The hitchhiker's guide to the Maldives.

The echeneid-host association between remoras and manta rays.

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All the photos used in this report, unless otherwise stated, belong to the Manta Trust.

ABSTRACT

The following study utilised the photographic data (over 38000 sightings) collected by the Maldivian Manta Ray Research Project (MMRP) during a period of over ten years. The study aimed to explore the patterns of association between manta rays and remoras. Factors influencing the number of the associated remoras, such as size, sex, pregnancy status and primary behaviour of the mantas, as well as the time and location of sightings, were investigated. A zero-inflated model was utilised to help reveal which factors best explained the variation in remora numbers. There was some degree of inter-dependency between the variables, so an analysis of the entire dataset using one model proved to be of a limited value. An approach that involved manipulating individual factors and looking for differences between these factors at specific locations was taken instead. The results showed that reef manta rays engaged in cleaning behaviour had significantly more remoras associated with them than those that were engaged in feeding. Females of both reef manta rays and oceanic manta rays appeared to carry more remoras than did the males. Pregnant manta rays had a significantly higher number of remoras associated with them, when compared to similar size, non-pregnant female manta rays. Within the Maldives archipelago, spatial distribution of remora associated with the mantas was not uniform. Specific atolls such as North Male and Ari had significantly higher ratios or remoras to mantas than for instance the Baa atoll. Analysis of temporal variation over a short time scale (days to weeks) revealed that the association between reef manta rays and *Echeneis naucrates* remoras was of a tentative nature. The number of remoras associated with a single individual fluctuated from day to day, and they appeared to be only loosely associated with their hosts. A more controlled observational study that would allow for unambiguous tests, would be required in the future, if the exact factors determining the presence of the remoras are to be revealed.

1. INTRODUCTION

1.1 The Maldivian Manta Ray Project (MMRP)

Since 2005, using photographic and video identification, the Maldivian Manta Ray Project (MMRP) has built a comprehensive database of over 38,000 sightings, contributing extensively to the knowledge of behaviour, population dynamics, and migration patterns of reef manta ray (*Manta alfredi*) in the Maldives. MMRP has already identified over 4,000 different mantas with new individuals being regularly sighted. The resident population of reef manta rays in the Republic of Maldives is likely to number around 5,000 individuals. (Manta Trust, 2016).

1.2 Study species

The following project focused on the patterns of associations between manta rays (hosts) and remoras that attach to them. The remora association with fish is still poorly documented and very little has been published regarding the relationship between manta rays and remoras.

1.2.1 Hosts

Manta rays (genus *Manta*) are pelagic planktivorous feeders and the largest batoid fishes in the world (Marshall et al., 2011). They have a circumglobal distribution in tropical and subtropical waters (Marshall et al., 2009). These cartilaginous elasmobranch fishes have recently been separated by Marshall et al., (2009) into two visually distinct species: *Manta alfredi* (Krefft, 1868) and *Manta birostris* (Walbaum, 1792).

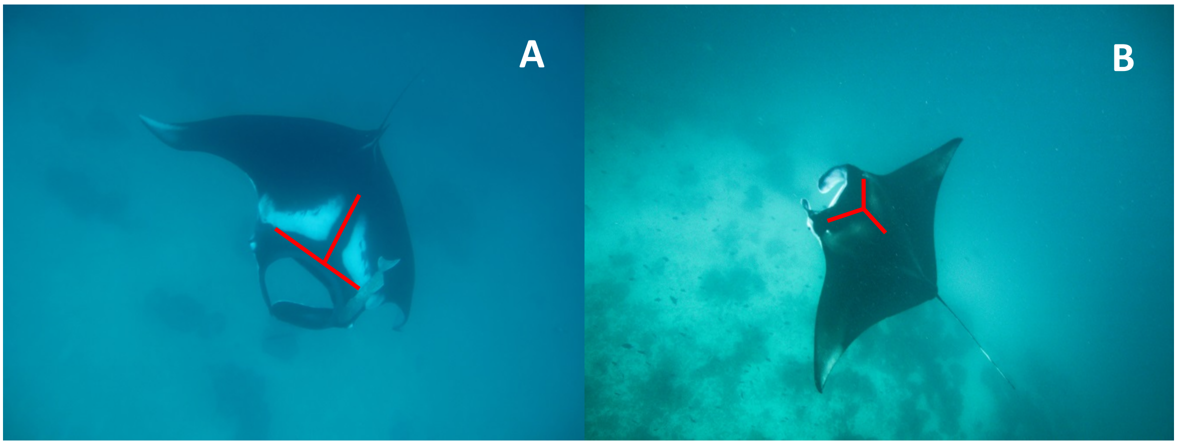


Figure 1 An oceanic manta ray (A) with a true remora attached to its dorsal side. A reef manta ray (B) with no visible remoras. The easiest way to tell the two species apart is by size (oceanic manta rays are larger), and also by the pattern of colouration on the dorsal sides, which in case of the oceanic manta rays forms a T-shape pattern, and in case of the reef manta rays is more of a Y-shape pattern (red lines).

