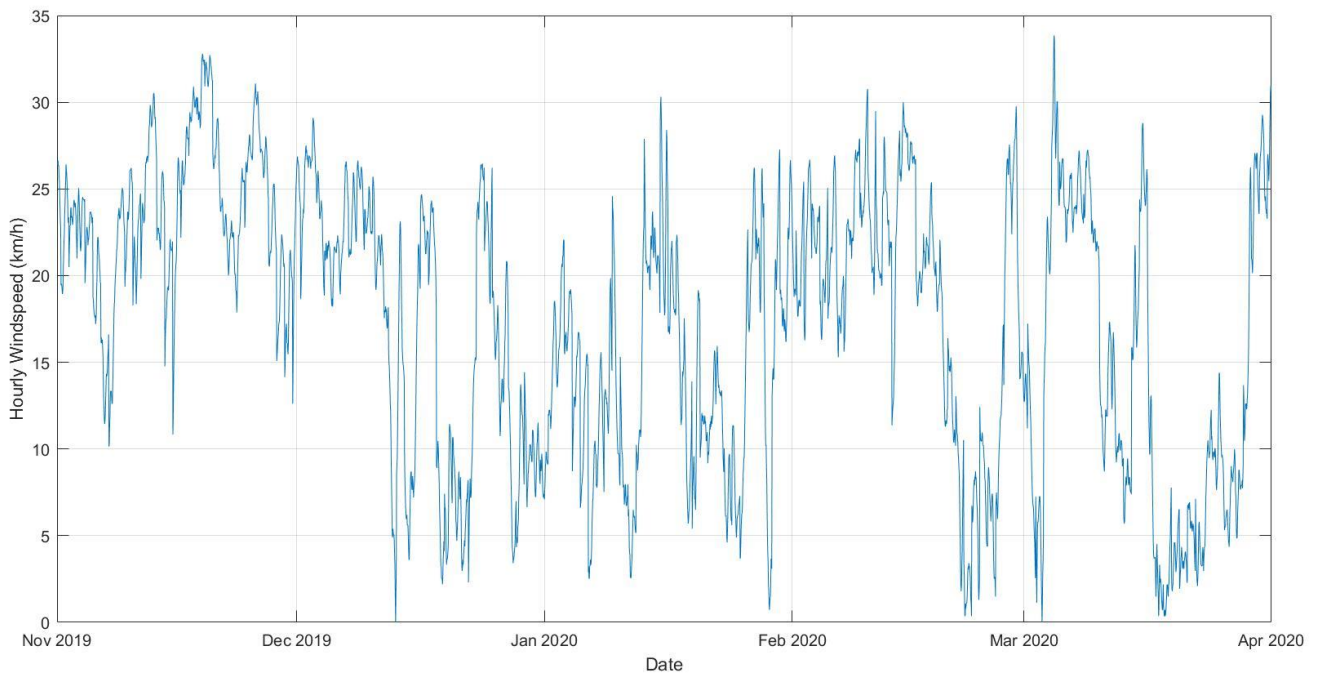


Figure A.3: Hourly windspeed in kilometres per hour (km/h) throughout the data collection period at the Egmont Atoll location

