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Quantifying social interactions and behaviours of *Mobula alfredi* through the use of Crittercams

Masters Dissertation

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Disclaimer

I, Nicole Pelletier, declare that all aspects of this dissertation are my original work. I have cited all sources used and have listed these in the reference section. I declare that my word count is 4999 excluding project title, disclaimer, acknowledgements, reference list, figure legends, tables and appendices.

Acknowledgments

I would like to thank Dr. Guy Stevens for all of his guidance through working on this project and for allowing me to use the Crittercam data for my dissertation. I would additionally like to thank Dr. Julie Hawkins for her support and guidance in editing drafts of my dissertation.

Many thanks are due to the Manta Trust and National Geographic researchers who developed the Crittercams and collected the data used in this project. Additionally, the time series graphs used throughout this dissertation would not have been possible without guidance from Josh Stewart of the Manta Trust. Thank you!

Abstract

Behavioural studies on animals in their natural habitats are important to help researchers understand the full ecology and habitat usage of a species. *Mobula alfredi* (reef manta rays) have been researched using observational studies and animal borne tagging devices, however research gaps exist where these methods present limitations. As an innovative research tool, Crittercam technology has evolved and can now be used to document the behaviours of *M. alfredi.* National Geographic and Manta Trust deployed Crittercams on *M. alfredi* which allowed for quantification of behaviours and social interactions through observation of the video footage. Courtship and feeding behaviours were recorded beyond 65 meters depth and Crittercams additionally recorded social interactions past 95 meters. The maximum depth reached by an individual was 329.5 meters at a corresponding minimum temperature of 12.7^{o}C. I documented interactions with *Mobula mobular* and *Carangidae* fish species to expand knowledge of species ecological roles within reef ecosystems. This study is the first time Crittercam technology has been used to research *M. alfredi*.

Introduction

Mobula alfredi (reef manta rays) are a large, plankton consuming elasmobranch species distributed amongst coral reefs in the Indo-West Pacific (Couturier *et al.,* 2012, Stevens *et al.,* 2018). Closely related to *Mobula birostris* (oceanic manta rays), *M. alfredi* has only been redefined as a distinct species since 2009 and officially under the genus Mobulid since 2017 (Marshall *et al.,* 2009; White *et al.,* 2018). Of the genus, *Mobula alfredi* is most studied and the focus has been to determine population sizes, describe their behaviour and understand their life history characteristics to improve conservation (Young *et al.,* 2006; Stevens, 2016). Innovative research is needed to fully understand the ecology of this species and its habitat use over a greater spatial and temporal scale.

M. alfredi seasonally aggregate in the presence of plankton which provides researchers with ideal opportunities to photograph and study these animals (Anderson *et al.,* 2011; Couturier *et al.,* 2012; Kitchen-Wheeler, 2010; Stevens, 2016). They additionally aggregate in shallow water at so called "cleaning stations" and allow small wrasse to remove parasites from their gill plates and skin (Losey, 1972; Kitchen-Wheeler, 2013). Photographs of their ventral markings serve as a tool for tracking movement and maturation rates as their markings do not change throughout their life and essentially function as a fingerprint (Marshall & Pierce, 2012). Through photo ID, the species has been determined as long lived and slow to reproduce with the greatest population in the Maldives at over 4,000 individuals (Couturier *et al.,* 2012; Stevens, 2016).

Observers at aggregation sites record behaviours related to feeding, courtship and cleaning however behavioural studies are limited by human observers and challenges associated with studying marine species such as depth, ocean currents and infrequent sightings of individuals (Couturier et al, 2012; Schofield *et al.,* 2007). Unable to match the depth and swimming speed of *M. alfredi*, observers are only able to record fragmented durations of time at relatively shallow locations.

To increase understanding of *M. alfredi* habitat range, acoustic tracking devices and satellite tags have been used to determine details about depth, movement and species distribution. Animal borne tags have revealed high sight fidelity, explored offshore habitat usage and extended the known depth range of *M. alfredi* to 400m (Clark, 2010; Braun *et al.,* 2014, 2015; Dewar *et al.,* 2008; Jaine *et al.,* 2014). Tracking devices have increased knowledge of *M. alfredi* movement however gaps remain in understanding spatial habitat usage and behaviour as they cannot be inferred from data logging devices (Braun *et al.,* 2014; Stewart *et al.,* 2016).

Technology is now available in the form of a Crittercam to address the spatial and temporal limitations of observational studies and observational limits of tracking studies (Marshall, 1998; Moll et al, 2007). Evolving over the last 30 years, Crittercams (Fig.1) have been used to record fine scale behaviour and habitat usage of sharks, whales and alligators amongst other species (Meynecke *et al.,* 2015; Nifong *et al*., 2014; Moll *et al.,* 2007; Heithaus *et al.,* 2001). Crittercams are now small enough to record *M. alfredi* behaviour without disturbance to the animal and durable enough to withstand pressure increases at depth (Marshall, 1998; Moll *et al.,* 2007; Stewart et al., 2016). As an additional benefit to using this technology, Crittercams are reusable. They are retrieved on the surface for data download and can be reattached to another individual of the same species.

Fig. 1 Crittercam Unit for *M. alfredi*

Crittercams contain a video camera, depth and temperature sensors and a VHF transmitter for retrieval in one specially designed species specific unit (Marshall, 1998; Moll *et al.,* 2007).

This research represents the first time Crittercams have been used on *M. alfredi* and the aims of my study are to:

- **Evaluate if Crittercams can be used to quantify social behaviours of *M. alfredi***
- **Increase understanding of *M. alfredi* behaviour beyond previously observable limits**
- **Determine if *M. alfredi* interacts with other marine species beyond aggregation sites**

Methods

For this dissertation, I was provided video files and environmental data by researchers from Manta Trust and National Geographic who utilized Crittercams to record *M. alfredi* behaviour.

Deployment of Crittercams

Manta trust researchers and National Geographic partnered to develop Crittercam attachments specially designed for *M. alfredi.* To maximize data collection, research was conducted in the Maldives where *M. alfredi* could be reliably located in a limited amount of time given their seasonal aggregation behaviour (Anderson *et al.,* 2011; Kitchen-Wheeler, 2013; Stevens, 2016). Raa Atoll (Fig.2), located 152km north of Male was designated as the study site based on logistics and known cleaning stations in the region. Cleaning stations at Kottefaru Beyru (5° 30'N, 73° 02'E) and Neyo Beyru (5° 29'N, 73° 02'E) were chosen after surveys indicated a presence of *M. alfredi*.

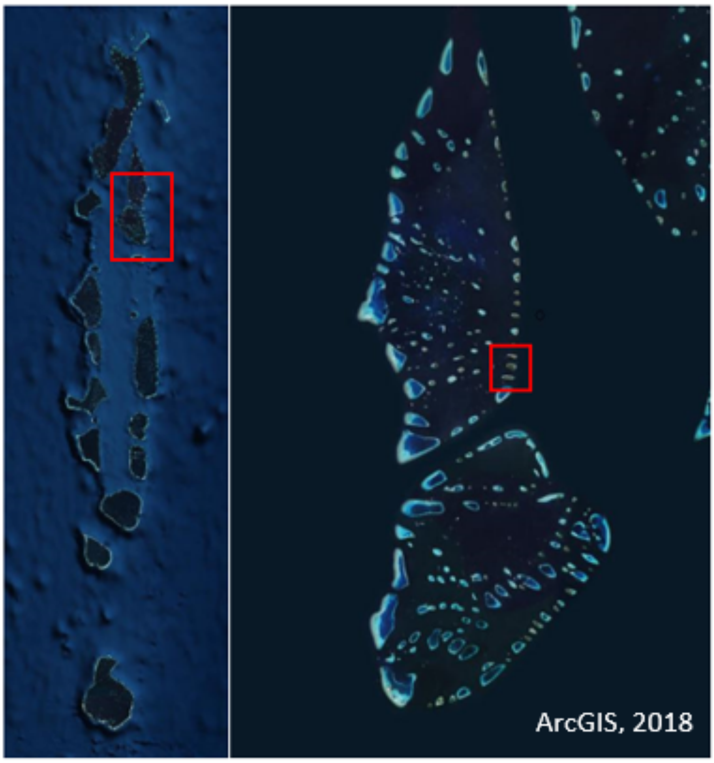


Fig 2. Map of the Maldives

Red box indicates study site within Raa Atoll

Crittercams were opportunistically attached to 11 female and 3 male *M. alfredi* with 1 additional individual of unknown sex over four non-consecutive days in a 7-day period (Oct. 7-12, 2016). Attachment was done via a suction cup and a plastic non-puncturing "hook" device by researchers who swam behind and over top of the *M. alfredi* while they were engaged in slow movements associated to cleaning (Fig.3). Researchers placed the hook under the upper jaw of each animal and pressed the suction cup into the back of the individual (G. Stevens, personal communication, 2018).

Fig 3. *M. alfredi* equipped with a Crittercam
Crittercam unit was connected via a non-invasive plastic hook and suction cup.

Crittercams were released when the animal dislodged the hook through breaching and upward swimming or when a release mechanism in the hook eventually disintegrated after approximately 5 hours. Crittercams floated to the surface and were retrieved by researchers using VHF transmitters.

Data Collection

Two Crittercam units identified as 277 and 475 were used throughout the study with videos stored on the camera memory in approximately one hour clips by date and time stamps. Crittercams recorded pressure in Barr at intervals of 5 seconds and water temperature at 30 second intervals in degrees Celsius.

The research team took photographs of individuals equipped with a Crittercam and recorded identification information. *M. alfredi* were identified by researchers using photographs of the unique markings on their ventral side (Fig.4A) (Marshall & Pierce, 2012; Town *et al.,* 2013). Their maturation status and sex was determined by visual observation and photos of reproductive organs (Fig. 4B-C) (Stevens, 2016; G. Stevens, personal communication, 2018).

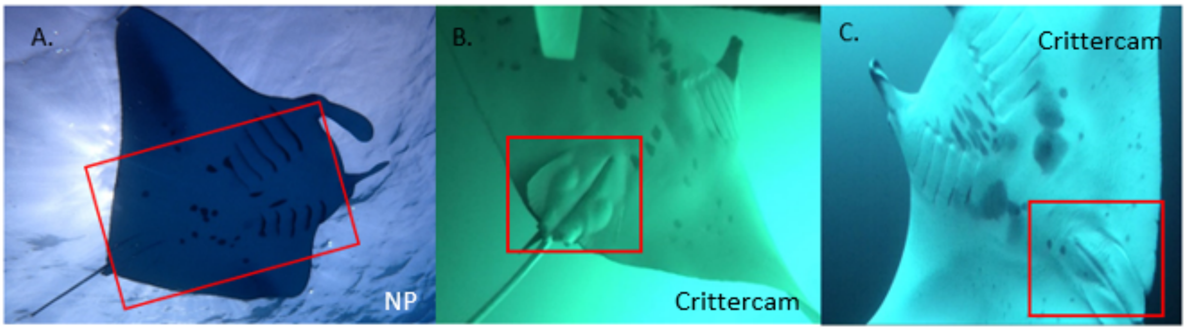


Fig 4: Identification Information Recorded

Image A: Ventral spot patterns (red box) such as the one shown can be used for photo identification of individuals. **Image B:** Claspers highlighted in red indicating male manta. **Image C:** Ventral spot pattern is visible and this individual is a female manta as shown by the absence of claspers.

Research Area

The research team recorded GPS coordinates when the Crittercam was deployed and retrieved on the surface. I used this data to create a map in ArcGIS and determined the distance between deployment and retrieval of the Crittercams.

Data Analysis

Given the provided data, I organized all videos and environmental data into discrete deployments, converted pressure in Barr to meters at a rate of 1 bar = 10 meters, and matched the time stamps of data recording devices to determine the exact time of camera attachment and release. I assigned each *M. alfredi* a deployment number based on the order in which the cameras were attached and will subsequently refer to an individual's collective videos and data as a "deployment".

I viewed all videos from attachment of the Crittercam to when the device surfaced and recorded qualitative observations of *M. alfredi* behaviour and video time stamps for each observation. I matched time stamps to the depth sensor to determine an exact time when behaviours occurred. While viewing the videos, I captured screenshots of interactions with other species.

Natural Behaviours

All *M. alfredi* had a similar reaction when the Crittercam was deployed and the animal was startled by attachment of the device. I termed this a "take off behaviour" which was characterized by fast swimming and quick depth changes. Throughout deployments, the attachment device was not fixed and moved slightly. This occasionally caused the animal to display side to side movements to move the Crittercam or to display a short reactionary behaviour similar to the "take off" behaviour. On three occasions, rolling caused the Crittercam to dislodge and two individuals breached which I considered attempts to remove the Crittercam as *M. alfredi* display similar behaviours to remove parasitic sucker fish (Stevens *et al.,* 2018). Behaviours in response to the camera were considered attachment reaction behaviours. The aim of using Crittercams for this study was to evaluate behaviour under natural conditions and therefore all behaviours that appeared to be in response to the Crittercam were not quantified.

The video component of the Crittercams malfunctioned or ran out of memory for 54 minutes over three deployments. Environmental data was recorded but these periods were not included in calculated behaviour durations. The total time of behaviours recorded without camera reactions and malfunctions was termed the "natural behaviour". Natural behaviour durations were used to determine all percentages of interaction and behaviour. Environmental data was used to determine temperature and depth statistics regardless of missing video segments.

Behaviour Classification

Behaviours were classified based on research from observational studies as well as expert advice from my dissertation supervisor, Dr. Guy Stevens, founder of the Manta Trust.

Cruising was classified as the primary behaviour of *M. alfredi* as the species is constantly in motion to move water over their gill plates for respiration (Tomita *et al.,* 2012). Periods of courtship, feeding and cleaning were determined by specific criteria as outlined in the sections below and summarized in Table 2. Unknown was recorded when a behaviour could not be attributed to a specific category but did not appear to be solely for cruising or when light depletion at depth ended my ability to determine behaviour. Durations of time were only classified under one category with criteria based categories chosen when applicable.

Cruising

Cruising behaviour (Fig.5) included all swimming that could not be definitively linked to courtship, feeding or cleaning. Swimming types outlined in Table 1 were not analysed separately as differentiation was not always possible.

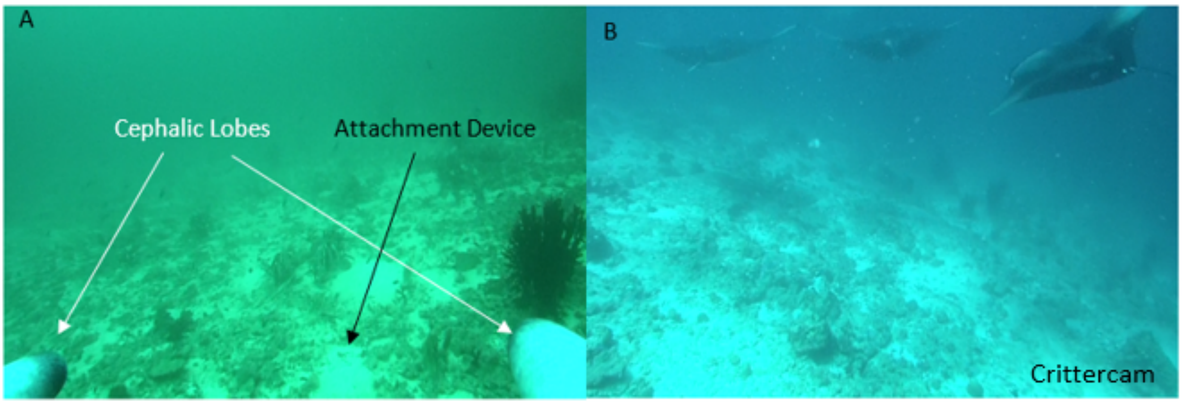


Fig 5. Cruising from the perspective of a Crittercam
Image A: *M. alfredi* cruising alone where the cephalic lobes of the individual can be seen. **Image B:** *M. alfredi* cruising with other individuals. The camera positioning did not allow for cephalic lobes to be visible in all videos.

Table I. *M. alfredi* Swimming Behaviors
M. alfredi employ different strategies to increase their efficiency and alter their position for depth changes. All swimming behavior was considered to be cruising.

Types of Cruising	Criteria
Shallow Cruising	Any form of swimming between 3-10m
Surface Cruising	Any form of swimming at depths of less than 3m
Glide Cruising	Calm movement through the water column with gradual depth change
Diving	Quick upward movement followed by a fast turn and diving
Active Swimming	Up and down motion recognizable on the camera from the wing beats of the animal
General Cruising	All types of swimming that could not be classified into the above categories

Feeding

M. alfredi forage on plankton using eight different feeding strategies, all of which include unfurling their cephalic lobes and opening their mouths to filter plankton from the water (Stevens, 2016; Stevens *et al.* 2018). To account for the forward facing direction of the camera, and to avoid misclassification of feeding in plankton filled waters, feeding was only recorded when other *M. alfredi* were visible with their cephalic lobes and mouths open (Fig.6).

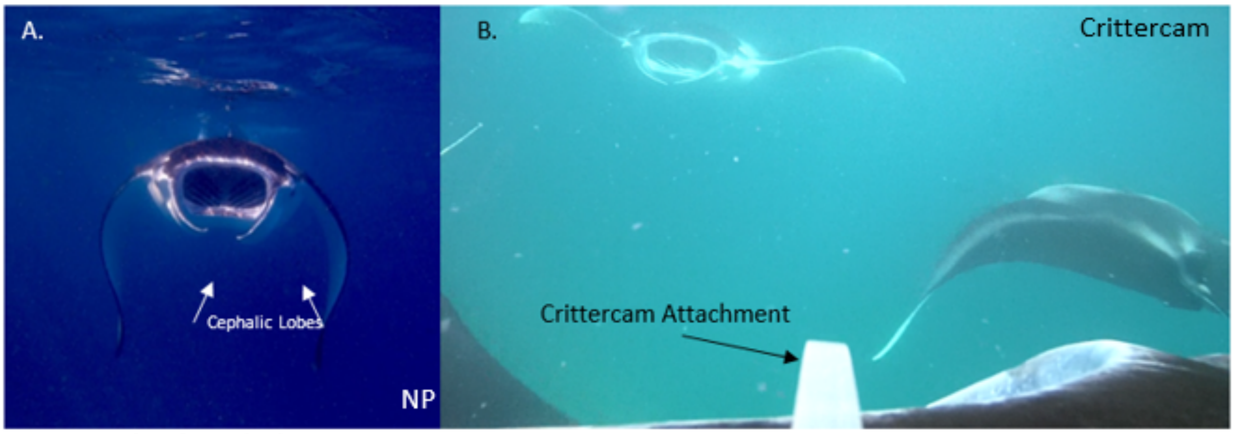


Fig 6. Feeding from dual perspectives

Image A: Taken by in water photographer, this *M. alfredi* is surface feeding with its mouth open and cephalic lobes unfurled. **Image B**: When recorded by the Crittercam, feeding was confirmed by visuals of other *M. alfredi* engaged in feeding.

Courtship

All *M. alfredi* courtship is social and has been described in research literature as a process by which female *M. alfredi* lead social groups of males in synchronized courtship trains (Fig.7) to test their suitability as a mate (Marshall and Bennett, 2010; Stevens 2016). As part of this, the swimming speed and positioning of *M. alfredi* in courtship trains is highly variable with the animals following one another and displaying rolling behaviours (Yano *et al.,* 1999; Marshall and Bennett, 2010; Deakos, 2012; Stevens, 2016; Stevens *et al.,* 2018). To avoid over-classification of ambiguous following which can be attributed to increased hydrodynamic efficiency or predator avoidance, courtship was only determined if *M. alfredi* engaged in forward rolling behaviour (Jacoby *et al.,* 2012; G. Stevens, personal communication, 2018). I included close following or chasing behaviour if it encompassed courtship rolling.