This late recognition of two separate species resulted in compromised knowledge on the Biology and ecology of mantas. Much of the current knowledge is attributed to information pertaining to *M. birostris,* leaving many biological attributes of *M. alfredi* unknown (Couturier et al., 2012). Since mantas are predominately zoo-planktivores, their distribution patterns reflects local productivity and food availability (Anderson et al., 2011). Mantas often aggregate to feed at specific locations, where plankton becomes concentrated. Those sites vary seasonally due the changes in current circulation patterns and water temperature (Couturier et al., 2012, Rohner et al., 2013). Manta rays will also often aggregate at locations functioning as cleaning stations, potential mating and birth sites, as well as nursery grounds (Couturier et al., 2012). In the Maldives, individuals of *M. alfredi* have been shown to migrate annually between the western and eastern sides of atolls with the changes in monsoons, most likely benefitting from enhanced productivity on the lee-sides of the atolls (Kitchen-Wheeler, 2013).

In the Maldives archipelago, the reef manta ray (*M. alfredi*) is the most commonly seen mobulid species. (Kitchen-Wheeler et al., 2012). Their high numbers attract tourists from around the world (Anderson et al. 2010) and thus contribute to the local economy.

This work focuses primarily on the reef manta ray (*M. alfredi)* since the majority of the data collected by the Maldivian Manta Ray Project regards this species.

1.2.2 Remoras

The superfamily Echeneoidea is comprised of three families of cosmopolitan marine fishes: the Echeneidae (remoras), two species of Coryphaenidae (dolphinfishes), and the monotypic Rachycentridae (cobia), (Gray et al., 2009).

The remoras (Teleostei, Echeneoidei, Echeneidae) are mainly small to medium fishes with circumglobal distribution in tropical and subtropical waters (O'Toole, 2002). The family Echeneidae contains eight recognised species in four genera (Lachner, 1966). These fishes are unique in that they possess a sucking disk, a modified dorsal fin, which they use to attach to their hosts. It is one of the most remarkable and most highly modified skeletal structures among vertebrates (Britz & Johnson, 2012). The sucking disks possess an immense adhesive power which is sometimes used by people in the tropics to catch sea turtles by attaching a fishing line to remoras' tails.

All of the eight species of Echeneidae rely on hitchhiking behaviour to varying degrees, with certain species exhibiting strong host preferences. *Echeneis naucrates*, also known as the 'live sharksucker', has been reported to attach to a variety of hosts (O'Toole, 2002; Sazima & Grossman, 2006).

Echeneis naucrates (Linnaeus, 1758) is the most abundant remora in warm waters. It is known to free swim and often associates with a variety of hosts within the reef environment. *E. naucrates* appears to undergo several stages of development of the relationship with their hosts. The first is a free swimming stage that occurs until the development of a sucking disc, at

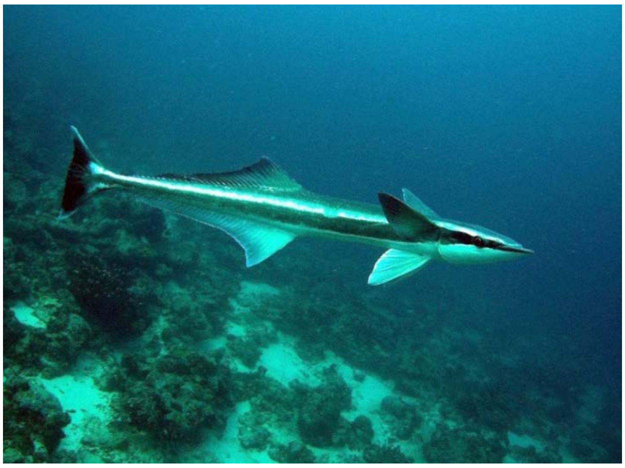


Figure 2 *Echeneis naucrates* (live sharksucker) individual, free swimming on a coral reef.

approximately 30mm length, according to (Nakajima et al., 1987). The next stage involves attachment of juvenile echeneids to hosts such as the slow moving reef fishes like ostraciids and scarids. In the final stage (echeneids larger than 200mm) reef sharks are the most commonly preferred hosts of *E. naucrates* (O'Toole, 2002). The relationship between *Echeneis naucrates* and its hosts is likely commensal.

Remora remora (Temmick and Schlegel, 1850) also known as the true remora, short remora or the brown remora is found in the pelagic environment and usually remains attached to the host (O'Toole, 2002), however free swimming individuals have occasionally been observed (Clark & Nelson, 1997). *R. remora* appears to be primarily a shark specialist exhibiting strong reliance on parasitic copoepods in the diet of juveniles, but this dependence lessens as the fish grows (O'Toole, 2002).

Figure 3 A whale shark (*Rhincodon typus*) with numerous remoras (*Remora remora*) attached

Remora albescens, known as manta sucker or white remora is almost exclusively found on manta rays, often residing inside the mantas and thus is not easily noticable. The literature search performed by O'Toole, (2002) on 30 records of *R. albescens* revealed that their predominant hosts (accounting for 90% of records) were mantas. This species of remora feeds very little, if at all, on external

Figure 4 The white suckerfish (Remora albescens) Source: fishbase.org (B).

copepods and commonly invades the mouth and gill chambers of manta rays (Cressey & Lachner, 1970).

Other associated fish, such as cobia, have also been documented following larger animals such as sharks and mantas. Cobias however do not attach to the host but simply follow it. Pilot fish (*Naucrates ductor*) and juvenile golden trevalley's (*Gnanthanodon speciosus*) are also commonly seen accompanying the mantas where they get shelter, protection and sustenance from the giants.