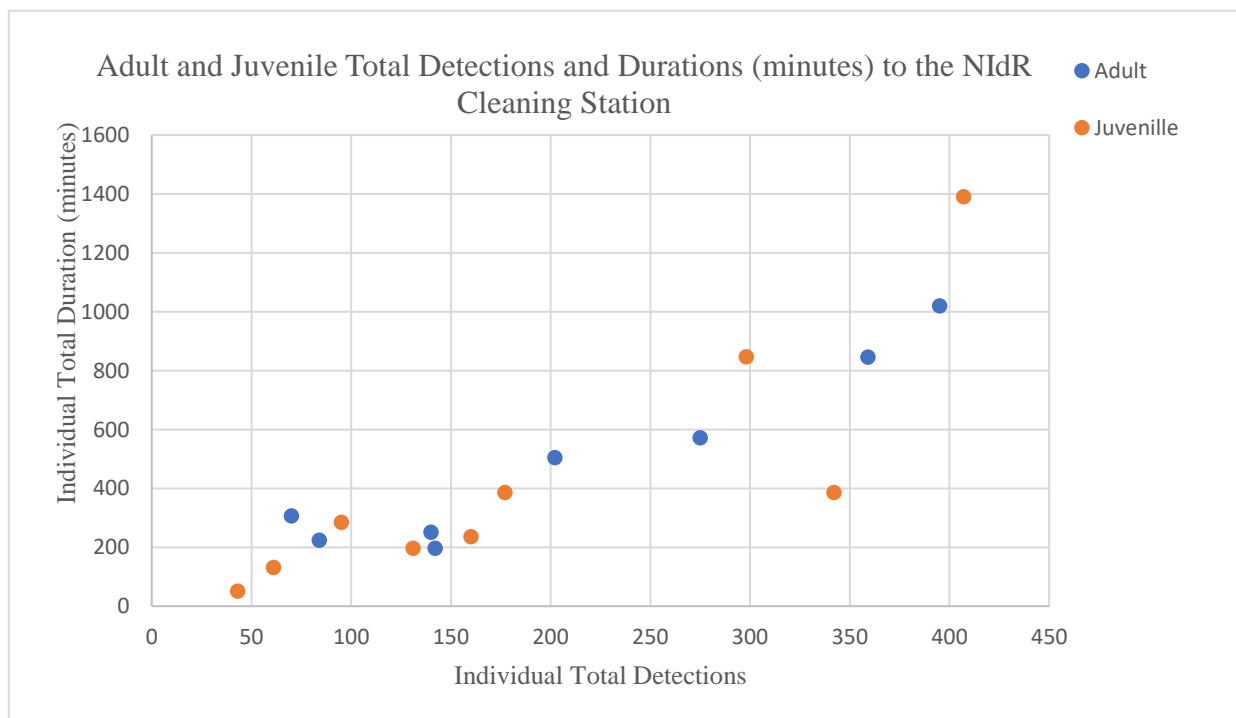


Figure A.4: Total detections (x axis) and total durations (minutes; y axis) of each *M. alfredi* individual across the data collection period. Juvenile individuals are indicated as orange circles and adults as blue.

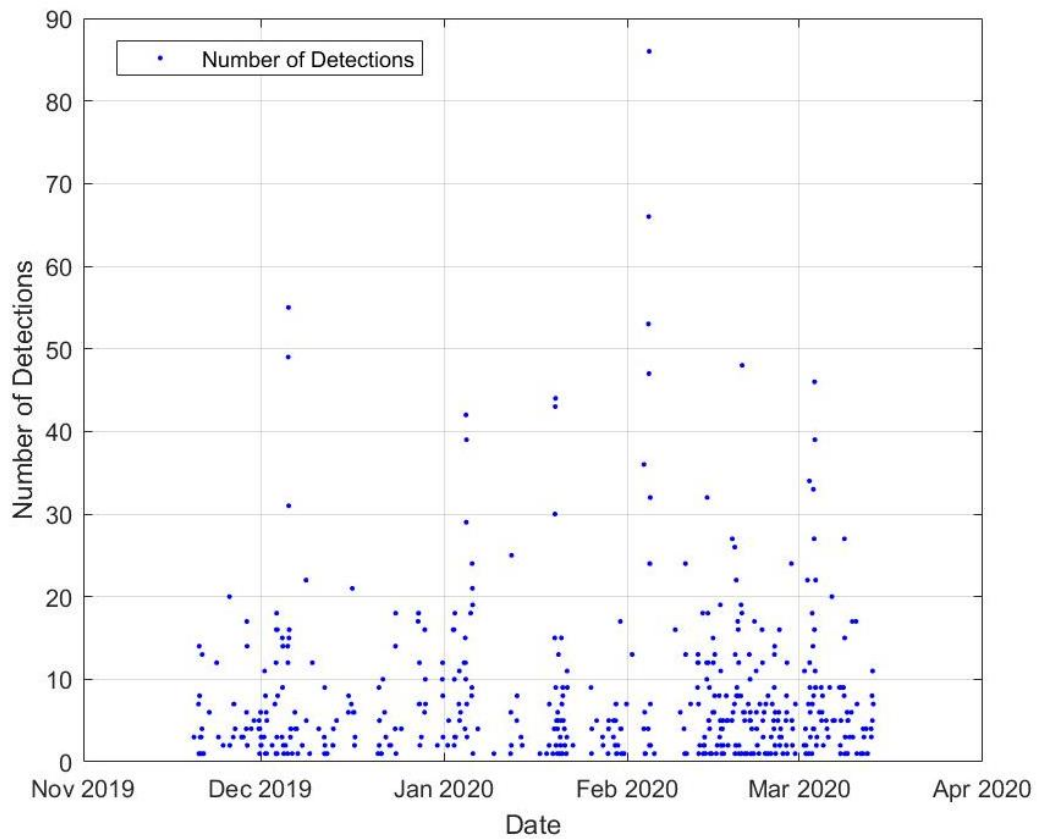


Figure A.5: Total number of detections (per hour) of *M. alfredi* across the data collection period