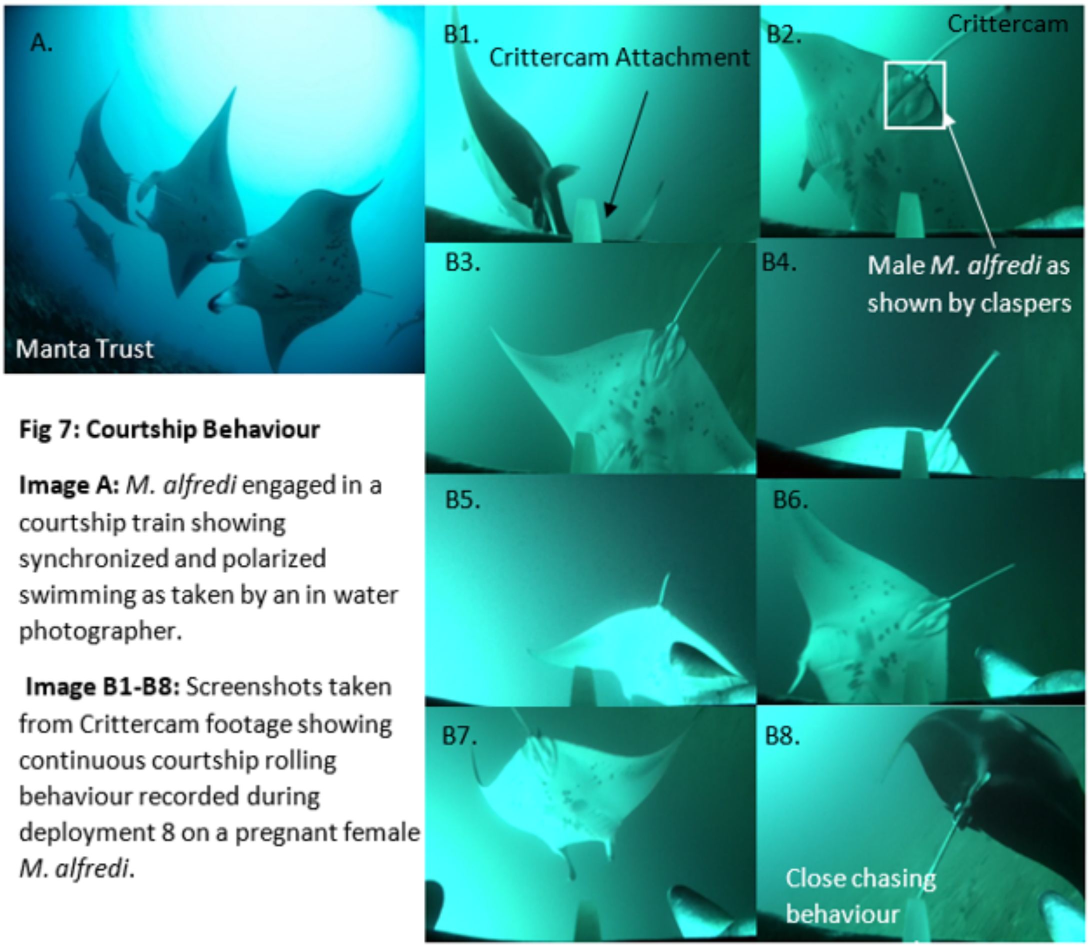


Fig 7: Courtship Behaviour

Image A: *M. alfredi* engaged in a courtship train showing synchronized and polarized swimming as taken by an in water photographer.

Image B1-B8: Screenshots taken from Crittercam footage showing continuous courtship rolling behaviour recorded during deployment 8 on a pregnant female *M. alfredi*.

Cleaning

M. alfredi cruise around "cleaning stations" for periods of time intermittently slowing down over reef structures to allow themselves to be cleaned (Losey, 1972; Jaine *et al.,* 2012; Kitchen-Wheeler, 2013; Stevens *et al.,* 2018). Cleaning stations were determined by the presence of *M. alfredi* in slow motionless behaviour and definitive reef structures providing habitat for cleaner wrasse. Researchers have suggested that cleaning stations could serve as a site to initiate social interactions and courtship (O'shea *et al.,* 2010; Stevens, 2016), however for this research, all visits to cleaning stations were considered for cleaning (Fig.8).

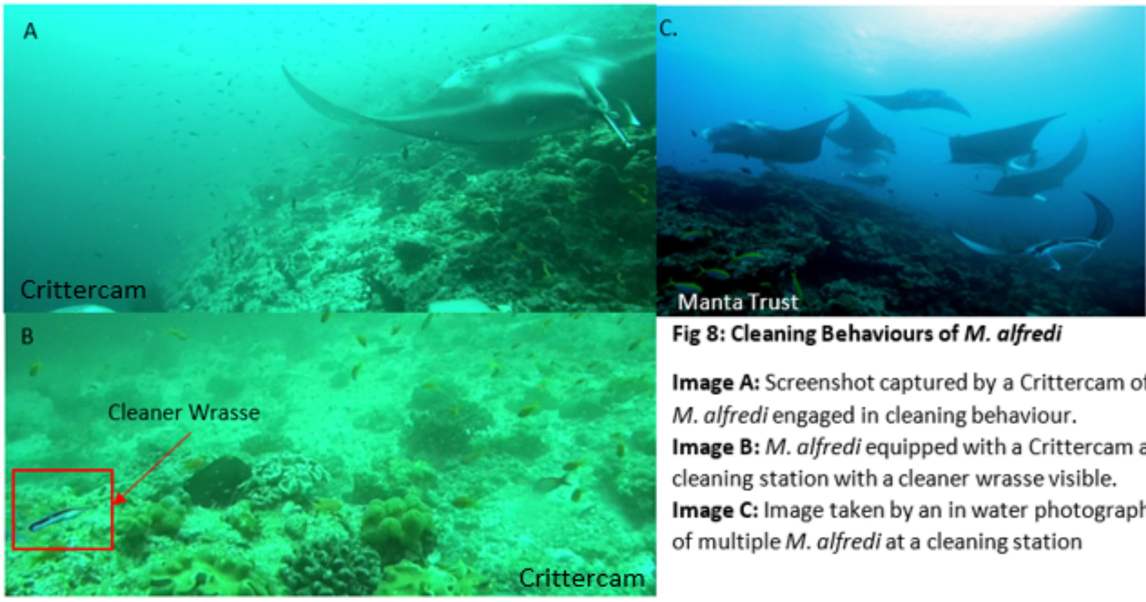


Fig 8: Cleaning Behaviours of *M. alfredi*

Image A: Screenshot captured by a Crittercam of *M. alfredi* engaged in cleaning behaviour.
Image B: *M. alfredi* equipped with a Crittercam at a cleaning station with a cleaner wrasse visible.
Image C: Image taken by an in water photographer of multiple *M. alfredi* at a cleaning station

Table 2: Summary of behavior classification criteria
Cruising was considered an innate behavior for the purpose of movement in the water with feeding, cleaning and courtship being criteria specific.

Category	Specific Criteria shown by *M. alfredi*
Cruising*	All types of swimming for the purpose of movement that did not meet the criteria of feeding, courtship and cleaning
Feeding	Other individuals with mouths open and cephalic lobes unfurled **
Courtship	Forward rolling and close distance following and chasing of other *M. alfredi* encompassing rolling
Cleaning	Cruising in the vicinity of a known cleaning station and slow, almost motionless movements associated with parasite removal by cleaner fish
Unknown	All other behaviours that did not appear to be a reaction to the camera or for the purpose of swimming and could not be definitively associated with feeding, courtship or cleaning.

*Cruising is recorded to a maximum possible duration. **See limitations on camera view.

Determining Interaction

Social behaviour in *M. alfredi* has received relatively little scientific study and the drivers of interaction within the species are not fully understood (Jacoby, 2012; Ari & D'Agostino, 2016; Stewart *et al.,* 2017). Interactions between individuals may occur for an array of reasons including courtship, predator avoidance, increased swimming efficiency, socialization and potentially information exchange (O'shea *et al.,* 2010; Jacoby *et al.,* 2012; Ari & D'Agostino, 2016; Stewart *et al.,* 2017). A level of interaction was assigned to each duration of behaviour based on criteria outlined in Table 3. During behaviours where an interaction ended, I used a period of 10 seconds where *M. alfredi* could return before the interaction became solitary.

Table 3. Summary of interaction criteria
Solitary levels are recorded to a maximum level with social and passive recorded to minimum levels as described in the limitations.

Level of Interaction*	Criteria shown by *M. alfredi*
Social	Distinct following behaviour Directional changes in response to another *M. alfredi* Synchronized swimming with others
Passive	Other M. *alfredi* are recorded by the camera No distinct interactions with other animals
Solitary*	No other *M. alfredi* recorded by video

*Solitary behaviors are recorded to a maximum value due to the limited camera view.

Interaction and behaviour classification

The two classifications scales were combined to create categories of behaviour interaction (Table 4). The entirety of the natural behaviour duration time (21:51:05) was categorized under one of the behaviour interactions in Excel and recorded to the nearest second.

Table 4. Dual Behaviour Interaction Categories
All recorded video was classified under only one interaction level and one behaviour category. These classification schemes were combined to determine a dual classification for each recorded observation.

Interaction	Behaviour	Criteria
Social	Cruising	Cruising behaviour where animals follow one another or use synchronized swimming patterns
	Courtship	All courtship behaviour is social since for the purpose of choosing a mate
	Cleaning	*M. alfredi* were cleaning while following the swimming pattern of or interacting with another *M. alfredi*
	Feeding	*M. alfredi* were following another animal or changed behaviour to follow a specific *M. alfredi*
	Unknown	Interactions between individuals where the purpose could not be determined
Passive	Cruising	*M. alfredi* were swimming near to another individual but not directly engaged in following or synchronized swimming behaviour
	Cleaning	Animals engaged in cleaning or circling around a cleaning station but showed no direct following or interaction with others present
	Feeding	*M. alfredi* was feeding with other manta individuals but no following behaviour or direct interaction
	Unknown	Other *M. alfredi* were present on video but no following, chasing or synchronized swimming occurred
Solitary	Cruising	*M. alfredi* was swimming and no other individuals present in the video
	Cleaning	*M. alfredi* was being cleaned with no other *M. alfredi* present
	Unknown	*M. alfredi* engaged in an undetermined behaviour and no other individuals in the video

Quantification of behaviours

Cumulative durations of behaviour interaction categories for each animal were determined in Excel. The behaviour interaction durations were divided by the natural duration to determine a percentage of time *M. alfredi* engaged in behaviours at each level of interaction. This was done for individual *M. alfredi* and the collective durations of all individuals.

Quantification of behaviour based on a factor

Deployments were split into two groups, early (8am-1pm) and late deployments (1pm-6pm). The same method for determining percentage of behaviours was used for each group to compare behaviours by time of day. This was additionally done to determine a comparison of animals by sex with the final deployment removed as the sex of this individual was not recorded.

Behaviour Events

Uninterrupted durations of criteria based behaviours (recorded to the second) were recorded as singular “behaviour events” (rounded to the nearest 5 seconds) regardless of interaction to match behaviour to depth and temperature (i.e. cleaning events, feeding events and courtship events). I recorded periods of combined social and passive interaction regardless of behaviour to the nearest minute and termed these “social events”.

Analysis by Depth

I recorded the maximum depth for each deployment and calculated the average depth for each individual**.** Using R software, I plotted the depth information recorded at 5 second intervals to create 15 time series graphs, one for each deployment. Behaviour events and social events were plotted on time series graphs to the nearest minute to indicate when events occurred. Graphs were visually analysed to determine a maximum and minimum depth for behaviours. Summary statistics for behaviour and social events were calculated using depth data points.

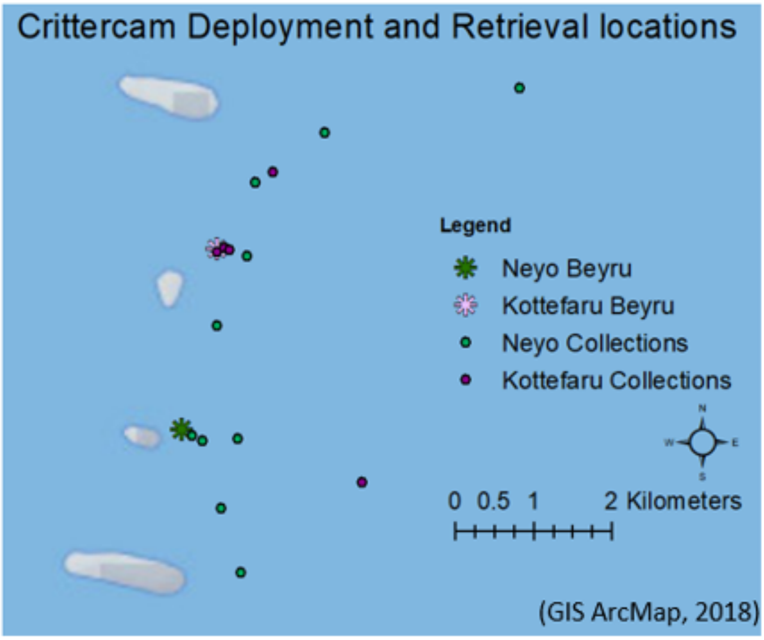
Analysis by Temperature

A second set of time series graphs were created to show temperature in relation to depth for each deployment. Behaviour and temperature graphs were visually compared to determine a temperature range for behaviours. Using temperature sensor data, I determined temperature summary statistics for behaviour events.

Results

GPS Coordinates

Six deployments were initiated at Kottefaru Beyru and nine at Neyo Beyru. The maximum distance between Crittercam deployment and retrieval locations was 4.23 km. Four Crittercams were retrieved at the same location where they were deployed (Fig.9).



Deployment	Distance (km)
0	0
1	3.39
2	1.97
3	1.07
4	0
5	0.15
6	1.2
7	0.54
8	4.23
9	2.42
10	0.2
11	1.42
12	0
13	4.42
14	3.42

Fig. 9 Crittercam deployment and retrieval locations in Raa Atoll, Maldives
Map shows deployment locations at Kottefaru Beyru and Neyo Beyru (indicated by stars) and the retrieval locations of Crittercams as indicated by points. The color of the points corresponds with the deployment location. The table shows the distance between deployment and retrieval of the Crittercams.

Recording Time

Crittercams recorded 23 hours and 22 minutes of data on the behaviour of *M. alfredi*. Camera attachment reaction behaviours accounted for 36 minutes and camera malfunctions totalled 54 minutes. Camera reaction time amounted to 2.62% of the total deployment time; Crittercams were considered to be of minimal disturbance to the natural behaviour. Durations of individual deployments and general study information is in table 5.

Table 5. Summary of Crittercam deployments

The duration of Crittercam deployments was varied over the 4 days of research. The camera reaction time and malfunction times are subtracted from the total duration to determine the duration of natural behaviours. Time and sex were the factors considered for a comparison of behaviour.

Deploy Number	ID	Sex*	Time[1]	Total Duration (mins)	Camera Reaction (mins)	Camera Malfunction[2] (mins)	Natural Duration (mins)
0	MV-MA-1035	F	L	14.45	0.73	--	13.72
1	MV-MA-1566	F	E[1]	156.22	1.05	--	155.17
2	MV-MA-1125	F	E	33.27	1.55	--	31.72
3	MV-MA-3929	F	E	36.02	6.17	--	29.85
4	MV-MA-3158	F	E	4.13	0.68	--	3.45
5	MV-MA-1822	F	E	40.53	1.40	--	39.13
6	MV-MA-1069	M	E	48.3	2.65	--	45.65
7	MV-MA-1308	F	L	44.12	1.90	--	42.22
8	MV-MA-0666	F**	L	250.55	1.83	35.88*	212.83
9	MV-MA-4169	F	E	175.95	6.55	--	169.40
10	MV-MA-1563	F*	E	32.33	1.27	--	31.07
11	Unknown A	M	L	60.93	1.37	--	59.57
12	MV-MA-2175	F	E	6.02	0.63	--	5.38
13	Unknown B	M	L	302.50	3.80	9.60**	289.15
14	Unknown C	UK***	L	196.47	5.20	8.48***	182.78

*All animals recorded were adults with the exception of deployment 10 being sub-adult **Third trimester pregnant female *M. alfredi*. ***Researchers unable to photograph this animal and could not confirm sex. Time[1] E = Early deployments (8:00-13:00) L = Late deployments (13:00-18:00) Time determined by the majority time recorded during each deployment. Camera Malfunction[2] = *Memory card space was depleted. **Camera malfunctioned at a depth of 239m and resumed recording at 70m. ***Unknown reason for camera malfunctioning between recordings.

Quantification of Behaviours and Interaction Levels

The total natural duration across all individuals was 1311.08 minutes for which behaviours and interactions were recorded. Cruising was the primary behaviour recorded during all deployments (n=906.5 minutes) with cleaning (n=159.08 minutes), feeding (n=73.18 minutes) and courtship (n=83.17 minutes) collectively accounting for 24% of behaviour (Fig.10A). Cleaning was recorded during 13 deployments and represented at all levels of interaction. Feeding was recorded as social and passive for two deployments with courtship recorded during five deployments. For individual durations by interaction, see Appendix 1, Table A1.1.

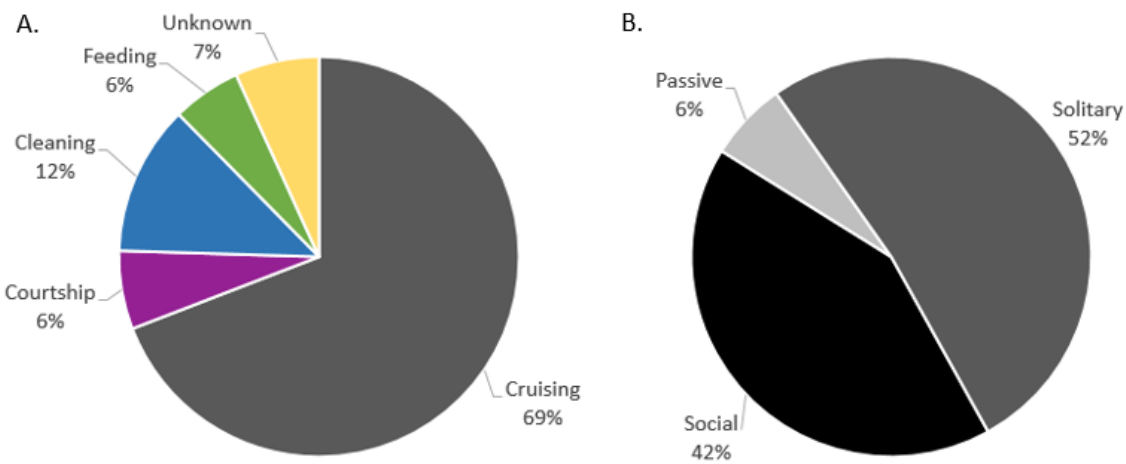


Fig. 10 Pie charts showing the percentage of duration recorded vs. individual classification scales Chart A: percentages of behaviour for all individuals (n=1311.08 minutes). **Chart B:** cumulative level of interaction across all behaviours (n=1311.08 minutes).

M. alfredi were recorded as solitary for the majority of the video footage (n=677.85 minutes) however they engaged in confirmed socialization across all behaviours for extended periods of time totalling almost half of the recordings (n=549.67 minutes) (Fig.10B). Classification scales were combined to determine the cumulative time all *M. alfredi* spent engaging in each behaviour at different levels of interaction as shown in Table 6.

Table 6. Durations of behaviour by interaction level for *M. alfredi* equipped with Crittercams
Cumulative durations of behaviour by each interaction level are presented for the entire research group of *M. alfredi*. The majority of behaviour was solitary cruising followed by social cruising.

Interaction	Behaviour	Cumulative Duration (minutes)
Social	Cruising*	332.97
	Courtship	83.17
	Cleaning	49.02
	Feeding	65.02
	Unknown	19.50
Passive	Cruising*	17.22
	Cleaning	46.85
	Feeding	8.17
	Unknown	11.33
Solitary*	Cruising*	556.33
	Cleaning	63.22
	Unknown	58.30

*Determined to a maximum possible duration for behaviour or interaction level. **Bold print signifies criteria based.**

When separated by interaction level, socialization was a component of all behaviours (Fig. 11) however cruising and cleaning were recorded at their highest levels as solitary. Interaction while cleaning was varied with social cleaning accounting for 31% and solitary cleaning accounting for 40% of the behaviour. Passive feeding accounted for <1% of the total behaviour but given the short duration of feeding, this was actually 11% of all feeding and significant to the study. Unknown behaviour interaction was varied at 58% solitary, 11% passive and 20% social.