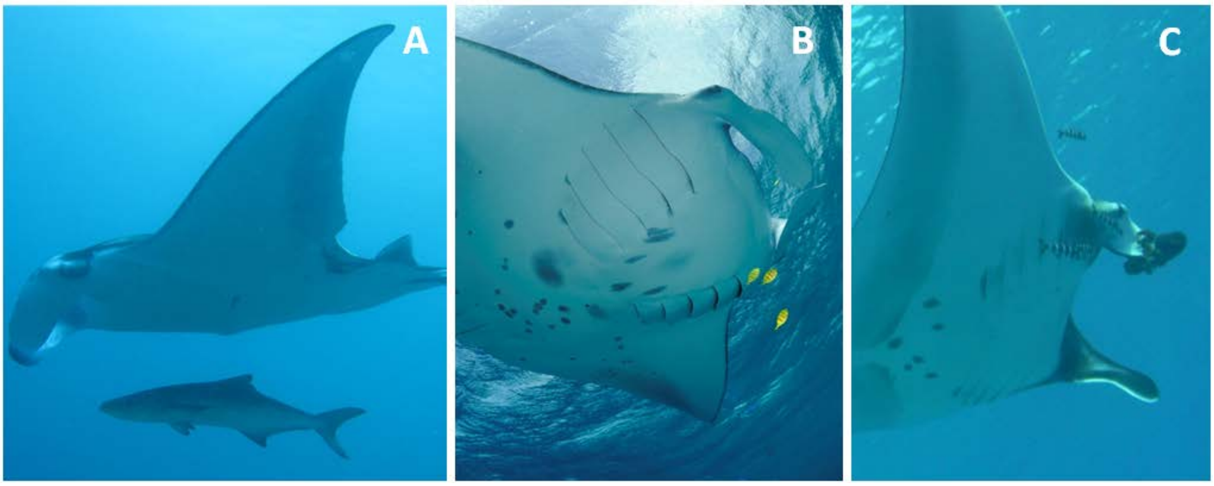


Figure 4 An adult male reef manta ray (Manta alfredi), accompanied by a large cobia (Rachycentron canadum) (A). Juvenile golden trevalley's (Gnathanodon speciosus) seeking shelter from predators in the vicinity of manta's body. Adults of this species are not seen associated with mantas. (B). Pilot fish (Naucrates doctor) associated with a manta ray (C).

This research focuses on the following species: *Echeneis naucrates and Remora remora,* and their associations with the reef manta ray and oceanic manta ray. *Remora albescens,* although a known associate of manta rays, was not often seen in the photographs, likely due to the fact that it mainly resides inside the mouths and gill chambers of its hosts.

1.3 The echeneid-host association in the literature.

Remoras have been observed to attach to a variety of hosts such as teleost fish (Cressey & Lachner, 1970), turtles (Sazima & Grossman, 2006), cetaceans, (Fertl & Landry, 1999), sirenrians (Williams et al., 2003), sharks (Brunnschweiler, 2006) and even conspecifics (Brunnschweiler & Sazima, 2008).

The echeneid-host association has been primarily studied for marine mammals, turtles and sharks, and although remoras have been observed on manta rays, this association is extremely poorly documented in the literature.

The relationship between remoras and their hosts has been known for centuries, present as part of myths and legends and the remoras have even been exploited by artisanal fishermen. The exact nature of the association however is still hard to define, with the costs and benefits of this interaction remaining poorly understood (Mucientes et al., 2008). In several behaviour studies it has been observed that sharksuckers, actively follow and attach to sharks (O'Toole 2002), so sharks seem obviously beneficial for sharksuckers (Cressey & Lachner, 1970). However it remains unclear whether or not the remoras themselves are beneficial or detrimental to their hosts, and to what degree. Remoras are considered hydrodynamic parasites as they potentially disrupt the flow over the host's body and add to the drag. (Fish et al., 2006). Sharks have been observed to occasionally attempt to dislodge sharksuckers or reposition them (Ritter, 2002; Ritter & Brunnschweiler, 2003). Hester et al. (1963) suggested that the purpose of the aerial manoeuvres executed by spinner dolphin is to aid the removal of remoras. Breaching behaviour of several elasmobranchs and cetaceans could potentially be linked to an attempted removal of remoras.

Attachment of remoras to certain sensitive areas such as sensory organs might result in a higher urge for their hosts to attempt to dislodge or reposition them (see Ritter, 2002 for this view for sharks), while their attachment to other areas such as the belly might go almost unnoticed.

The table below presents a literature view on potential costs and benefits of an association between echeneids and their hosts.

Table 1 Potential costs and benefits of an association between remoras and their hosts.

Benefits to remoras	Benefits to hosts	Costs to hosts
Increased feeding opportunities (i.e. parasites and dead skin (Strasburg 1959), food scraps (Strasburg, 1962) vomits and faeces (Sazima et al., 2003))	Cleaning and removal of diseased or injured tissue (Mucientes et al., 2008)	Attachment can irritate the skin of the host (Schwartz, 1977; Schwartz, 1992).
Protection from predators (i.e.. Fertl & Landry, 1999)	Echeneids are reported to feed, to a certain extent, on ectoparasites. However the relative importance of parasites in their diet varies with the echeneid species involved and can change during different life stages of the remoras (Cressey & Lachner, 1970; O'Toole, 2002)	Hydrodynamic interference - adding to the resistance of swimming (Weihs et al., 2007). The greater the remora/host ratio, the greater the hydro-dynamic drag the attached fish exerts on its host. (Sazima & Grossman, 2006) Additional drag may arise when the remoras attach to a body region where it affects the structure of the boundary layer (Vogel, 1994)
Constant flow of water across its gills (Strasburg, 1957)		
Transport - riding or hitchhiking as an energy-saving behaviour (e.g. O'Toole, 2002)		
Increased reproduction potential through finding mates when the hosts aggregate, especially if the hosts are social animals (Silva-jr & Sazima, 2003)		