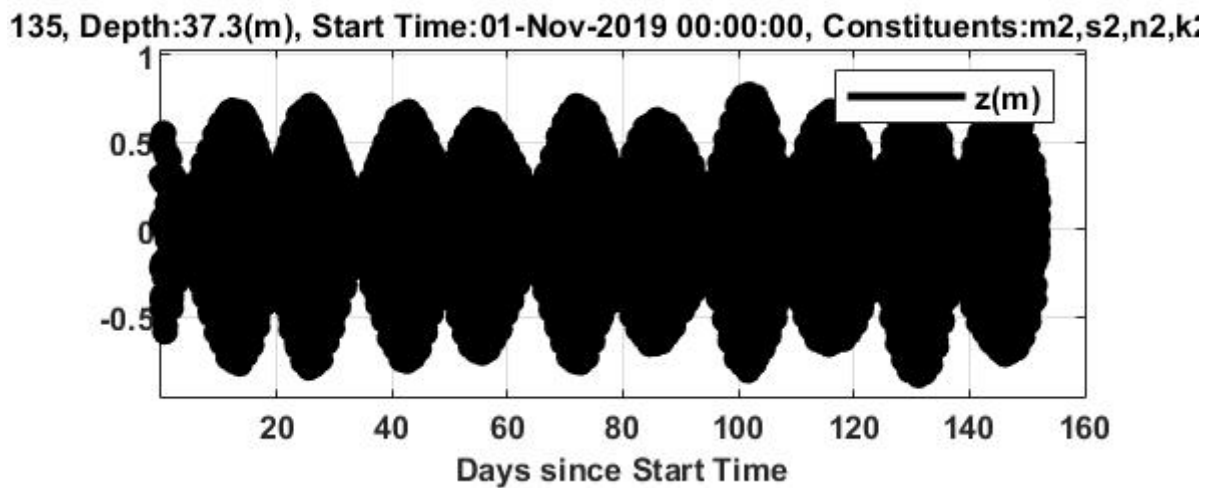


Figure A.6: Tidal Model Driver (TMD package, Matlab®) created of tide predictions at the study location (Chagos).

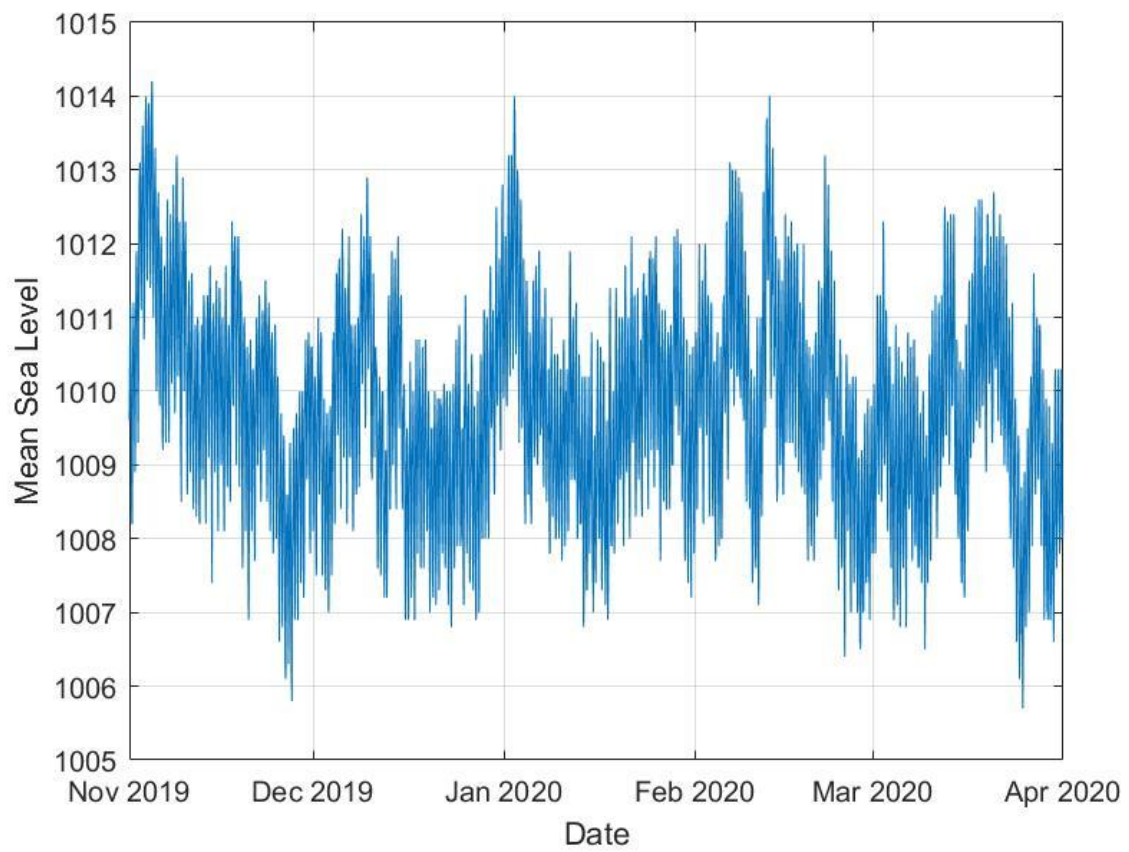


Figure A.7: Mean Sea Level changes at the study location throughout the data collection period