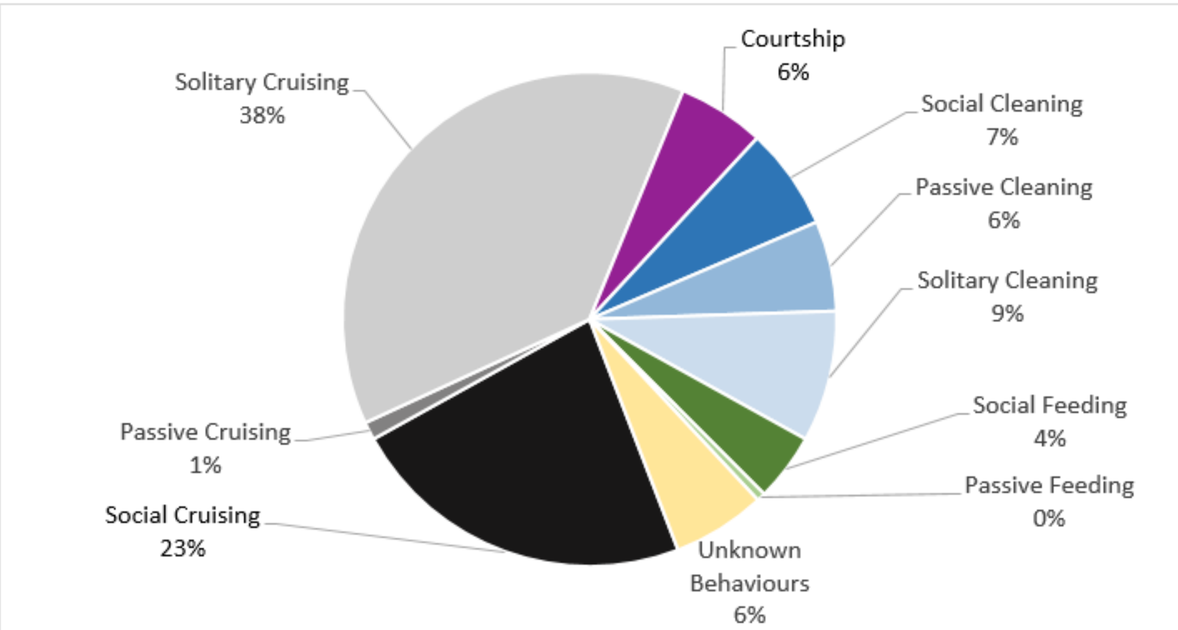


Fig. 11 - Cumulative percentages of behaviour split by interaction level for all *M. alfredi*
Durations of time were recorded under one level of interaction and one behaviour category before being merged. This figure visually represents the varying levels of interaction across all behaviours.

Quantification by Factor

M. alfredi recorded videos from 8am-6pm with 10 deployments (n= 570.38 minutes) occurring from 8am-1pm. Five deployments (n= 740.70 minutes) took place between 1pm-6pm however these deployments provided 23% more footage than early deployments.

Percentage of cruising behaviour was relatively consistent across both time factors, 71.5% for early deployments and 67% for late deployments. Courtship behaviour accounted for an 8% greater part of the late duration while feeding behaviour was only recorded during late deployments. Cleaning behaviours recorded in the early duration totalled 113.28 minutes with cleaning in the late duration totalling only 45.8 minutes. *M. alfredi* engaged in solitary interactions for 56% of the early deployments and 48% of the late deployments. With the increase in criteria based behaviour, there was an increase in social interactions accounting for 46% of the late deployments compared to 36% of the early deployments. The full breakdown of behaviours and interaction by time of day are shown in Fig 12.

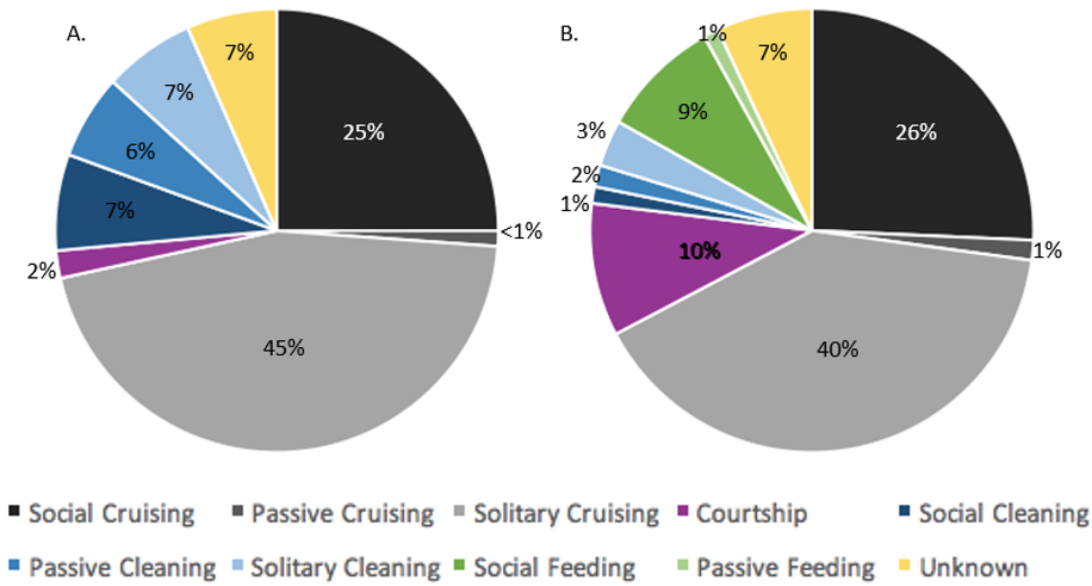


Fig. 12 Behaviour and Interactions of *M. alfredi* by Time of Day:

Chart A: percentage of behaviour and interactions occurring between 8am and 1pm over 10 deployments (n=570.38 minutes). **Chart B**: percentage of behaviour and interactions occurring between 1pm and 6pm over 5 deployments (n=740.70 minutes).

Figures showing *M. alfredi* behaviour by sex are in Appendix 1, Fig A1.1. I did not include these in the results as sex was not determined for all individuals involved in the research which significantly reduced the durations that could be compared.

Environmental Data

The maximum depth recorded across all Crittercam deployments was 329.5 meters with only two individuals exceeding depths of 100m. The average depth over all deployments was 34.64 meters (n=15 deployments). The minimum temperature recorded by the Crittercam sensors was 12.7^{o}C with two individuals reaching temperatures below 20^{o}C. Summary information for all deployments is in table 7.

Table 7. Temperature and Depth Statistics for all *M. alfredi* deployments

Summary statistics are provided for depth and temperature over each deployment and as a cumulative value for all deployments.

Deploy No	Average Depth (meters)	Depth Range (meters)	Average Temp (°C)	Temperature Range (°C)
0	7.33	1.3 - 18.7	28.4	28.3 - 29.0
1	38.4	6.6 - 91.8	27.5	24.6 - 28.8
2	15.7	0.1 - 40.7	28.4	28.2 - 29.5
3	37.2	6.4 - 68.3	28.3	28.1 - 28.5
4	14.4	9.5 - 23.1	28.4	28.4 - 28.5
5	19.2	3.5 - 67.2	28.5	28.0 - 28.6
6	65.4	3.4 - 96.6	25.3	21.9 - 28.6
7	43.7	4.8 - 80.8	27.2	23.8 - 28.7
8	34.5	-0.1 - 89.6	27.2	20.5 - 29.0
9	57.3	5.6 - 157.7	26.1	16.9 - 28.6
10	35.5	7.0 - 79.6	27.9	23.8 - 28.6
11	43.1	2.9 - 91.0	26.9	24.3 - 28.8
12	20.3	0.4 - 44.7	28.5	27.5 - 28.7
13	45.2	-0.2 - 329.5	26.0	12.7 - 29.0
14	46.2	2.2 -85.7	26.0	20.9 - 28.9
Total	**42.7***	**-0.2 - 329.5**	**26.7***	**12.7 – 29.5**

*Values presented in the table for average depth and average temperature are weighted, using all available data points recorded throughout deployments

Events by Depth

Social events were recorded during fourteen deployments with a total duration of 10 hrs and 33mins. Social events occurred across all depths from the surface to 96.6m and uninterrupted socialization ranged in duration from 11 seconds to 54.22 minutes**.** Twenty-two cleaning events, nineteen courtship events and thirteen feeding events were observed across all deployments (Appendix 2, Tables A2.1-A2.3). Summary statistics for behaviour events are shown in table 8. Behaviour events were plotted to the time series of depth and social events were highlighted in R as shown on the following pages in Figs 14-16 and in Appendix II.

Table 7: Summary Statistics for Criteria Behaviours
Temperature and depth statistics were determined for each event and additionally determined as a weighted value using all environmental data points recorded during behaviour duration.

Event	No. of Events	Mean Duration (n=no. events)	Duration Range	Mean Depth (n= events)	Max Depth	Mean Depth **(weighted)**	**Mean Temp**	**Temp Range (c)**
Cleaning	22	7.04 mins	0.25m-22.08m	13.23m	37.1m	13.24m	28.57	27.9-28.9
Feeding	13	5.70 mins	0.75m-30.08m	20.89m*	69.8m	13.61m*	28.05	24.1-29.0
Courtship	19	4.35 mins	0.17m-16.25m	50.12m	86.3m	49.38m	26.55	21.9-28.8

*Average depth for feeding is presented however this value does not reflect the mean. Deployment 8 (duration= 55mins, mean depth = 1.5m) and deployment 14 (duration = 19.08mins, mean depth = 43.5m).

Temperature

Temperatures of behaviours were determined through a comparison of time series graphs (Fig 13) and the detailed temperature sensor data. Time series graphs of behaviour and temperature for all deployments are in appendix II, Figs A2.1-A2.30. Summary temperatures for criteria behaviours are listed in Table 7.

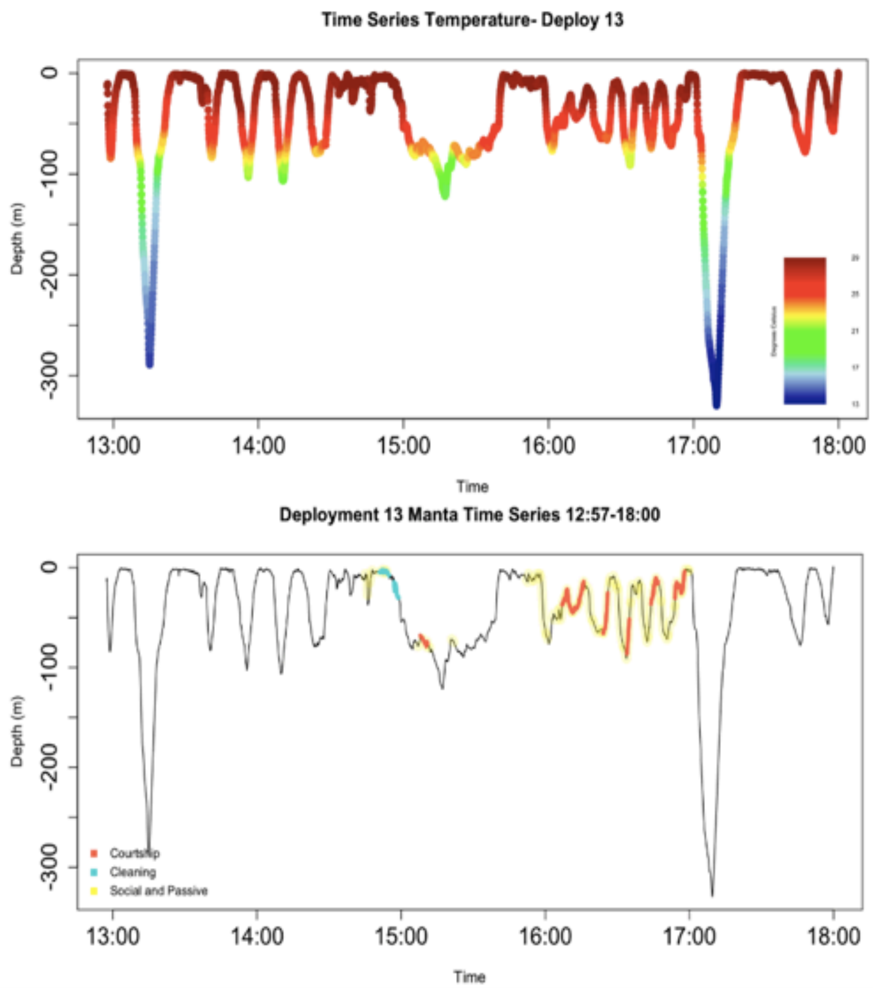


Fig 13: Temperature Time Series

A visual comparison of plot A to plot B shows courtship occurring from 21.9°C – 28.7°C. Crittercam deployment 13 recorded the lowest temperature reached by an individual at 12.7°C. Temperature plots were created for all individuals for comparison with behaviour plots.

Cleaning = Blue

Courtship = Orange

Feeding = Purple

Social and Passive = Yellow

Temperature legend differs by plot.

Larger versions of these plots are shown in Appendix 2.

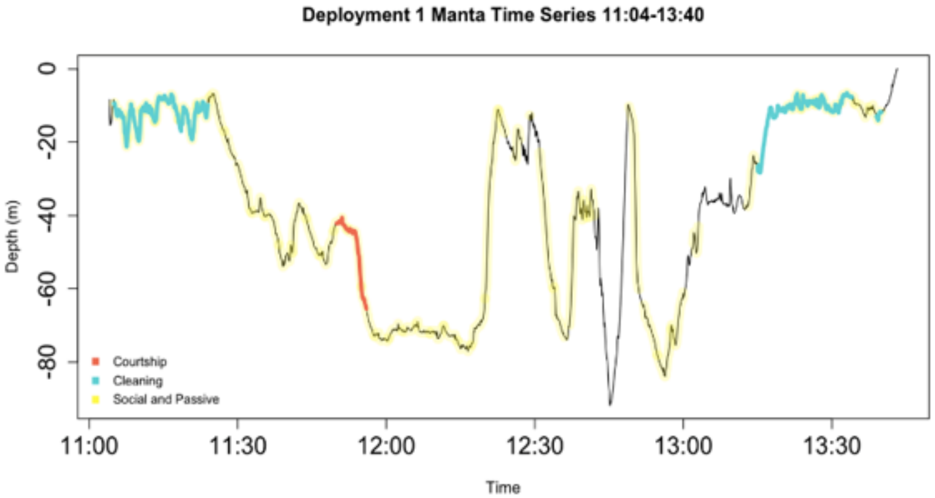


Fig 14. Time series graph for Deployment 1

Behaviour and social event plotting for deployment 1 shows the longest uninterrupted cleaning behaviour (00:22:05) and the longest consecutive social event at (00:54:00).

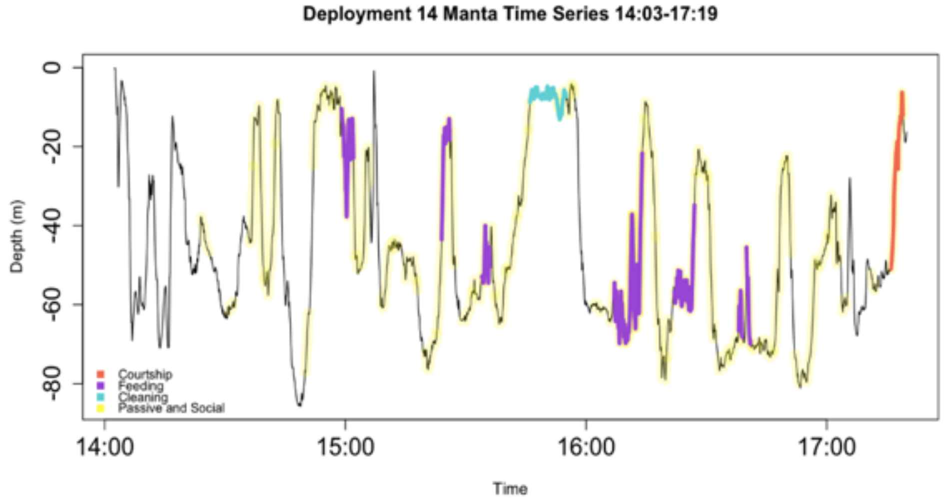


Fig 15. Time series graph for Deployment 14

Behaviour and social event plotting for deployment 14 shows the deepest feeding behaviour at 69.9m and social events occurring to 79.5m.

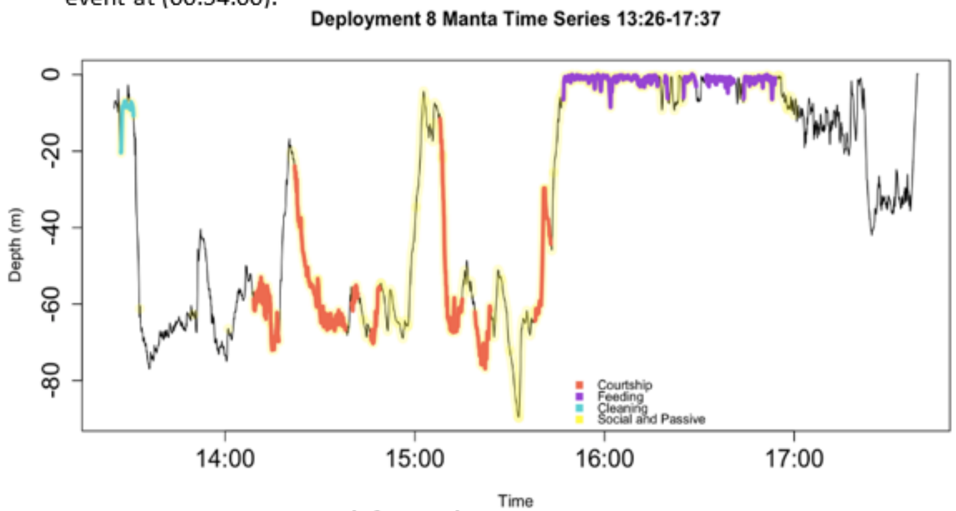


Fig 16. Time series graph for Deployment 8

Deployment 8 shows the longest feeding event recorded (00:30:05) and the longest recorded courtship event (00:16:05).

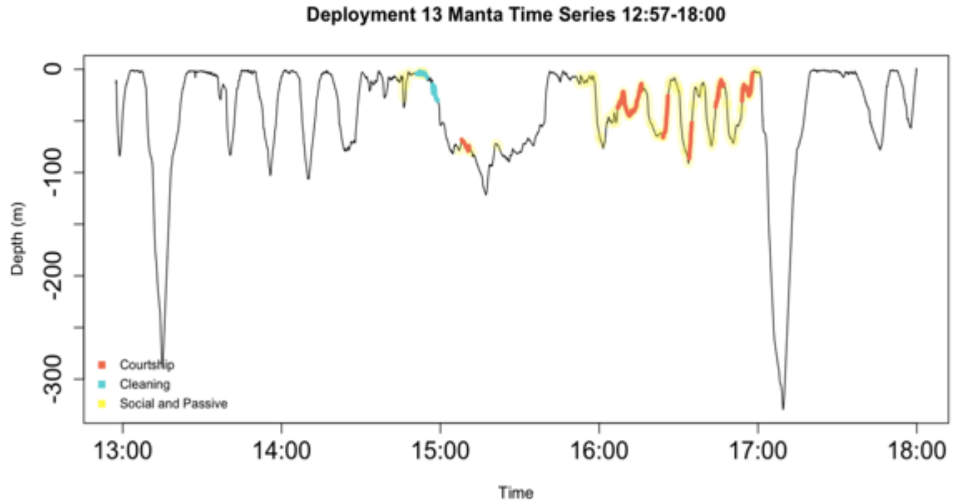


Fig 17. Time series graph for Deployment 13

Deployment 13 shows the deepest courtship event recorded to 86.3m, the deepest dive by *M. alfredi* to 329.5m and social activity occurring to 91.1m

Interactions with other species

M. alfredi were recorded interacting with *Mobula mobular* during deployment one for 3 minutes and 36 seconds between 33.1 meters and 40.6 meters. Crittercams captured at least 10 different interactions where fish species from the *Carangidae* family (jacks) were interacting with and traveling underneath *M. alfredi* as shown in Fig 18. Interactions were recorded between 37 and 81 meters as described in Appendix 3.