1.3.1 The mechanism of suction and suction disk performance

It has been suggested that echeneid fish’s hitch-hiking on sharkskin is not free of cost (Bandyopadhyay et al., 2008). Remora's suckers are likely to be active and not passive. Although they do not seem to have plumbing or centralized suction pumps, it is possible that numerous minute local pneumatic pumps exist. Fulcher & Motta (2006) noted that the ‘suction disk of *E. naucrates* and *E. neucratoides* is supported and controlled by a series of muscles and skeletal elements that function to erect and depress the numerous spinule-bearing laminae. The loosely attached, fleshy marginal lip provides a pliable seal around the suction disk’. Additionally, through the use of spinules (small tooth-like projections of mineralized tissue) remora’s increase the resistance to slippage and thereby enhance friction to maintain attachment to a moving host (Beckert et al., 2015). Recent experiments on remora’s suction pad attachment have revealed that spinules are primarily responsible for friction enhancement on rough host topologies such as shark skin (Beckert et al., 2015).

1.3.2 The diet of the remoras

Smith (1950) proposed that sharksuckers are coprophagous and feed on feces of their hosts. Strasburg (1959) contradicted this view and established the modern opinion of remoras feeding opportunistically on host-scraps, plankton and parasites. In reality it seems that depending on circumstances, remoras indeed feed on a variety of food, including the fecal material. Remoras have often been observed feeding on offal material (Sazima et al., 2003) of their hosts and additional stomach content studies suggested that they feed on pre-digested material (Williams et al., 2003). At least one species of remora has been observed to feed on plankton bloom and filtering plankton by ram-feeding (Clark & Nelson, 1997).

1.3.3 Remoras as parasite pickers?

Parasitic copepods seem to constitute an important part of diet among echeneid fishes, but this dependence varies among the different species of remoras. Certain remora species feed on parasitic copepods found externally or in the mouth and gill chambers of their hosts (O'Toole, 2002). In a study of stomach contents performed by Cressey & Lachner, (1970) over 70% of stomachs of *Remora remora* containing food had parasitic copepods present in them. Younger individuals of *R. remora* seemed to be more active as parasite pickers, and parasites constituted a major part of their diet. The live sharksucker appeared to rely on parasitic copepods to a much lesser degree. Only one of the smallest individuals of *Echeneis naucrates* contained parasitic copepods, and the highest percentage of those were found in middle sized specimens. There were no parasites found in the stomachs of *E. naucrates* larger than 311 mm, even though 30 larger specimens have been examined. This indicates that there might be a change in remora's diet associated with its life history and once again the nature of the association cannot easily be defined and given a label such as 'commensalism' or 'mutualism'.

In case of the *R. albescens*, its role as a parasite picker could not be established from the study of 17 specimens examined by Cressey & Lachner (1970). It is possible that this specie is not involved with parasite picking so a mutualistic relationship is absent (Cressey & Lachner 1970).

For the hosts, the potential benefit of living in an association with a parasite picker could be demonstrated by comparing an ectoparasitic load of those hosts that carry remoras with those that do not have remoras associated with them. Doubilet et al., (1990) reported a heavily parasite infested sleeper shark living at depths without a remora. Lamnid sharks rarely carry any remoras and are commonly infested by parasitic copepods (O'Toole, 2002). Mucientes et al., (2008) investigated the relationship between the ectoparasite load of sharks and the presence of echeneid fish. They found that in case of the mako sharks, the number of ectoparasites was indeed negatively correlated with the number of associated echeneids.

1.4 Aims and hypotheses of this report

The main aim of this report is to investigate the patterns in the association between the reef manta ray and the remoras from the Maldives archipelago, and to suggest what factors make the mantas more likely to acquire remoras.

Spacio-temporal influence, as well as the characteristics of the hosts (such as the sex, size, pregnancy and behaviour) will be analysed to see whether they significantly impact the number of remoras that associate with the mantas.

The following null hypotheses were tested in this investigation:

1. **H_0** There is no significant difference between the average number of remoras associated with manta rays engaged in cleaning and those engaged in feeding.

2. **H_0** Male and female manta rays have a similar average number of associated remoras.

3. **H_0** Pregnant manta rays do not have a higher number of remoras associated with them, when compared to similar size, non-pregnant females.

4. **H_0** The ratios of remoras to manta rays are not influenced by the size of the mantas.

5. **H_0** The average number of remoras associated with reef manta rays stays similar between different locations (atolls within the Maldives archipelago).

6. **H_0** The number of remoras associated with an individual manta ray does not change over a short time scale (day to day).

7. **H_0** The ratio of remoras to mantas shows no variation between the seasons.

8. **H_0** The average number of remoras associated with manta rays stays more or less constant between the years.

2. METHODS

2.1 Study site

The Maldives archipelago is composed entirely of coral atolls, which form a chain running from north to south from about 7^{o}N to about 0.5^{o}S (Figure 5). In the north and south of the archipelago the atoll chain is single but it splits into a double chain in its central part. Maximum depths within the atolls are typically 50–60 m but vary from about 10–100 m (Anderson et al., 2011). Outside the atolls, the reef typically slopes drop steeply away to about 2000–3000 m, with the exception in the area between the double chain of atolls in the central Maldives, where bottom depths are of the order of 200–500 m.

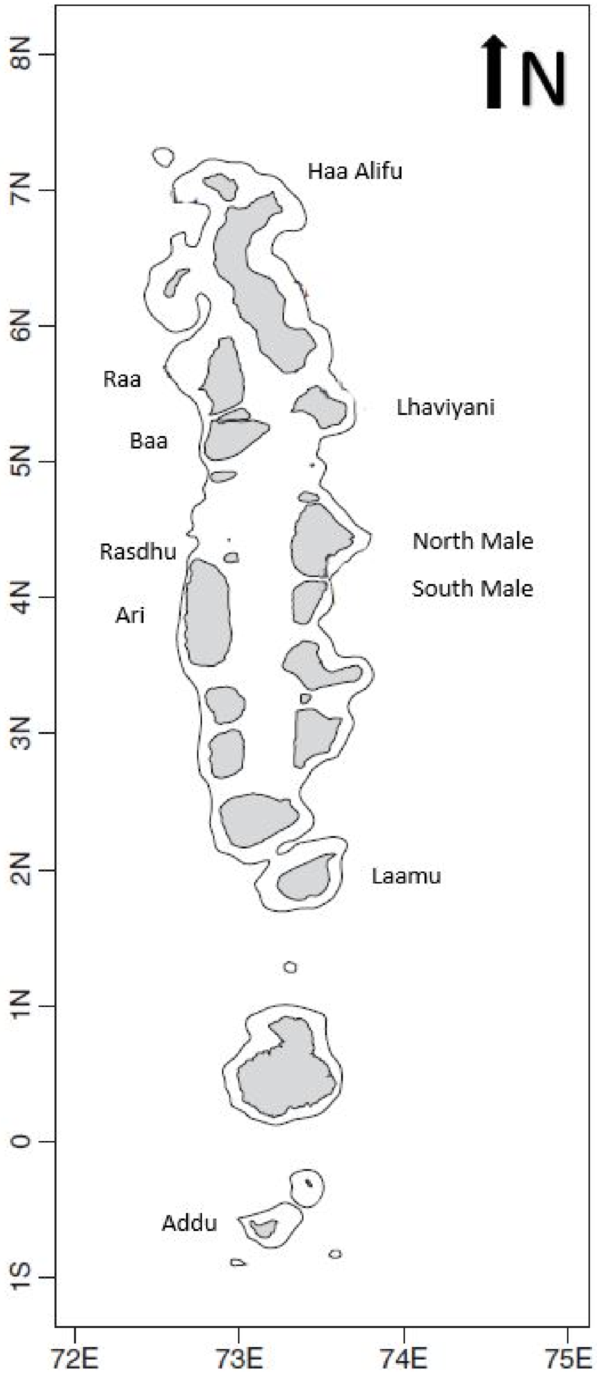


Figure 5 Map of the Maldives, showing some of the main atolls mentioned in this study. The outer line marks the position of the 1000m depth contour.

2.1.1 Weather patterns, current and tides

Kitchen-Wheeler (2013) provides a thorough description of weather in the Maldives. The Archipelago experiences tropical-oceanic climate with small daily and yearly temperature variations. The Maldives lie in the monsoon belt of the northern Indian Ocean, and experiences a dry north-east monsoon and a wet south-west monsoon.

The NE monsoon lasts from mid-December to the end of April. During that time the rainfall averages 75 mm per month and monthly sunshine averages 256 hours. The SW monsoon lasts from May to November, during which the rainfall averages 215 mm per month and sunshine averages 208 hours per month. The months of April and November are change-over months, when winds are typically not very intense or variable.

2.2 Data acquisition

The Maldivian Manta Ray Project (MMRP) database with over 4000 individual manta rays and over 38000 sightings was used in this study.

After each snorkel or dive the photographic documentation of encountered mantas was added to the database and identified with use of the photoID software. Each new individual was assigned a number. For each sighting, the date, time, location (atoll and more specific, such as the name of the reef), sex of the individual and its size class were noted. If an individual was observed to be pregnant this was also noted down and the individuals with very large bulgy abdomens were described as ‘heavily pregnant’. The heavily pregnant individuals were most often in their last 1-2 months of pregnancy.

2.2.1 Photo identification

Photo identification of manta rays relies on the unique spot patterns on their bellies. Photograph of each individuals were taken, compulsory of the ventral side, ideally encompassing an entire animal, so that the sex can also be determined. The sex of the animal was determined through the presence or absence of male reproductive organs (claspers) located on the pelvic fins. The spot pattern was then compared to a database of previously photographed individuals (Arzoumanian et al., 2005).