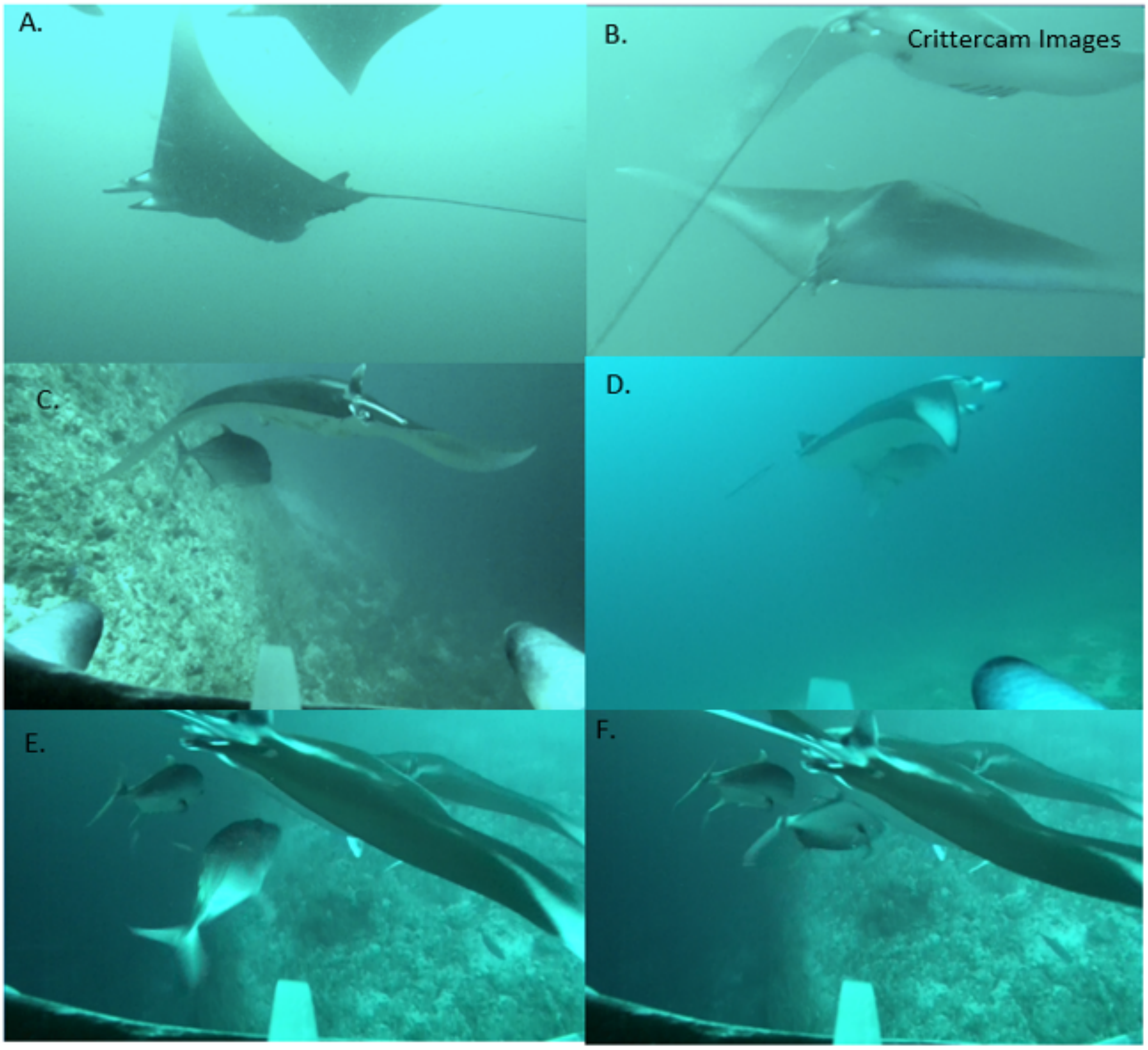


Fig 18. *M. alfredi* interacting with other species- Image A & B: Interactions with *Mobula mobular* occurred during the first deployment. **Images C & D:** Jacks were filmed traveling under *M. alfredi* during multiple deployments. **Images E & F**: Two jacks were filmed under the same *M. alfredi*. One jack completely inverted itself to hit the ventral side of the *M. alfredi* who showed no reaction to the contact from the fish.

Discussion

Crittercams can be used to quantify social behaviours of *M. alfredi* beyond observational and tracking limits however the technology comes with limitations that must be considered to fully understand the research.

Quantification of Behaviours

Feeding and courtship behaviours were most difficult to define as they were not limited to a particular habitat range such as a cleaning station. On multiple occasions, *M. alfredi* equipped with a Crittercam were in plankton filled waters but the mouths of other *M. alfredi* indicated they were not feeding. Due to the placement of the camera, I was unable to visualize the mouth of the research individual and could not quantify solitary feeding. With observational research periods being fragmented in duration, it is unknown how much time *M. alfredi* spends feeding on average and therefore I cannot say if the amount of feeding recorded was an accurate representation of *M. alfredi* behaviour.

Courtship activity varies intra-annually and inter-annually depending on the fecundity of individuals within a regional population (Stevens, 2016). Manta trust researchers reported far higher levels of courtship during the study than I recorded when viewing the video footage. Courtship was observed taking place beyond the view of the camera as photographed by researchers (Fig.18A) and the forward direction of the camera (Fig. 18B) would have led this behaviour to be classified as solitary cruising.

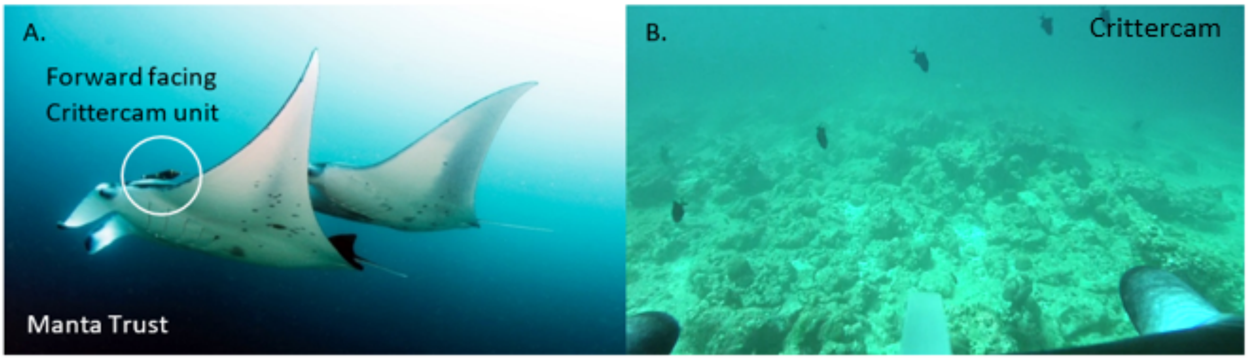


Fig 18. Comparison of behaviours based on camera perspective

Image A: *M. alfredi* equipped with a Crittercam and engaged in courtship behaviour from the perspective of an in water photographer. **Image B**: Same behaviour from the perspective of the Crittercam; this would have been recorded as solitary cruising behaviour. The camera view was a limiting factor in fully quantifying behavioural interactions.

Courtship was most difficult to quantify given the complexity of the behaviour and diverse swimming patterns associated with the process (Marshall & Bennett, 2010; Stevens, 2016). Following behind pregnant females is often a sign of courtship however I categorized this as social following when no rolling behaviours were observed. Given other potential drivers of group cruising such as predator avoidance (Jacoby *et al.,* 2012), determining all social following to be courtship would have been over-classification of ambiguous behaviour.

For this research, cleaning was defined by a habitat requirement however research on these sites suggests that *M. alfredi* may be driven to cleaning stations by environmental factors and potential social exchange which I could not consider in my criteria classification (Kitchen-Wheeler, 2013; Jaine *et al.,* 2012). O'shea (2010) suggested that socialization was not the primary driver of cleaning station visits when 84% of the individuals he researched were solitary at cleaning stations in Australia. From my observations, 39% of cleaning was solitary, a significant difference from levels reported in the literature. The discrepancy in interaction may be due to potential courtship initiated at cleaning stations or simply due to the higher population and therefore opportunity for socialization in the Maldives. The cleaning duration presented should be interpreted with the knowledge that multiple behaviours may have been taking place at these sites.

Within my unknown duration, multiple individuals exhibited a series of behaviours by which they would cruise for a short duration (close to the reef or seafloor) and demonstrate intermittent “flinch” behaviours lasting 8-20 seconds. These “flinch” behaviours were characterized by a very minor upward movement as if bitten by a cleaner fish, unfurling of cephalic lobes and a subsequent minor depth change to return to cruising. While “flinching” may have been caused by bites from cleaner fish, the consistent duration of the behaviour between periods of cruising led me to classify this as unknown. Research gaps exist in understanding *M. alfredi* behaviour and I could not determine if the “flinch” was for a specific purpose. It was only possible to recognize the pattern of “flinching” and cruising through uninterrupted recording of individuals with Crittercams; studies should continue using this technology to further evaluate this behaviour.

Behaviour in *M. alfredi* populations is known to vary seasonally, due to tides and potentially with time of day (Anderson *et al.,* 2011; Jaine *et al.,* 2012; Armstrong et al., 2016). Although a comparison was made for behaviours by time of the day, the sample size was uneven and the duration of the study was not enough to test for correlations of significance in this research. Extended Crittercam research has the potential to determine how behavioural variations may be linked to lunar, tidal and temporal factors.

Social interactions, courtship and feeding were quantified to a minimum, however increased knowledge of behaviour at any level is valuable for increasing research available on *M. alfredi*. The single direction camera view was the primary limitation in my ability to quantify behaviour however the available footage allowed me to increase research beyond previously observable limits as discussed in the following sections.

Interactions with Other Species

Crittercams recorded numerous fish species, cephalopods, elasmobranchs and a turtle but *M. alfredi* had no interactions with these species. Ecological interactions were only observed with two species, *Mobula mobular* and fish in the *Carangidae* family, commonly known as trevally or jacks. Interaction with *M. mobular* appeared to be purely social in mid-water with none of the animals exhibiting a particular type of behaviour. Interactions with *M. mobular* have been recorded by observers however the drivers between interaction are unknown (G. Stevens, personal communication, 2018).

Crittercams recorded *M. alfredi* interactions with jacks at depths greater than 35m where we have little knowledge of behaviour. Researchers have recorded jacks traveling under *M. alfredi* at Maldivian sites where they come from deeper waters (G. Stevens, personal communication, 2018). In the Crittercam interactions, Jacks may have been using *M. alfredi* to hide and predate on food sources that may be disoriented by mantas over the reef (Potts, 1981) or potentially to increase the efficiency of their swimming. One jack was recorded inverting itself and hitting the ventral side of *M. alfredi*; this behaviour may be an interaction where the fish is rubbing against the rough skin of M. alfredi (Marshall et al., 2009) to remove parasites. A similar behaviour has been observed by silky sharks who use the skin of whale sharks for parasite removal (BBC, 2018).

While interaction numbers were limited over the course of the study, Crittercams are a useful tool for observing all species in an ecosystem regardless of interaction with the research species. IUCN (2009) lists the lower depth limit of grey reef sharks as 50m however the Crittercam footage from deployment one recorded the species beyond 74m. These devices could provide a wealth of information about ecosystems and species biodiversity without the influence of human observers. Continued Crittercam studies in the Maldives where *M. alfredi* are often found with other planktivorous species such as whale sharks may reveal additional ecological interactions.

Beyond Observable Limits

While vertical movements patterns are unknown in the Maldives*, M. alfredi* populations in Indonesia, the Red Sea and Australia exhibit diurnal tendencies where they spend daytime hours near shallow reef habitats and dive at night using offshore habitats to potentially take advantage of plankton at depth (Anderson *et al.,* 2011; Braun *et al.,* 2014, Jaine *et al.,* 2014; Dewar *et al.,* 2008). Crittercams revealed that dives by *M. alfredi* in the Maldives were not limited to set hours with individuals recording depths greater than 80m throughout the day. The deepest dive was completed after 5pm which is consistent with tracking research on *M. alfredi* (Dewar *et al.,* 2008). From the depth data provided by the Crittercams, it appears as though *M. alfredi* in the Maldives share the same depth preferences as populations in the Red Sea frequently inhabiting the upper epipelagic layer (<60m) (Braun *et al.,* 2014).

Braun *et al.,* (2014) was the first to record deep diving behaviour in *M. alfredi* to 400m but he could not infer behaviour beyond assumptions of feeding. Stewart *et al.,* (2016) highlighted the same challenges with determining behaviour for *M. birostris* without an observable video component and confirmed feeding at depth for the species using submersible video cameras. This Crittercam research confirmed social feeding to a depth of 69.8m and provided video evidence that diving behaviour in the Maldives may not be for the sole purpose of feeding.

Courtship behaviours were also recorded to 86.3m with the longest uninterrupted duration of courtship being 16 minutes. Courtship was the deepest criteria based behaviour however social interactions were recorded to a maximum depth of 96.6m. Observational study limitations would not have allowed these behaviours to be described without a Crittercam.

Crittercams recorded a minimum temperature of 12.7^{o}C with one individual remaining below 20^{o}C for greater than nine minutes. Braun *et al.,* (2014) recorded the minimum temperature range of *M. alfredi* in the Red Sea to be 21.6^{o}C and elasmobranch research has shown these animals prefer temperatures greater than 20^{o}C (Thums *et al.,* 2013). Deployment 13 recorded the lowest temperature and deepest dive (329.5 m) however a definitive reason for the dive is unknown. The individual was engaged in courtship prior to diving but video at depth was unavailable as light was diminished past approximately 100m and the camera malfunctioned beyond 260m. The *M. alfredi* immediately returned to warmer water after the dive which was consistent with research on diving and thermoregulation in elasmobranchs (Thums *et al.,* 2013).

Jaine *et al.,* (2012) found foraging increased between 21 and 23^{o}C however feeding in this study only occurred above 24^{o}C. No correlations were determined for temperature and behaviour in the Maldives however this is an important an area of research to continue exploring with habitats in the region threatened by global climate change (Church *et al.,* 2006). Temperature has been directly linked to zooplankton accumulations and climate change has the potential to drastically modify plankton abundance and consequentially, *M. alfredi* distribution (Richardson, 2008).

Future Directions

Overall, this study has shown that a Crittercam is an effective tool via which to increase spatial and temporal knowledge about *M. alfredi* and in the future it appears that Crittercam technology will play an increasingly important role in expanding knowledge of migratory marine species and ecosystem interactions. Understanding the flexibility of behaviours at depth as well as the temperature range of animals can help provide baseline data to inform conservation measures and predict how climate change and anthropogenic impacts may shift a species habitat range.

References

Anderson, R. C., Adam, M. S. and Goes, J. I. (2011) 'From monsoons to mantas: Seasonal distribution of Manta alfredi in the Maldives', *Fisheries Oceanography*, 20(2), pp. 104–113. doi: 10.1111/j.1365-2419.2011.00571.x.

Ari, C. and D'Agostino, D. P. (2016) 'Contingency checking and self-directed behaviors in giant manta rays: Do elasmobranchs have self-awareness?', *Journal of Ethology*, 34(2), pp. 167–174. doi: 10.1007/s10164-016-0462-z.

Armstrong, A. O. *et al.* (2016) 'Prey Density Threshold and Tidal Influence on Reef Manta Ray Foraging at an Aggregation Site on the Great Barrier Reef', *PloS one*, 11(5), p. e0153393. doi: 10.1371/journal.pone.0153393.

BBC *Blue Planet II*, 2017. Big Blue, BBC. Nov 19.

Braun, C. D. *et al.* (2014) 'Diving behavior of the reef manta ray links coral reefs with adjacent deep pelagic habitats', *PLoS ONE*, 9(2). doi: 10.1371/journal.pone.0088170.

Braun, C. D. *et al.* (2015) 'Movements of the reef manta ray (Manta alfredi) in the Red Sea using satellite and acoustic telemetry', *Marine Biology*, 162(12), pp. 2351–2362. doi: 10.1007/s00227-015-2760-3.

Calambokidis, J., G.S. Schorr, G.H. Steiger, J. Francis, M. Bakhtiari, G.J. Marshall, E.M. Oleson, D. Gendron, K. Robertson. (2007) Insights into the underwater diving, feeding, and calling behavior of the blue whales from a suction-cup-attached video-imaging tag (Crittercam). *Marine Technology Society Journal*. 41(4): 19-29.

Church, J. A., White, N. J. and Hunter, J. R. (2006) 'Sea-level rise at tropical Pacific and Indian Ocean islands', *Global and Planetary Change*, 53(3), pp. 155–168. doi: 10.1016/j.gloplacha.2006.04.001.

Couturier, L. I. E. *et al.* (2011) 'Distribution, site affinity and regional movements of the manta ray, Manta alfredi (Krefft, 1868), along the east coast of Australia', in *Marine and Freshwater Research*, pp. 628–637. doi: 10.1071/MF10148.

Couturier, L. I. E. *et al.* (2012) 'Biology, ecology and conservation of the Mobulidae', *Journal of Fish Biology*, 80(5), pp. 1075–1119. doi: 10.1111/j.1095-8649.2012.03264.x.

Dewar, H. *et al.* (2008) 'Movements and site fidelity of the giant manta ray, Manta birostris, in the Komodo Marine Park, Indonesia', *Marine Biology*, 155(2), pp. 121–133. doi: 10.1007/s00227-008-0988-x.

Heithaus, M. R. *et al.* (2001) 'Employing Crittercam to study habitat use and behavior of large sharks', *Marine Ecology Progress Series*, 209, pp. 307–310. doi: 10.3354/meps209307.

Heithaus, M. R. *et al.* (2002) 'Habitat use and foraging behavior of tiger sharks (Galeocerdo cuvier) in a seagrass ecosystem', *Marine Biology*, 140(2), pp. 237–248. doi: 10.1007/s00227-001-0711-7.

Jacoby, D. M. P., Croft, D. P. and Sims, D. W. (2012) 'Social behaviour in sharks and rays: analysis, patterns and implications for conservation', *Fish and Fisheries*. doi: 10.1111/j.1467-2979.2011.00436.x.

Jaine, F. R. A. *et al.* (2012) 'When Giants Turn Up: Sighting Trends, Environmental Influences and Habitat Use of the Manta Ray Manta alfredi at a Coral Reef', *PLoS ONE*, 7(10). doi: 10.1371/journal.pone.0046170.

Jaine, F. R. A. *et al.* (2014) 'Movements and habitat use of reef manta rays off eastern Australia: Offshore excursions, deep diving and eddy affinity revealed by satellite telemetry', *Marine Ecology Progress Series*, 510, pp. 73–86. doi: 10.3354/meps10910.

Kitchen-Wheeler, A.-M. (2013) *The* behaviour *and ecology of Alfred mantas (Manta alfredi) in the Maldives*. Newcastle University.

Marshall, A. D. and Bennett, M. B. (2010) 'Reproductive ecology of the reef manta ray Manta alfredi in southern Mozambique', *Journal of Fish Biology*, 77(1), pp. 169–190. doi: 10.1111/j.1095-8649.2010.02669.x.

Marshall, A. D., Compagno, L. J. V and Bennett, M. B. (2009) 'Redescription of the genus Manta with resurrection of Manta alfredi (Krefft, 1868) (Chondrichthyes; Myliobatoidei; Mobulidae)', *Zootaxa*, (2301), pp. 1–28. doi: 10.3161/000345409X484856.