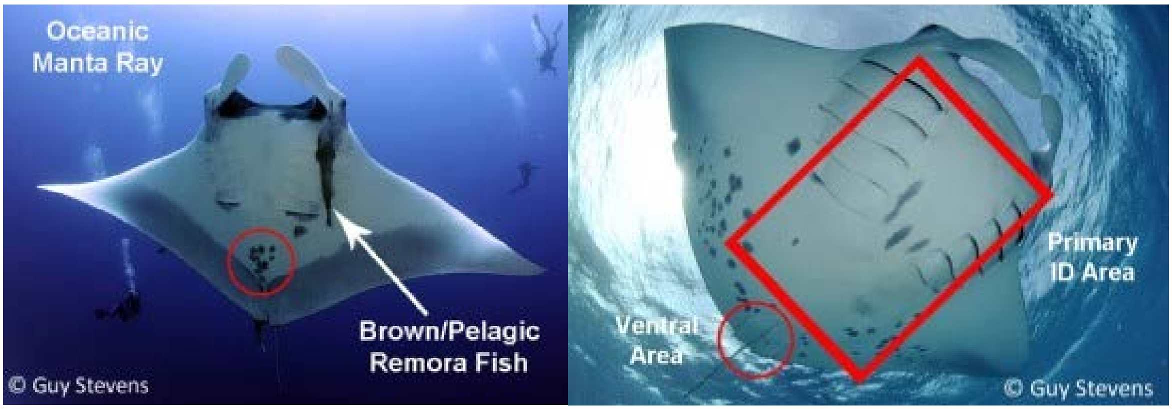


Figure 5 The primary ID area for spot comparisons differ between the species. Oceanic manta ray(left) and the reef manta ray (right).

2.2.2 Obtaining size estimates

A size estimate of the animal was obtained from appropriate photographs. Several techniques have been used over the time. Disc width (DW), the distance between the tips of the pectoral fins, was often estimated based on to a nearby diver size. Direct measurements have also been obtained using measuring tape, however mantas were very wary of measuring tapes laid on the reef or in the water column so calibrating visual estimates was difficult. Attempting to measure the mantas using a tape held across the dorsal/ventral surface could cause significant disruption to their behaviour, so this technique was not employed often.

Paired-laser photogrammetry was occasionally utilised and provided simple, non-invasive, accurate, and precise measurements of free-ranging manta rays (Deakos, 2010). When photographs of the dorsal or ventral surface of the manta ray were taken flat-on, two small green dots, 50 cm apart, were visible in the resulting image, allowing the approximate size of the manta ray to be extrapolated. Since the project employed different techniques and methods for obtaining disc width estimates, the estimates were binned into four size classes and those were further analysed and compared. Four size classes have been established; female within all four classes have been recorded, while the male individuals never attained the 4th class (Table 1).

Table 2 Size classes for reef manta ray individuals. The asterisk signs show size classes at which males and females reach maturity.

FEMALE		MALE	
Size class	Size (m)	Size class	Size (m)
1	1.5 - 2.3	1	1.5 - 2.3
2	2.4 - 2.6	2	2.4 - 2.6
3	2.7 - 3.1	3*	2.7 - 3.1
4*	3.2 - 3.6	N/A	N/A

2.2.3 Identification and counting of the remoras

The remoras were visually identified based on their external characters (body colour, shape, and proportions – see e.g. Robins & Ray, (1986); Froese & Pauly, (2016). The number of remoras associated with a given manta ray during a given day at a given location was derived from still photographs. Often multiple photos were available and then the highest number of remoras present in a single picture was reported. For the reef manta rays, only the ventral side was considered, as this is where most of the remoras attach and very few mantas had photographs of both ventral and dorsal sides. In case of the oceanic manta ray and the true remora association, only the images of the mantas' dorsal side were taken into consideration.

When the counting was problematic due to large number of remoras, the software ImageJ was used to mark individual remoras with a red dot and thus avoid double counting (Figure 6)

When the identification from photos to species level was not feasible; the ID was performed on a group level (remoras) and still added to the count.

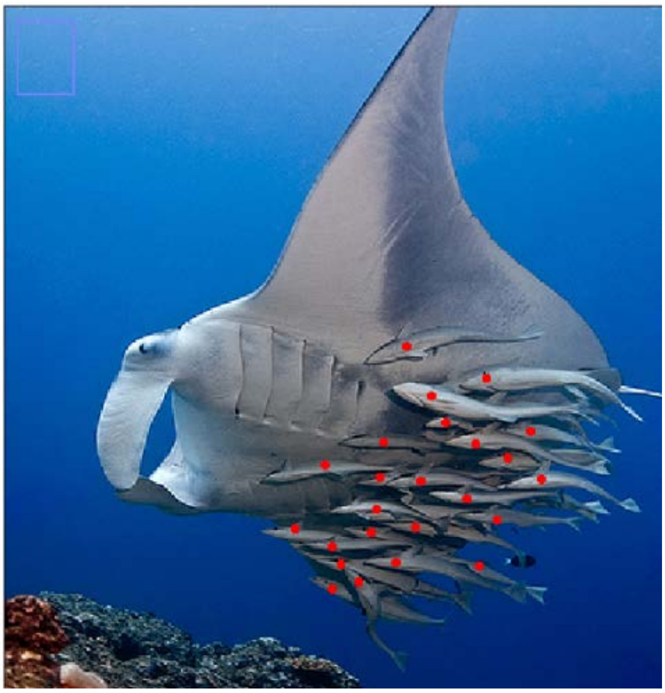


Figure 6 Use of the ImageJ software to aid the counting process by marking individual remoras.

2.2.4 Data quality monitoring.

The photos that were illegible or did not show enough of the manta's body to effectively count the number or remoras were removed from subsequent analysis. Mantas whose gender was unknown were also removed from the analysis. In case of the oceanic manta rays, mantas with only ventral photos were removed from analysis.