Marshall, A. D. and Pierce, S. J. (2012) 'The use and abuse of photographic identification in sharks and rays', *Journal of Fish Biology*. doi: 10.1111/j.1095-8649.2012.03244.x.

Meynecke, J.-O., Abernathy, K. and Marshall, G. (2015) 'In Murky Waters: Crittercam on Juvenile Bull Sharks (Carcharhinus leucas)', *Marine Technology Society Journal*, 49(5), pp. 25–30.

Moll, R. J. *et al.* (2007) 'A new "view" of ecology and conservation through animal-borne video systems', *Trends in Ecology and Evolution*. doi: 10.1016/j.tree.2007.09.007.

Nifong, J. C. *et al.* (2014) 'Animal-borne imaging reveals novel insights into the foraging behaviors and diel activity of a large-bodied apex predator, the American alligator (Alligator Mississippiensis)', *PLoS ONE*. doi: 10.1371/journal.pone.0083953.

O'Shea, O. R., Kingsford, M. J. and Seymour, J. (2010) 'Tide-related periodicity of manta rays and sharks to cleaning stations on a coral reef', *Marine and Freshwater Research*, 61(1), pp. 65–73. doi: 10.1071/MF08301.

Potts, G. W. (1981) 'Behavioural interactions between the Carangidae (Pisces) and their prey on the fore-reef slope of Aldabra, with notes on other predators', *Journal of Zoology*, 195(3), pp. 385–404. doi: 10.1111/j.1469-7998.1981.tb03472.x.

Richardson, A. J. (2008) 'In hot water: Zooplankton and climate change', in *ICES Journal of Marine Science*, pp. 279–295. doi: 10.1093/icesjms/fsn028.

Schofield, G. and Katselidis, K. (2007) 'Behaviour analysis of the loggerhead sea turtle Caretta caretta from direct in-water observation', *Endangered Species Research*, 2(December), pp. 71–79. doi: 10.3354/esr002071.

Smale, M.J. 2009. *Carcharhinus amblyrhynchos*. The IUCN Red List of Threatened Species 2009: e.T39365A10216946. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T39365A10216946.en. Downloaded on **20 June 2018**.

Stevens, G. *et al.* (2018) *Guide to the Manta & Devil Rays of the World*. Plymouth: Wild Nature Press.

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

Stewart, J. D. *et al.* (2016) 'Deep-water feeding and behavioral plasticity in Manta birostris revealed by archival tags and submersible observations', *Zoology*. doi: 10.1016/j.zool.2016.05.010.

Stewart, J. D. *et al.* (2017) 'Are mantas self aware or simply social? A response to Ari and D'Agostino 2016', *Journal of Ethology*, pp. 145–147. doi: 10.1007/s10164-016-0491-7.

Thums, M. *et al.* (2013) 'Evidence for behavioural thermoregulation by the world's largest fish', *Journal of the Royal Society Interface*, 10(78). doi: 10.1098/rsif.2012.0477.

Tomita, T. *et al.* (2012) 'Live-bearing manta ray: How the embryo acquires oxygen without placenta and umbilical cord', *Biology Letters*, 8(5), pp. 721–724. doi: 10.1098/rsbl.2012.0288.

Town, C., Marshall, A. and Sethasathien, N. (2013) 'Manta Matcher: Automated photographic identification of manta rays using keypoint features', *Ecology and Evolution*, 3(7), pp. 1902–1914. doi: 10.1002/ece3.587.

Ware, C. *et al.* (2014) 'Bottom side-roll feeding by humpback whales (Megaptera novaeangliae) in the southern Gulf of Maine, U.S.A', *Marine Mammal Science*, 30(2), pp. 494–511. doi: 10.1111/mms.12053.

White, W. T. *et al.* (2018) 'Phylogeny of the manta and devilrays (Chondrichthyes: Mobulidae), with an updated taxonomic arrangement for the family', *Zoological Journal of the Linnean Society*, 182(1), pp. 50–75. doi: 10.1093/zoolinnean/zlx018.

Yano, K., Sato, F. and Takahashi, T. (1999) 'Observations of mating behavior of the manta ray, *Manta birostris*, at the Ogasawara Islands, Japan', *Ichthyological Research*. doi: 10.1007/BF02678515.

Appendix 1: Quantification of Behaviours

Table A1.1 Durations of Behaviour by interaction level for each *M. alfredi*

Table shows deployment numbers and corresponding durations of behaviour recorded by each level of interaction (social, passive, solitary). Durations are in HH:MM:SS with the cumulative duration of each behaviour in the final row.

Deployment	*Social Cruising*	*Passive Cruising*	*Solitary Cruising*	*Social Cleaning*	*Passive Cleaning*	*Solitary Cleaning*	*Social Feeding*	*Passive Feeding*	*Courtship*	*Social Unknown*	*Passive Unknown*	*Solitary Unknown*
0	--	--	00:01:09	--	00:00:20	00:12:03	--	--	--	--	--	00:00:11
1	01:10:37	00:01:32	00:26:14	00:23:02	00:07:54	00:10:59	--	--	00:05:25	00:06:19	00:00:48	00:02:20
2	--	--	00:30:05	--	--	--	--	--	--	--	--	00:01:38
3	00:04:55	--	00:24:29	--	--	--	--	--	--	--	00:00:07	00:00:20
4	--	00:00:14	00:01:00	--	00:00:34	00:01:29	--	--	--	--	--	00:00:10
5	00:01:09	--	00:04:35	00:10:35	00:14:37	00:03:39	--	--	--	00:01:09	00:00:15	00:03:09
6	00:19:09	00:02:07	00:17:54	--	--	--	--	--	00:06:00	00:00:21	00:00:00	00:00:08
7	00:13:04	00:03:15	00:10:02	00:04:05	00:02:51	00:07:52	--	--	--	00:00:21	00:00:11	00:00:32
8	00:59:34	00:02:09	00:32:16	00:01:30	--	00:00:13	00:46:22	00:07:42	00:44:25	00:02:12	00:00:07	00:16:20
9	00:03:10	00:02:04	02:11:17	--	00:02:23	00:11:13	--	--	--	00:02:38	00:01:44	00:14:55
10	00:02:51	--	00:16:23	00:02:40	00:03:12	00:04:32	--	--	--	--	--	00:01:26
11	00:40:48	--	00:04:45	00:03:43	00:03:57	00:06:18	--	--	--	--	--	00:00:03
12	--	00:02:57	00:02:26	--	00:02:30	--	--	--	--	--	--	--
13	00:41:26	00:00:42	00:04:43	00:02:54	00:01:05	00:03:52	--	--	00:25:16	00:01:23	00:01:53	00:09:09
14	01:16:15	03:21:29	00:52:16	00:00:32	00:07:28	00:01:03	00:18:39	00:00:28	00:02:04	00:05:07	00:06:15	00:07:57
TOTAL	05:32:58	00:17:13	09:16:20	00:49:01	00:46:51	01:03:13	01:05:01	00:08:10	01:23:10	00:19:30	00:11:20	00:58:15

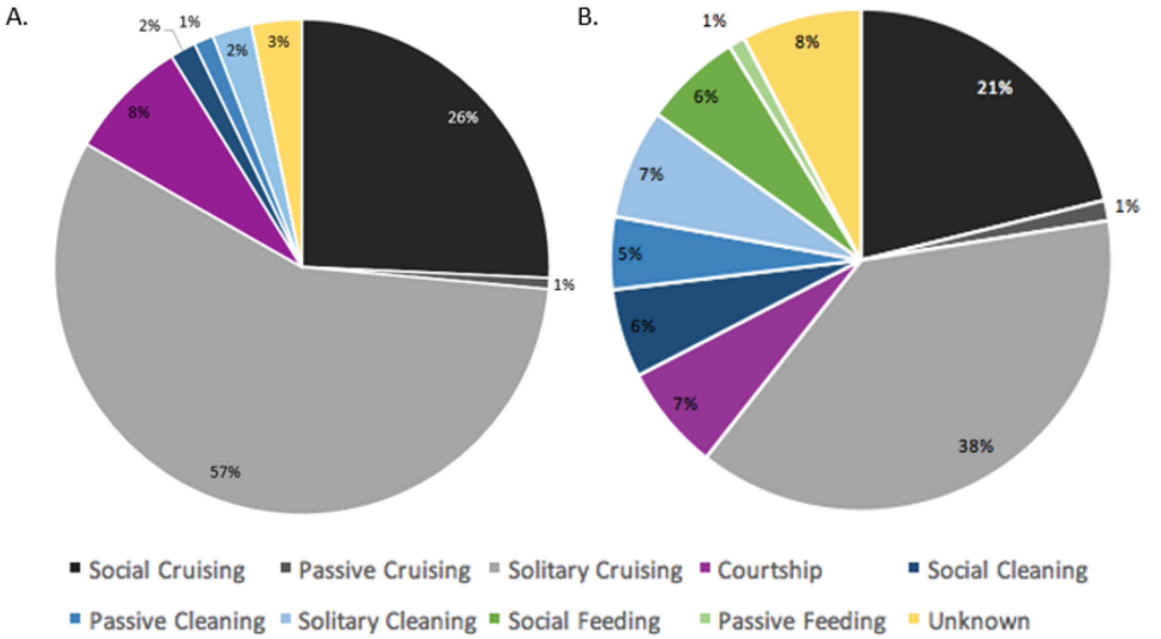


Fig. A1.1 **Behaviour and Interactions of *M. alfredi* by Sex**
Chart A: percentage of behaviour and interactions occurring during deployments for three male mantas (n=394.37 minutes). **Chart B:** percentage of behaviour and interactions occurring for eleven female mantas (n= 733.93 minutes).

Appendix II. Beyond Observable Limits

Table A2.1 Summary statistics for all cleaning events

Deploy	Type	Duration (hh:mm:ss)	Minutes	Max Depth (meters)	Avg Depth (meters)	Min Depth (meters)	Max Temp °C	Average Temp °C	Min Temp °C
0	Cleaning	00:12:25	12.42	11	7.33	1.3	28.63	28.37	28.26
1	Cleaning	00:13:55	13.92	21.2	11.9	7	28.79	28.71	28.64
1	Cleaning	00:07:45	7.75	19.2	11.58	6.7	28.64	28.6	28.59
1	Cleaning	00:22:05	22.08	28.3	11.23	6.6	28.61	28.43	27.95
4	Cleaning	00:02:05	2.08	16.1	11.88	9.5	28.43	28.43	28.4
5	Cleaning	00:06:25	6.42	18.3	15.22	11.9	28.61	28.58	28.56
5	Cleaning	00:04:35	4.58	25.6	16.43	10.5	28.56	28.53	28.53
5	Cleaning	00:10:05	10.08	22	17.7	11.7	28.53	28.53	28.5
5	Cleaning	00:08:15	8.25	23.5	18.28	14.6	28.53	28.52	28.5
7	Cleaning	00:01:00	1.00	11.4	6.97	4.8	28.7	28.67	28.56
7	Cleaning	00:00:15	0.25	10.9	10	9.4	28.7	28.7	28.7
7	Cleaning	00:14:30	14.50	26	17.3	11.5	28.7	28.52	28.38
8	Cleaning	00:01:25	1.42	8.6	7.52	5	28.69	28.68	28.66
8	Cleaning	00:00:25	0.42	9.1	8.08	7.3	28.69	28.69	28.69
9	Cleaning	00:06:15	6.25	16.9	10.95	5.6	28.63	28.62	28.57
9	Cleaning	00:03:05	3.08	27.5	23.52	20.9	28.6	28.6	28.6
9	Cleaning	00:03:50	3.83	34.7	16.91	7.2	28.46	28.27	28.11
10	Cleaning	00:10:25	10.42	35.2	15.47	7.9	28.64	28.63	28.61
11	Cleaning	00:13:30	13.50	17.7	12.48	8.5	28.79	28.73	28.67
12	Cleaning	00:02:30	2.50	18.1	14.7	12.1	28.64	28.59	28.47
13	Cleaning	00:04:45	4.75	11.1	5.17	2.1	28.72	28.7	28.66
13	Cleaning	00:02:35	2.58	37.1	25.93	16.3	28.66	28.55	28.08
14	Cleaning	00:09:45	9.75	13.1	7.68	4.8	28.85	28.8	28.76

Table A2.2 Summary statistics for all feeding events

Deploy	Type	Duration (hh:mm:ss)	Minutes	Max Depth (meters)	Avg Depth (meters)	Min Depth (meters)	Max Temp °C	Average Temp °C	Min Temp °C
8	Feeding	00:30:05	30.08	8.5	1.13	0	29.01	28.84	28.52
8	Feeding	00:00:45	0.75	5.7	1.9	0.3	28.92	28.91	28.89
8	Feeding	00:00:55	0.92	7	2.61	0.4	28.81	28.81	28.81
8	Feeding	00:03:10	3.17	2.3	0.8	0	28.92	28.89	28.84
8	Feeding	00:00:45	0.75	2.1	1.31	0.8	28.87	28.83	28.81
8	Feeding	00:09:30	9.50	4.6	1.45	0.3	28.95	28.89	28.81
8	Feeding	00:09:50	9.83	6.4	1.32	0	28.92	28.88	28.84
14	Feeding	00:02:15	2.25	32.8	18.1	13.1	28.53	28.27	28.01
14	Feeding	00:01:55	1.92	54.6	47.1	40.4	26.76	26.49	26.24
14	Feeding	00:07:05	7.08	69.8	58.74	27.6	26.91	24.82	24.19
14	Feeding	00:04:15	4.25	61.5	56.32	43.3	26.39	24.96	24.37
14	Feeding	00:01:10	1.17	69.9	59.38	45.5	25.98	25.4	24.42
14	Feeding	00:02:25	2.42	37.7	21.36	13	28.38	27.97	26.76

Table A2.3 Summary statistics for all courtship events

Deploy	Type	Duration (hh:mm:ss)	Minutes	Max Depth (meters)	Avg Depth (meters)	Min Depth (meters)	Max Temp °C	Average Temp °C	Min Temp °C
1	Courtship	00:05:30	5.50	66.6	49.24	40.6	28.24	28.24	28.21
6	Courtship	00:00:55	0.92	79.1	77.48	75.5	24.78	24.7	24.64
6	Courtship	00:05:20	5.33	62.8	46.57	30.9	27.97	27.73	26.84
8	Courtship	00:07:15	7.25	72	61.32	53	28.28	26.97	23.13
8	Courtship	00:16:15	16.25	66.9	56.62	25.3	28.23	26.17	24.49
8	Courtship	00:00:25	0.42	63.6	59.64	57.5	25.65	25.65	25.65
8	Courtship	00:01:30	1.50	59.5	57.13	55	27.94	27.81	27.68
8	Courtship	00:02:20	2.33	70.2	62.84	55.6	26.72	24.87	22.95
8	Courtship	00:07:10	7.17	67.3	56.1	16.4	28.34	26.33	23.94
8	Courtship	00:04:25	4.42	76.9	70.31	60.6	26.17	23.28	21.97
8	Courtship	00:05:45	5.75	64.6	48.64	29.7	27.94	26.61	25.16
13	Courtship	00:09:20	9.33	44.8	32.64	14	28.66	27.25	26.2
13	Courtship	00:01:50	1.83	61.4	42.19	19.1	27.27	25.15	24.23
13	Courtship	00:01:15	1.25	79.4	73.75	67.1	25.27	23.95	22.78
13	Courtship	00:01:20	1.33	86.3	64.75	43.5	24.43	22.94	21.88
13	Courtship	00:05:25	5.42	74.2	32.85	10.4	28.75	26.72	23.74
13	Courtship	00:04:10	4.17	38.3	19.7	4.1	28.63	28.2	26.43
14	Courtship	00:02:15	2.25	43.4	22.16	8.6	28.7	27.77	24.89
14	Courtship	00:00:10	0.17	18.7	18.3	17.9	28.79	28.79	28.79

Table A2.4 Summary of Social Events

Summary statistics are shown for social events (social and passive interactions) by deployment. Plotting for the graphs was completed to the nearest minute however these statistics were calculated to the nearest second. The count value represents the number of times uninterrupted periods of social/passive interaction were recorded.

Deployment	Max Depth of a social event*	Max Duration of a singular social event (calculated to second)* (HH:MM:SS)	Count of uninterrupted social and passive behaviours**
0	10	00:00:11	1
1	83.8	00:54:13	20
2	--	--	0
3	64	00:01:40	8
4	12.1	00:00:48	1
5	25.6	00:07:39	11
6	89.9	00:04:39	14
7	80.5	00:05:21	12
8	89.4	00:49:43	24
9	96.6	00:01:35	24
10	77.3	00:05:52	5
11	91	00:26:05	14
12	35.3	00:02:57	1
13	91.1	00:19:56	17
14	79.5	00:13:23	44

*Calculated using data points from depth loggers. These are plotted to the nearest minute. **Determined from qualitative observation record.