2.3 Model choice and construction

The initial data exploration of the count data revealed that the negative binomial distribution model provides a good fit to the data (see figure x). The negative binomial distribution can be used as an alternative to the Poisson distribution and is especially useful for discrete data over an unbounded positive range in which sample variance exceeds the sample mean (Crawley, 2005). In such cases, the observations are over dispersed with respect to a Poisson distribution.

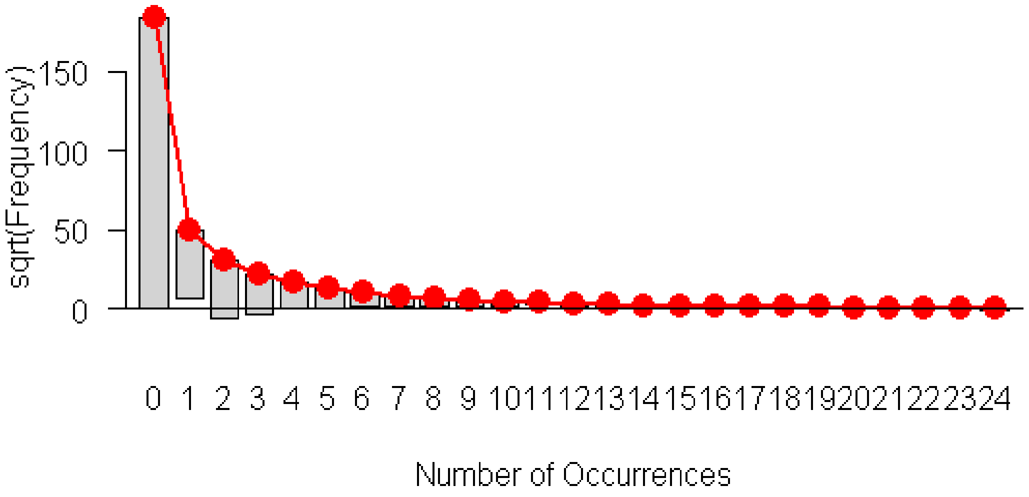


Figure 7 A fit of the negative binomial distribution model to the count data using a hanging rootgram. The comparison between the observed data (grey bars) and the fitted distribution curve is made easier by 'hanging' the observed results from the theoretical curve, so that the discrepancies are seen by comparison with the horizontal axis. The vertical axis is scaled to the square-root of the frequencies so as to draw attention to potential discrepancies in the tails of the distribution (Upton & Cook, 2008).

Since the histogram of all of the count data revealed that the majority of the count data was zeros, the zero inflated model was chosen to examine the effect of different factors in predicting the number of the remoras. The parameter for testing over dispersion of the data was positive and significant (log(theta)= 0.4228), further confirming that the negative binomial distribution should be specified in the model, instead of the Poisson distribution.

Defining the variables within the model

The dependent variable was the number of remoras associated with mantas.

The predictors (independent variables) were manta's sex, size class, behaviour, pregnancy status as well as the location (atoll).

The excess zeros in the data were due to extremely low number of remoras present at the Baa atoll, where the majority of the data in present study came from.

In order to account for some of the interdependence in the data, binary factors have been created in the model (for instance heavily pregnant mantas were grouped together with pregnant ones and compared against the non-pregnant mantas). The factors specified in the model as predictors included sex (female vs male), size class (juvenile vs mature), pregnancy status (pregnant vs non-pregnant) and primary behaviour (cleaning vs feeding).

The analysis was performed using the software package ' R, version 3.1.3' (R Core Team, 2015).

3. RESULTS

3.1 Differences between the two species of manta rays

Echeneis naucrates, the live sharksucker remora, was the most commonly observed echeneid fish associated with *Manta alfredi,* the reef manta ray. Other echeneid species such as *Remora brachyptera* were only sporadically observed with the reef manta rays.

In all but two cases (n=173), if there were remoras associated with the oceanic manta rays, they were identified as true remoras (*Remora remora*). The two exceptions were oceanic mantas carrying two and three sharksucker remoras (*E. naucrates)* each. The highest number of true remoras associated with oceanic mantas was two. Oceanic female manta rays carried more remoras (remora to manta ratio = 1.0235) than did the males (remora to manta ratio = 0.6571). A uniformly most powerful unbiased (UMPU) test on the ratio of rates of two Poisson counts reported this finding to be significant. (Exact rate ratio test, **p** = 0.0171, 95% CI [1.08, 2.28])

For the 38618 sightings of reef manta rays, a total of 9887 remoras have been reported.

The number of remoras associated with mantas ranged from 0 to 24. (*i.e.* the highest amount of remoras counted in a single picture of a manta ray individual was 24). Most of the sightings (88.3%) had zero visible remoras. When the remoras were present, their number associated with each manta was most often 1-2.