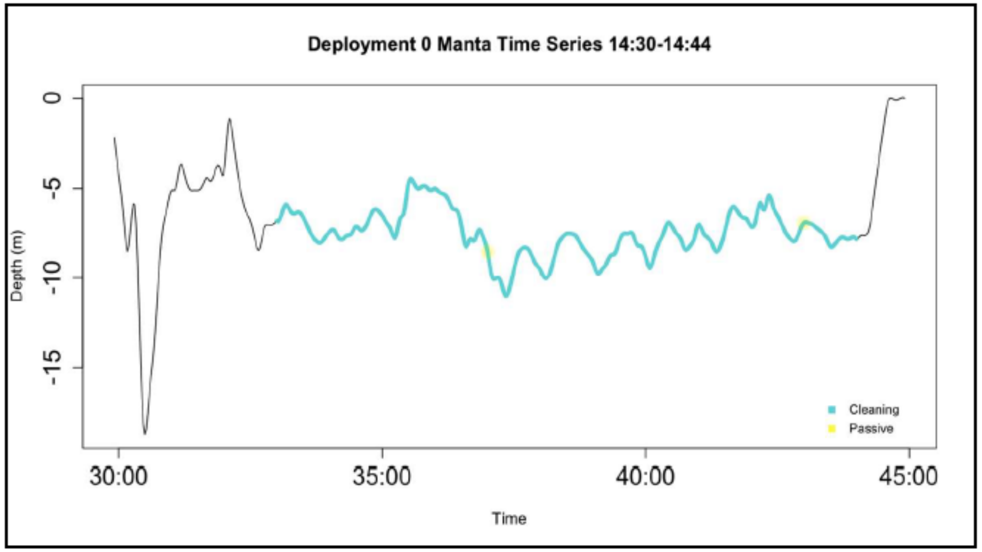


Fig A2.1 Behaviour Time Series Graph for Deployment 0

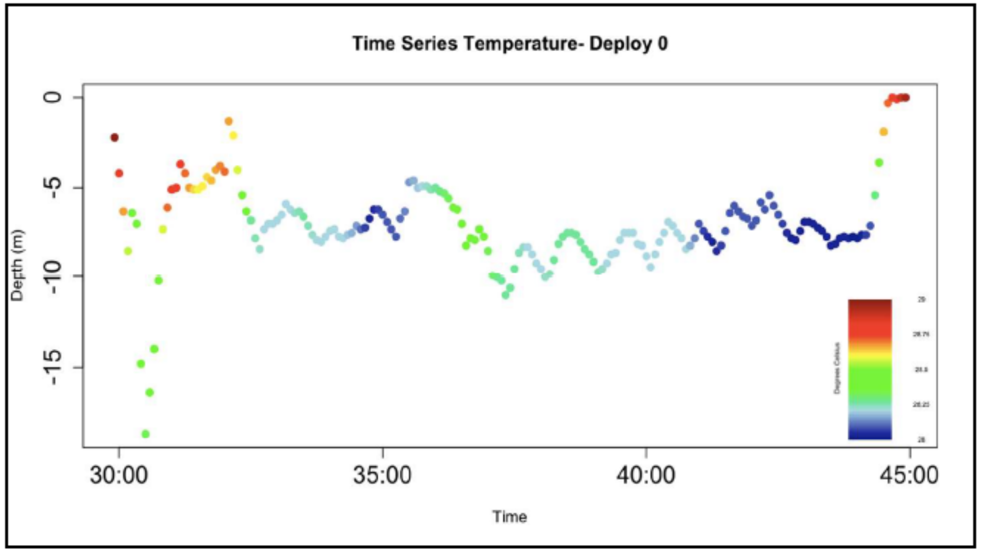


Fig A2. 2 Temperature Time Series Graph for Deployment 0

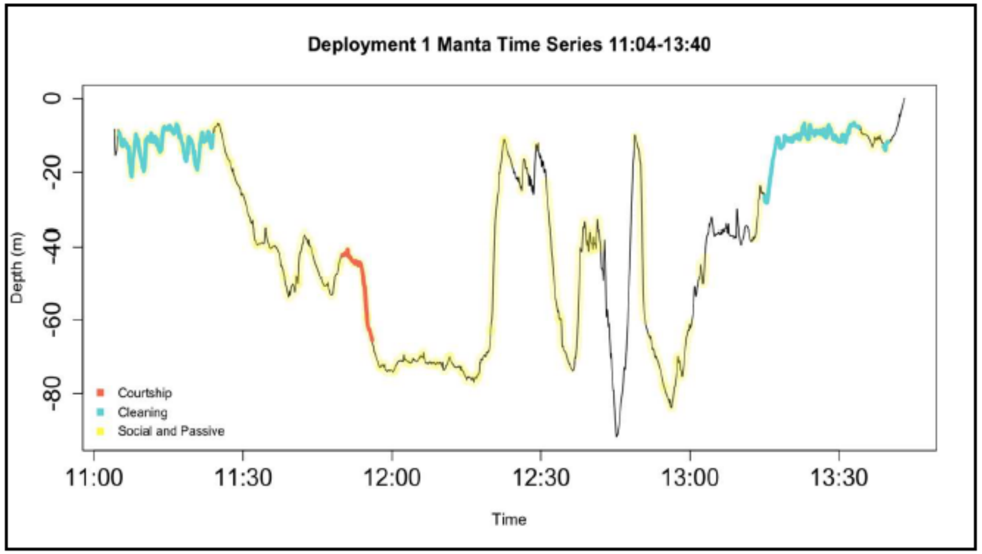


Fig A2.3 Behaviour Time Series Graph for Deployment 1

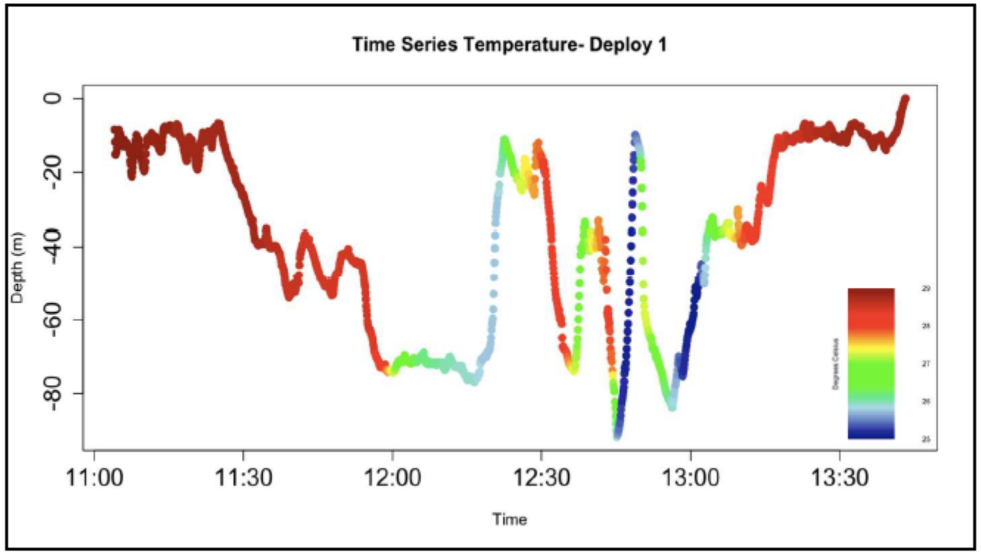


Fig A2. 4 Temperature Time Series Graph for Deployment 1

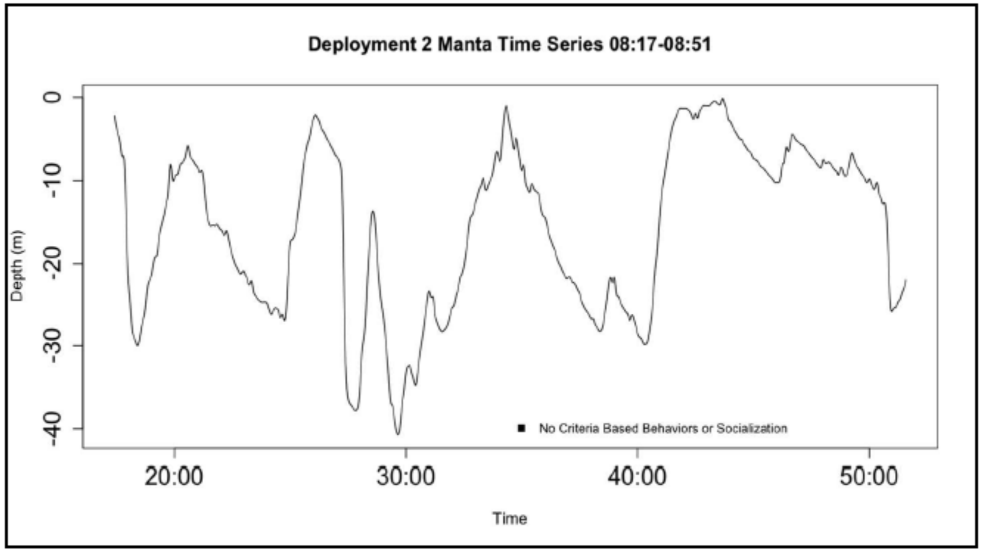


Fig A2.5 Behaviour Time Series Graph for Deployment 2

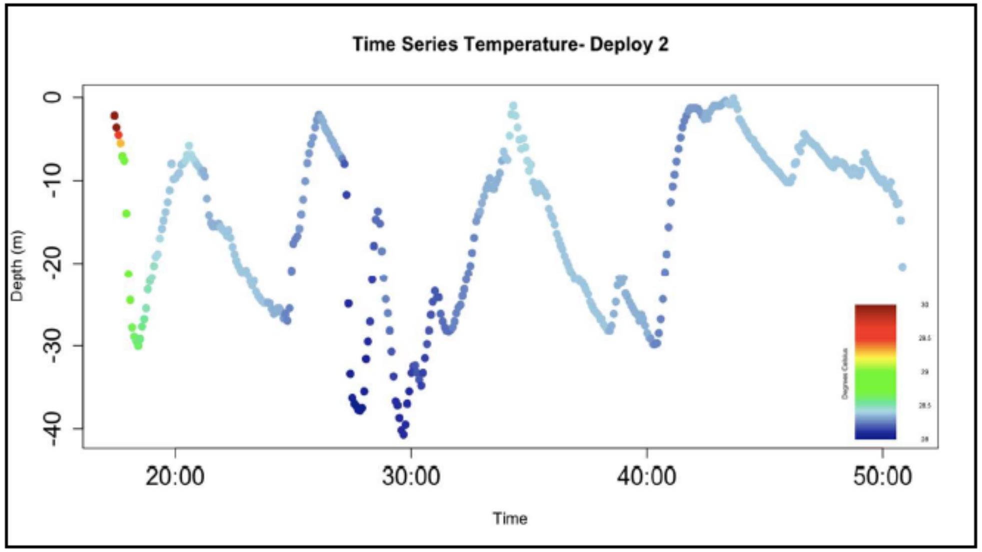


Fig A2. 6 Temperature Time Series Graph for Deployment 2

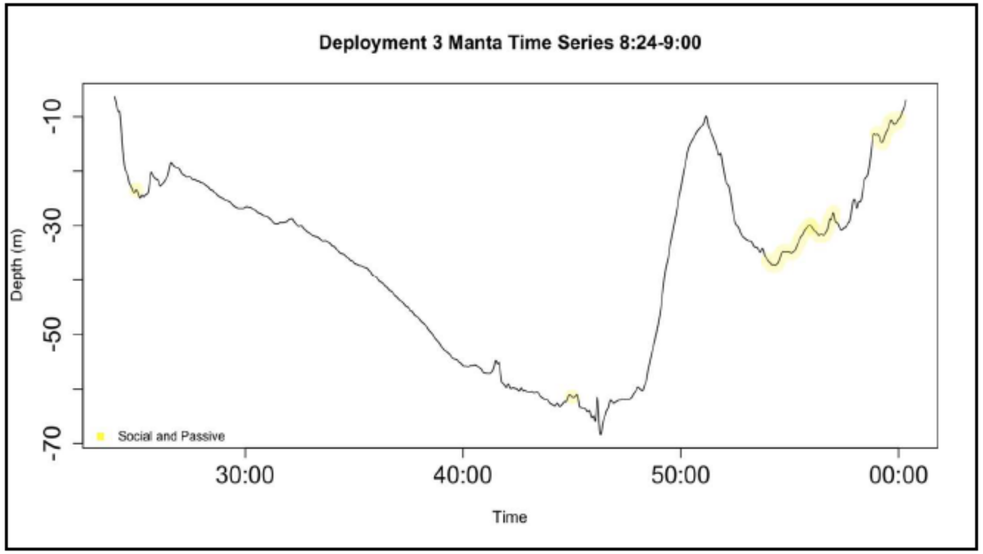


Fig A2.7 Behaviour Time Series Graph for Deployment 3

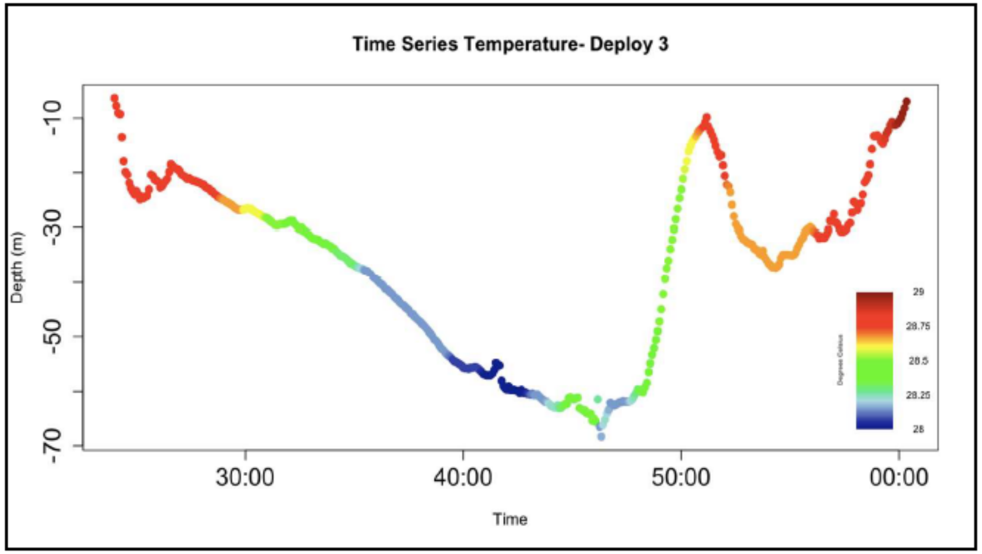
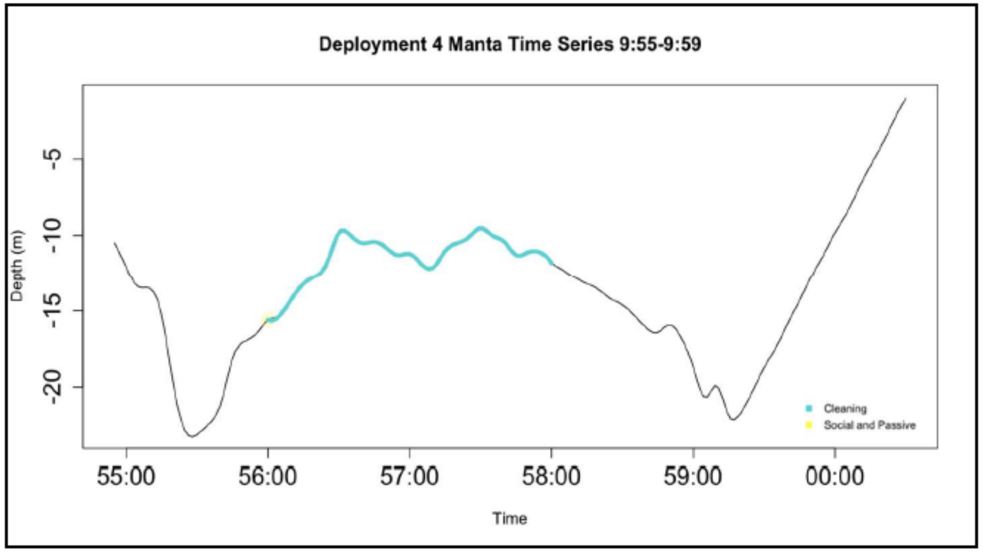


Fig A2. 8 Temperature Time Series Graph for Deployment 3



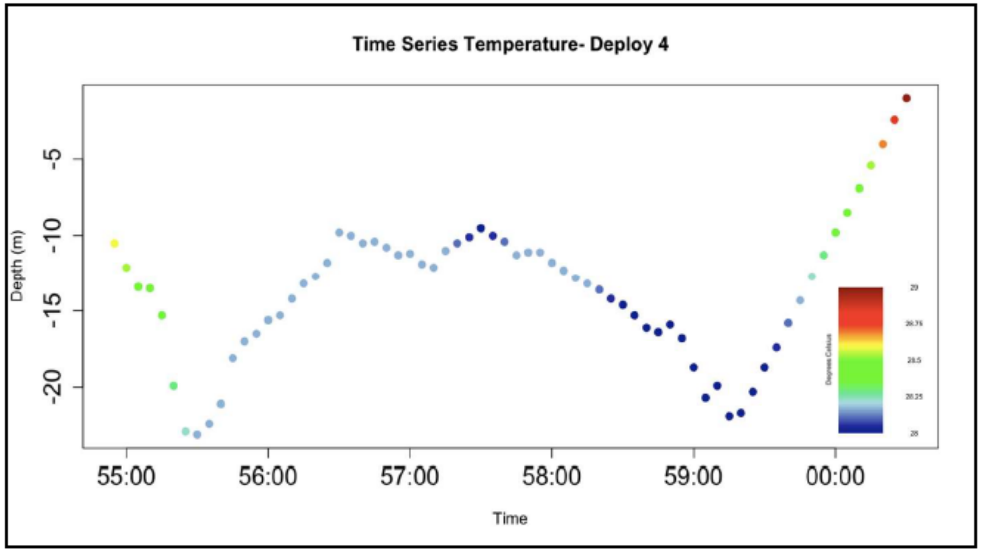
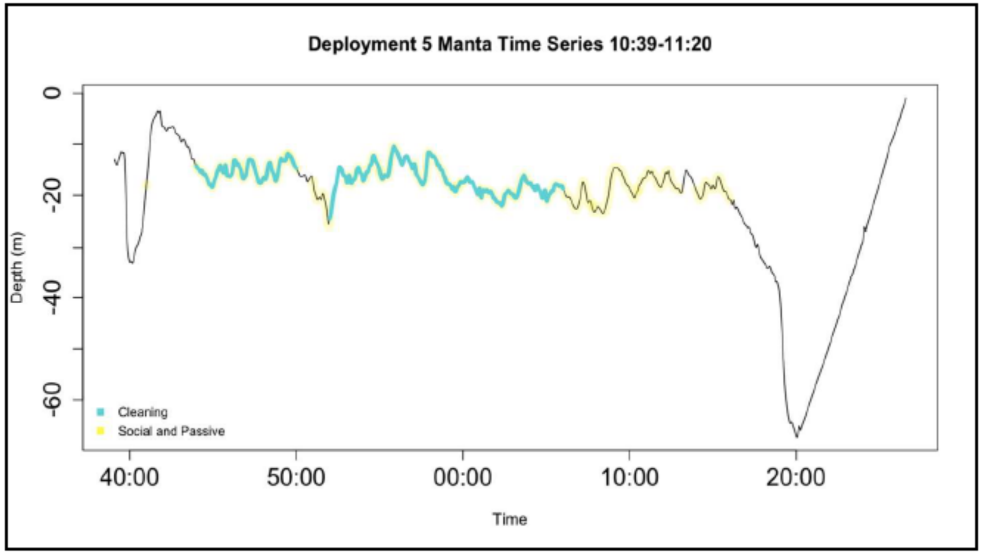


Fig A2.9 Behaviour Time Series Graph for Deployment 4

Fig A2.10 Temperature Time Series Graph for Deployment 4



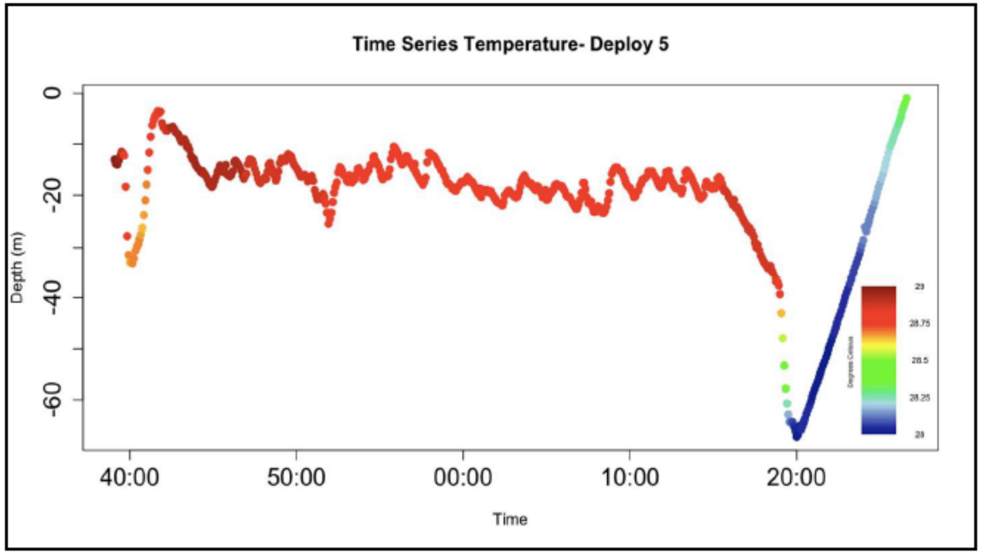


Fig A2.11 Behaviour Time Series Graph for Deployment 5

Fig A2.12 Temperature Time Series Graph for Deployment 5

Y

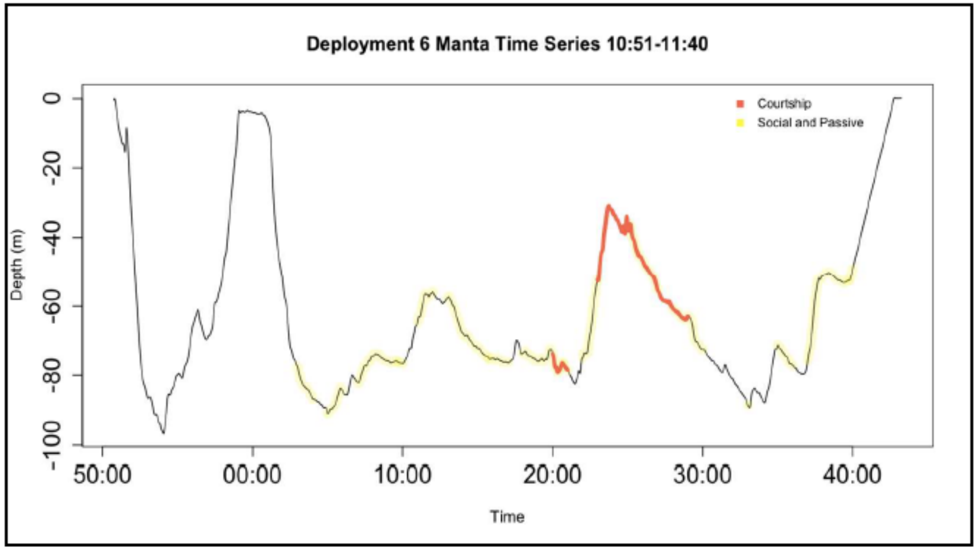


Fig A2.13 Behaviour Time Series Graph for Deployment 6

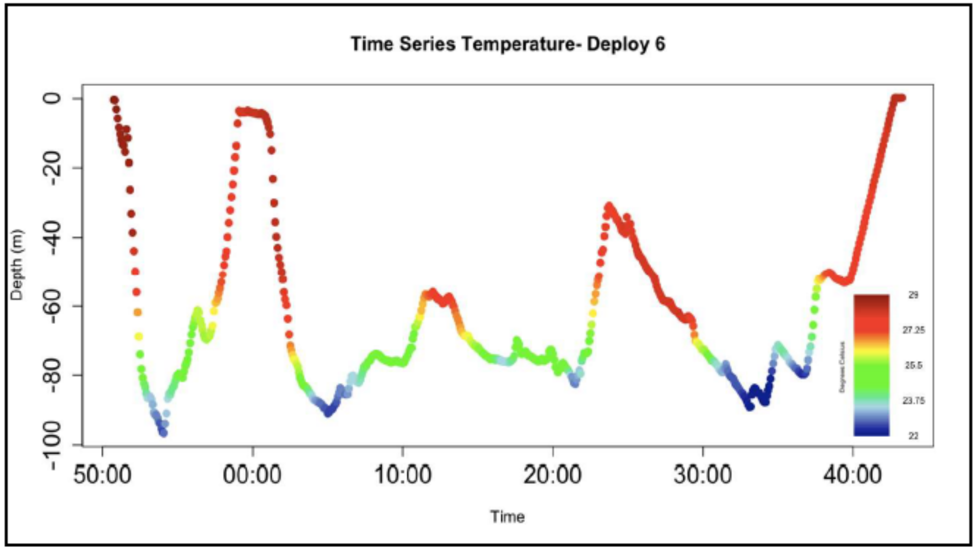


Fig A2.14 Temperature Time Series Graph for Deployment 6

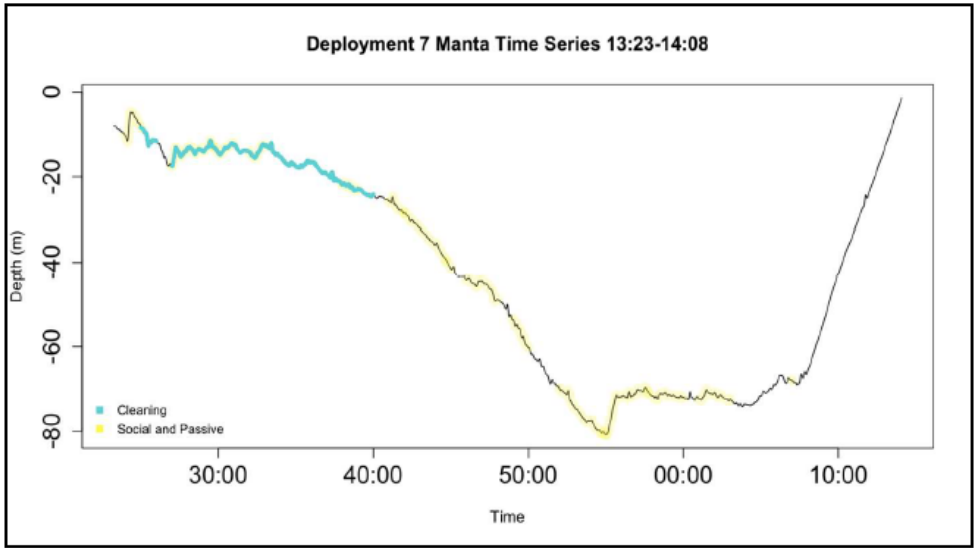


Fig A2.15 Behaviour Time Series Graph for Deployment 7

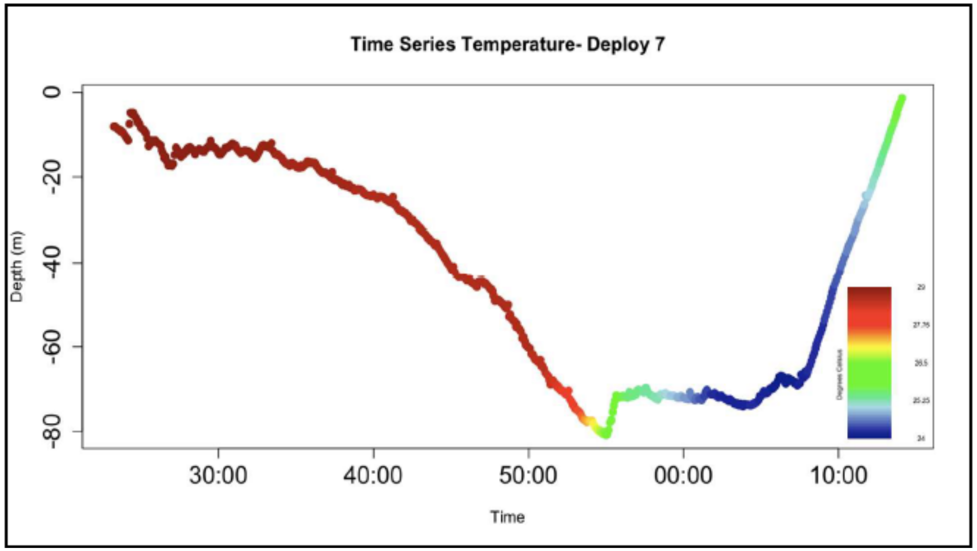
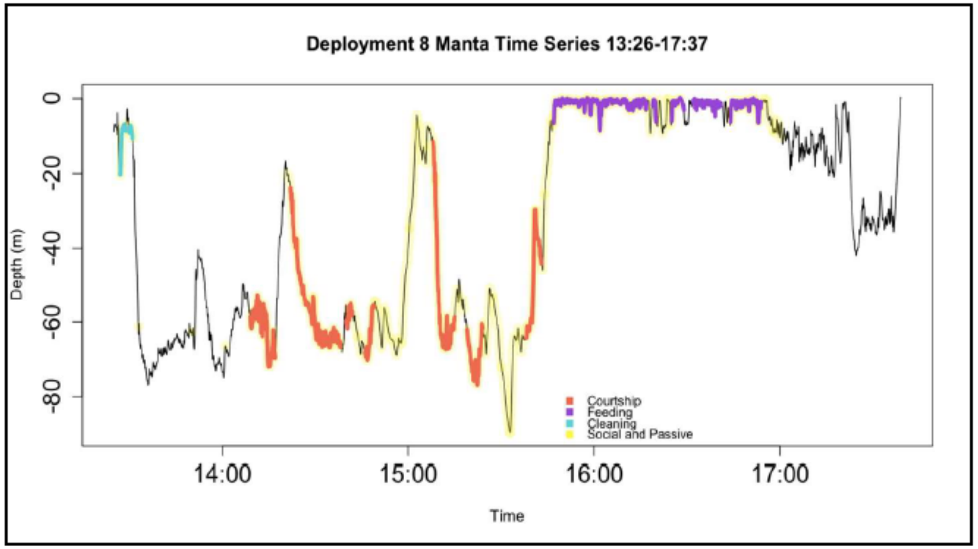


Fig A2.16 Temperature Time Series Graph for Deployment 7



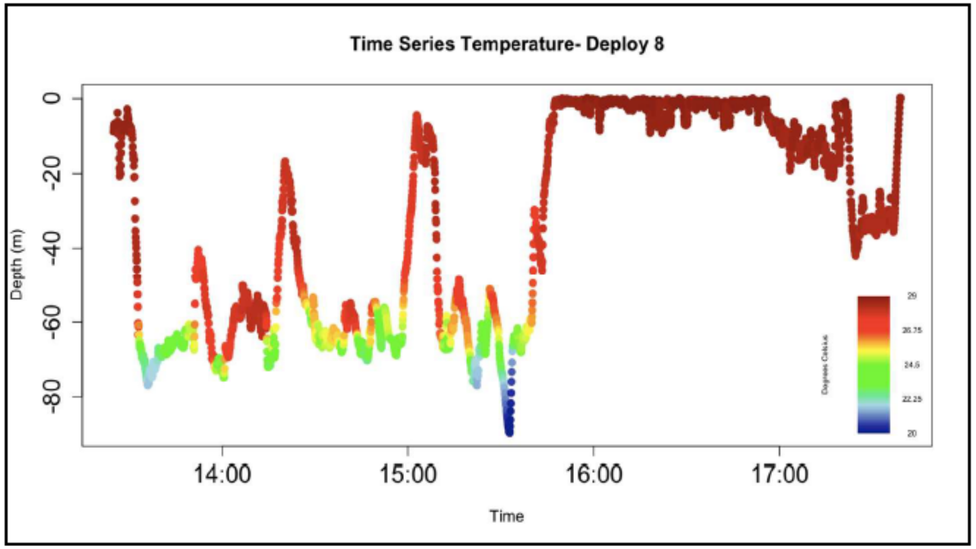
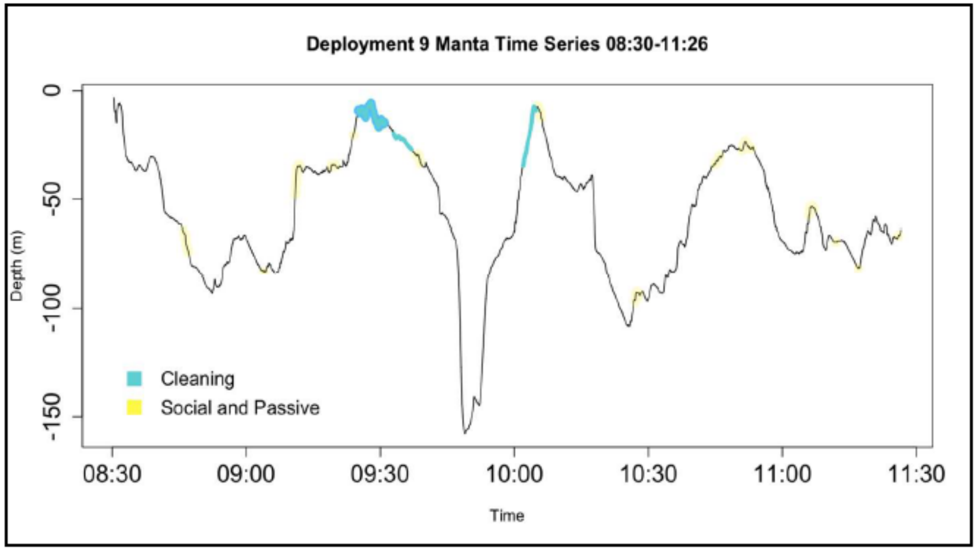


Fig A2.17 Behaviour Time Series Graph for Deployment 8

Fig A2.18 Temperature Time Series Graph for Deployment 8



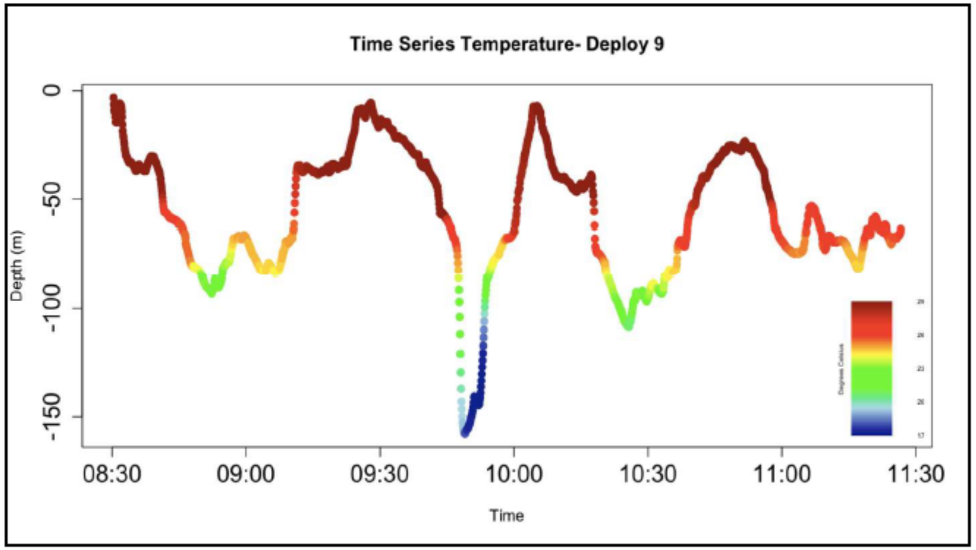


Fig A2.19 Behaviour Time Series Graph for Deployment 9

Fig A2.20 Temperature Time Series Graph for Deployment 9

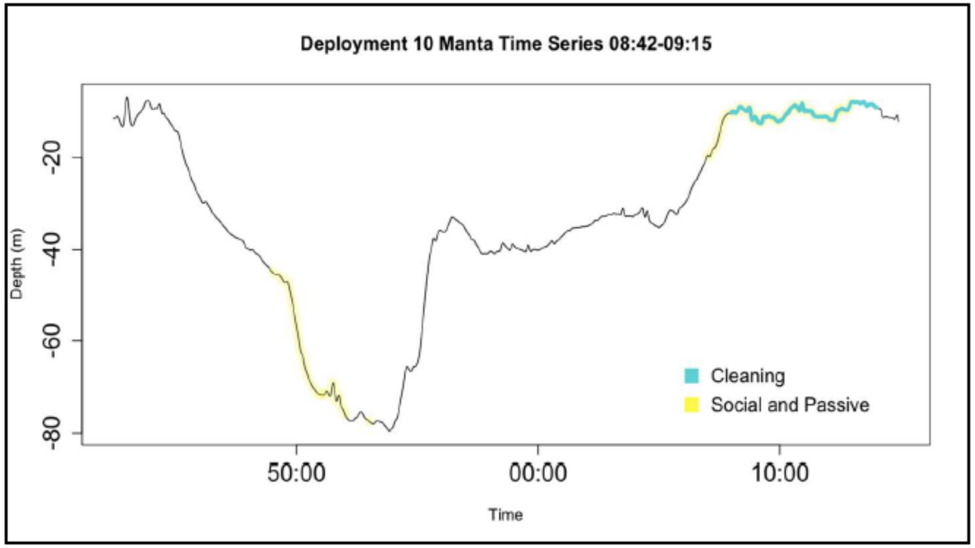


Fig A2.21 Behaviour Time Series Graph for Deployment 10

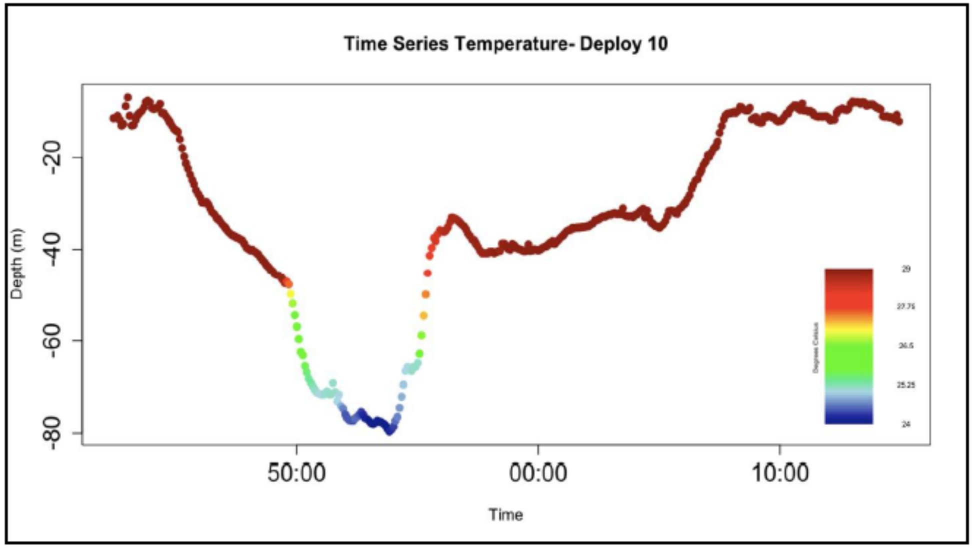


Fig A2.22 Temperature Time Series Graph for Deployment 10

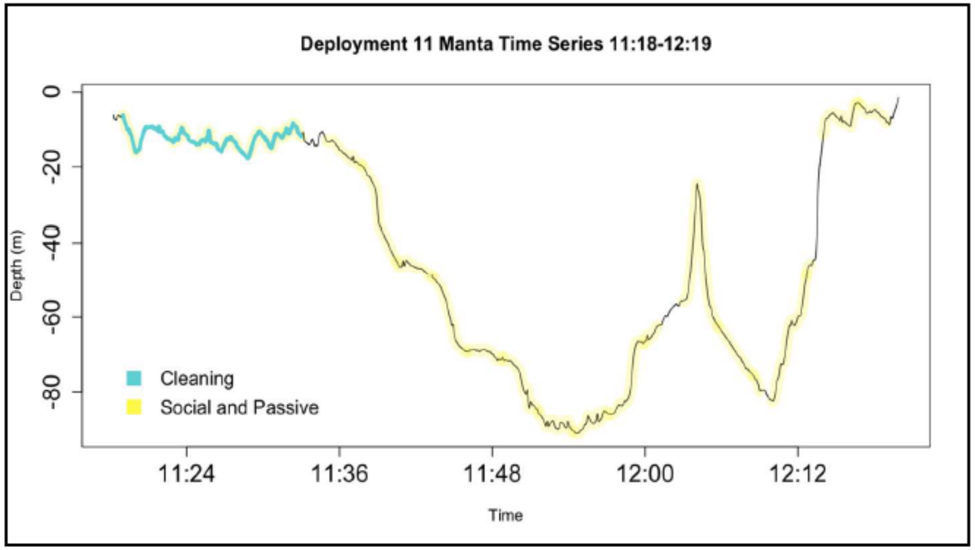


Fig A2.23 Behaviour Time Series Graph for Deployment 11

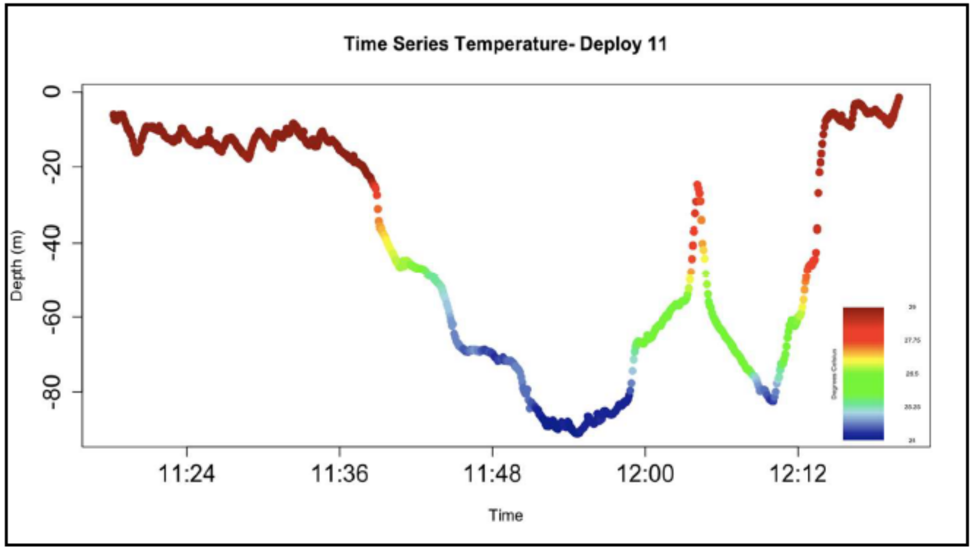


Fig A2.24 Temperature Time Series Graph for Deployment 11

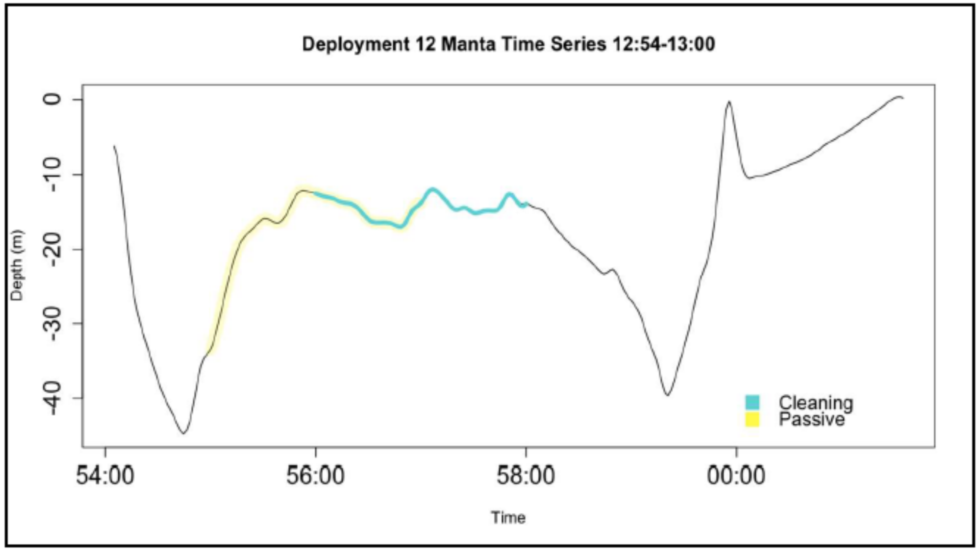


Fig A2.25 Behaviour Time Series Graph for Deployment 12

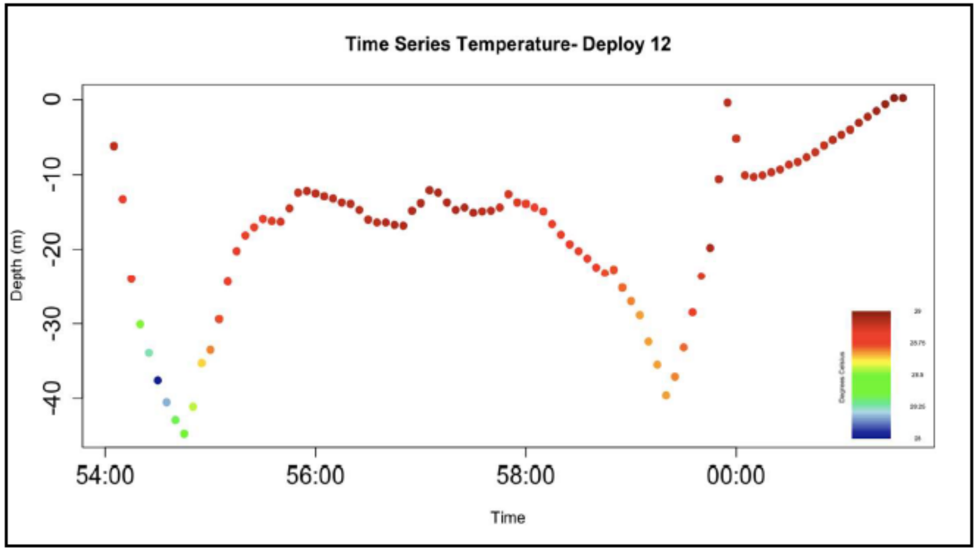


Fig A2.26 Temperature Time Series Graph for Deployment 12

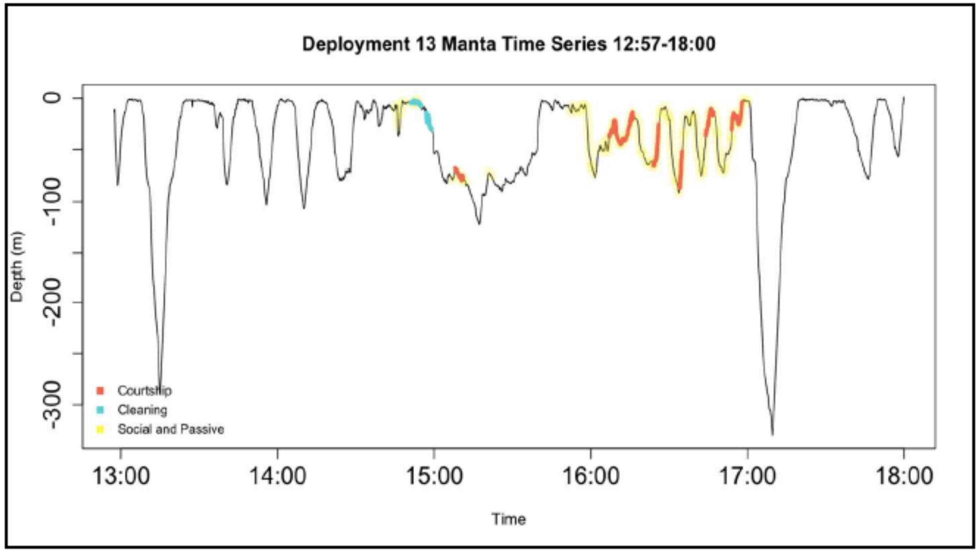


Fig A2.27 Behaviour Time Series Graph for Deployment 13

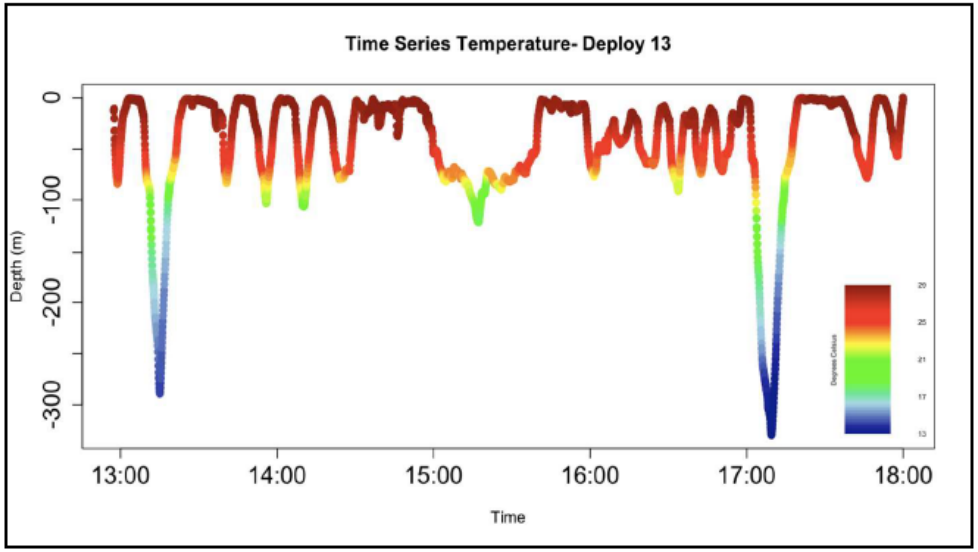


Fig A2.28 Temperature Time Series Graph for Deployment 13

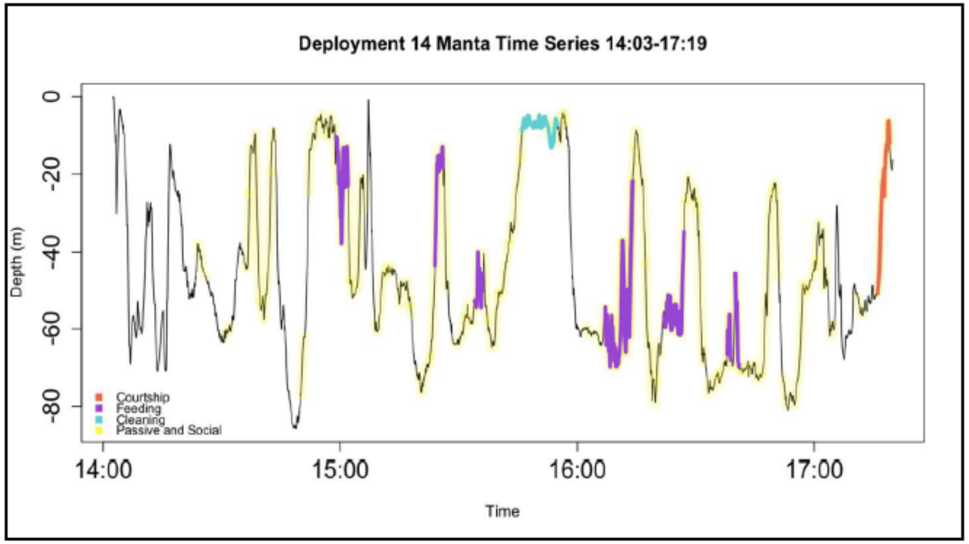


Fig A2.29 Behaviour Time Series Graph for Deployment 14

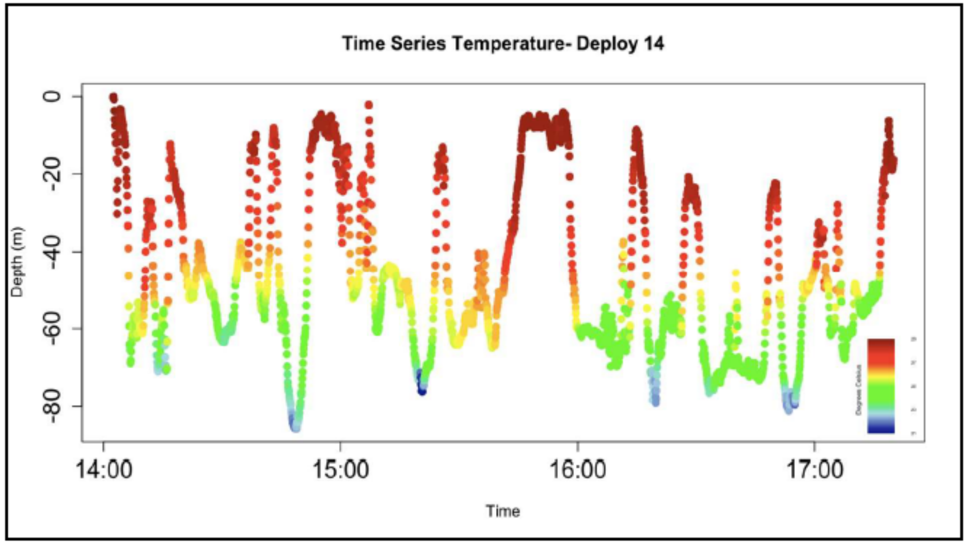
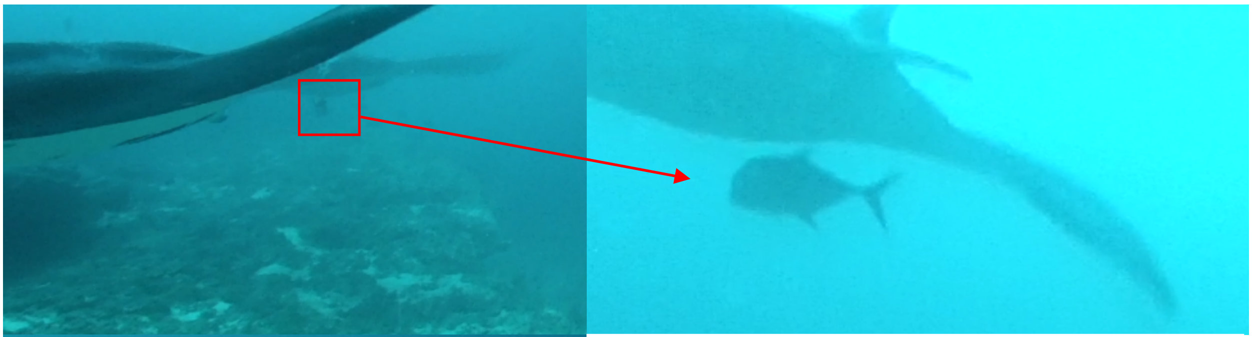


Fig A2.30 Temperature Time Series Graph for Deployment 14

Appendix III. Interactions with Other Species



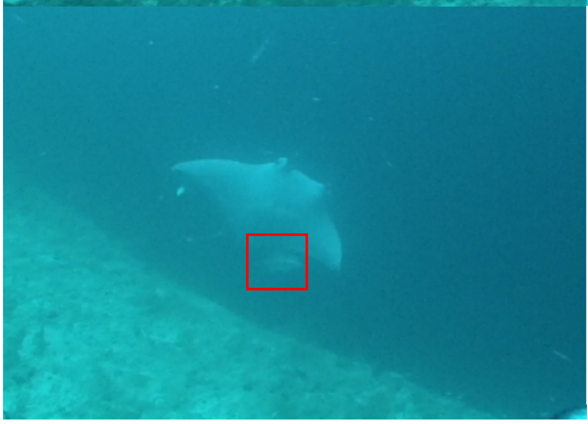


Fig A3.1: Deployment one interactions with *Carangidae* family of fish

Red boxes indicate fish recorded that were difficult to identify with resolution of video. Two interactions (above and left) occurred between 73.5 meters and 73.9 meters for a maximum duration of 15 seconds.

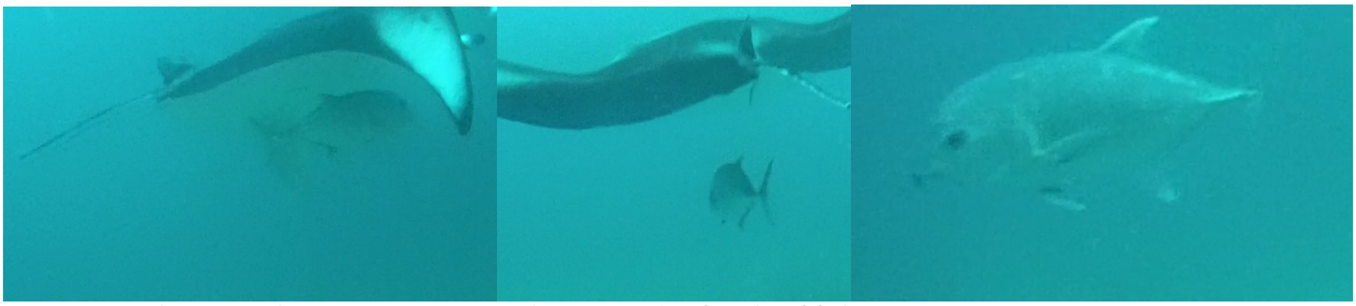


Fig A3.2: Deployment three interactions with *Carangidae* family of fish

Interaction occurred for 47 seconds between 34.8 meters and 37.2 meters. Images show progression of interaction.

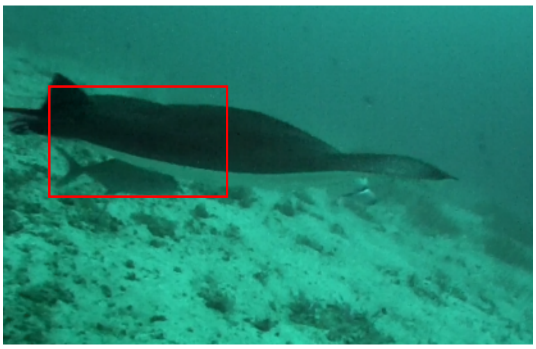


Fig A3.3: Deployment seven interactions with *Carangidae* family of fish

Interaction occurred for eleven seconds where the Crittercam recorded a fish swimming under another *M. alfredi* between 72.3 meters -72.4 meters

Fig A3.4 Interaction during Deployment 8

The Crittercam simultaneously recorded fish under two different *M. alfredi* for a recorded duration of sixteen seconds between 63.5 meters and 65.3 meters.

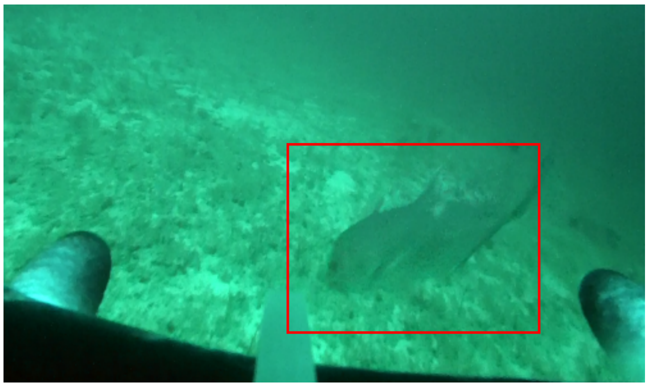


Fig A3.5 Interaction during Deployment 8 (Left)

Fish swam under research individual at 59.5 meters and *M. alfredi* subsequently displayed an unknown flinch reaction (see discussion). The driver of flinch behaviours was not determined and it is unknown whether the fish and behaviour are correlated.

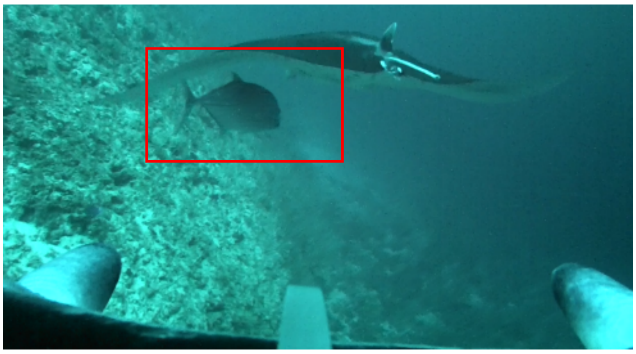


Fig A3.6 Interaction during Deployment 8 (Right)

Fish swam under recorded *M. alfredi* for two mins and 56 seconds between depths of 57.5 and 66.9 meters.

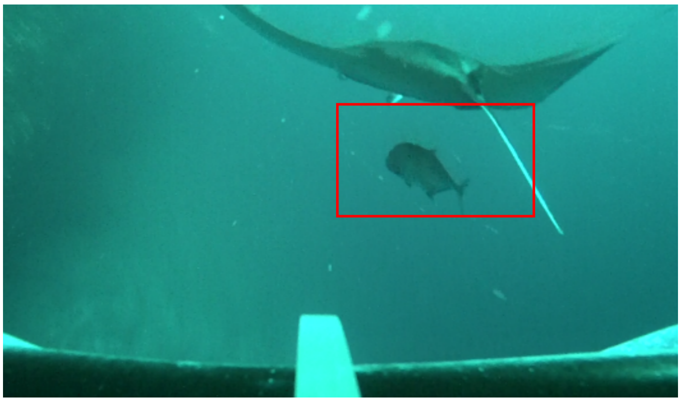
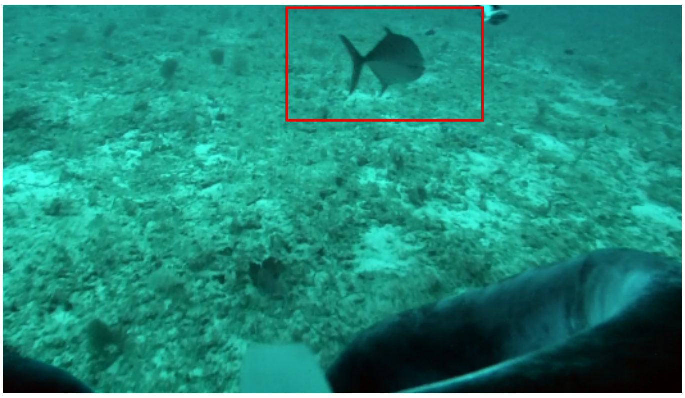


Fig A3.7 Interaction during Deployment 9

Screen shot from a Crittercam recording of a *Carangidae* under *M. alfredi* between 53.1meters -54.3meters for one minute.



A3.8 Interaction during Deployment 11

Crittercam recorded a jack fish under *M. alfredi* for four seconds.