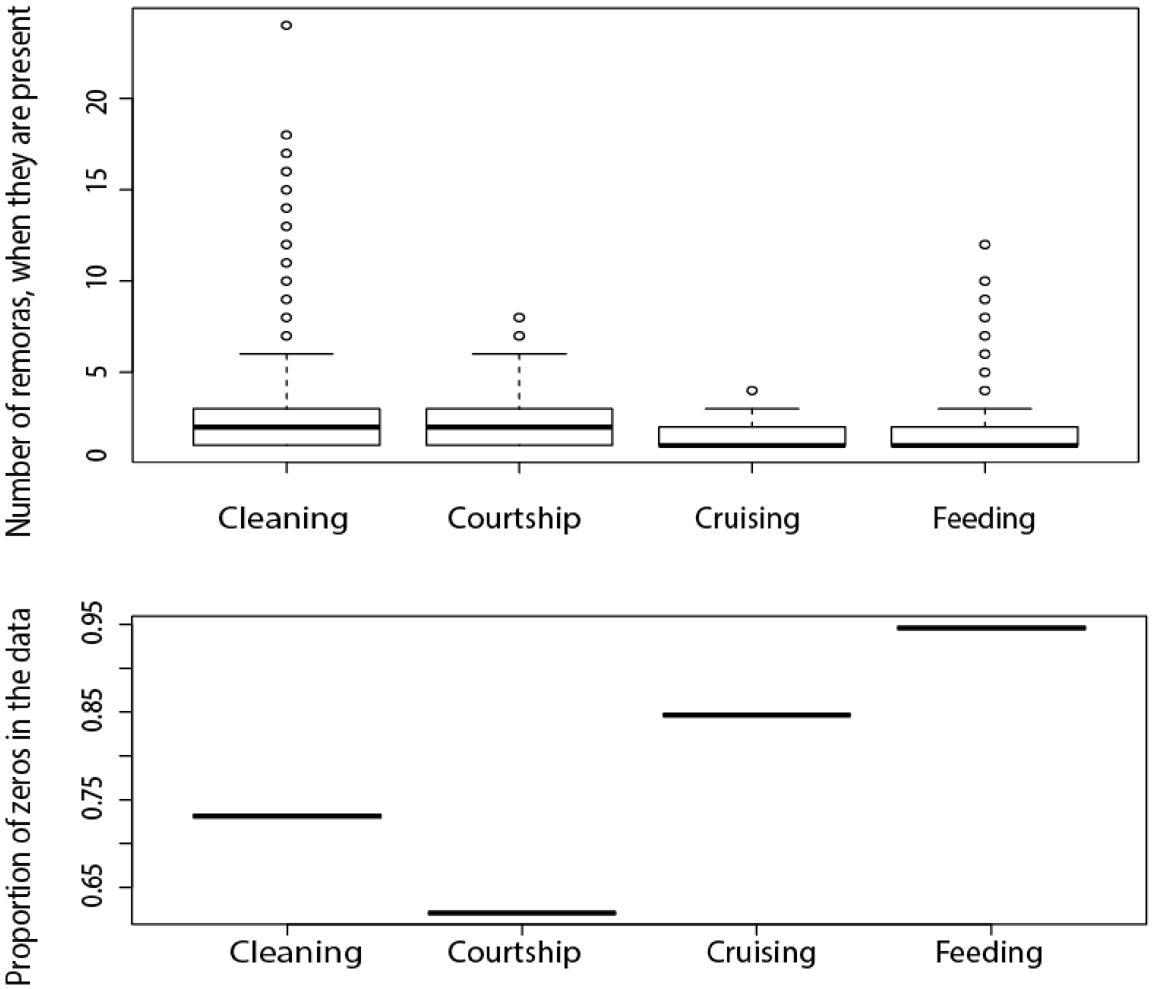
Only 35.8 % of manta ray individuals have at least once been observed in association with at least one remora. This number is much higher for the oceanic manta rays, with 61.5% of individuals having been observed at least once with at least one remora attached.

Since the amount of sightings for the oceanic manta rays was much smaller than the reef manta rays (173 as opposed to over 38000), only the reef manta rays were analysed further. The following results and the zero inflated model pertain only to the reef manta ray (*M. alfredi*).

3.2 Factors influencing the association between the reef manta rays and remoras

3.2.1 Primary behaviour

The ‘primary behaviour’ consisted of four categories; cleaning, courtship, cruising and feeding. The individual category within the primary behaviour appeared to influence the number of the remoras; however the trend was more visible in the differences between proportions of zeros in the data (Figure 8).



Total number of sightings in each category			
n=10194	n=538	n=710	n=27176

Figure 8 Variation in the number of remoras associated with manta rays engaged in different behaviour. The box plots display the variation where the remoras are actually present (ignoring the absence data), the middle figure shows the proportion of zeros in the data and the table underneath displays the number of sightings.

Mantas involved in courtship had the lowest proportion of zeros in the data. The number of observations for each of the factors was not equal, with most observations being recorded on feeding stations (n=27176). To account for this bias, a uniformly most powerful unbiased (UMPU) test on the ratio of rates of two Poisson counts was performed.

Mantas engaged in cleaning had significantly more remoras that those engaged in feeding. (Exact rate ratio test, **p** <0.001, 95% CI [0.125, 0.138])

3.2.2 Gender

The variance in the number of remoras that were associated with female manta rays was much higher than the variance for males (1.04 as opposed to 0.378). To take out the effect of multiple confounding variables, the most commonly attended cleaning station 'Lankan Beyru' reef in 'North Male' atoll has been investigated separately from all other locations. All the mature mantas (size class 3 and 4) that frequently visited this site (have been observed there over 20 times) have been taken out of the database. The number of remoras associated with mature females was significantly higher than the number of remoras associated with mature males. (Exact rate ratio test, p <0.001, 95% CI [0.314, 0.416])

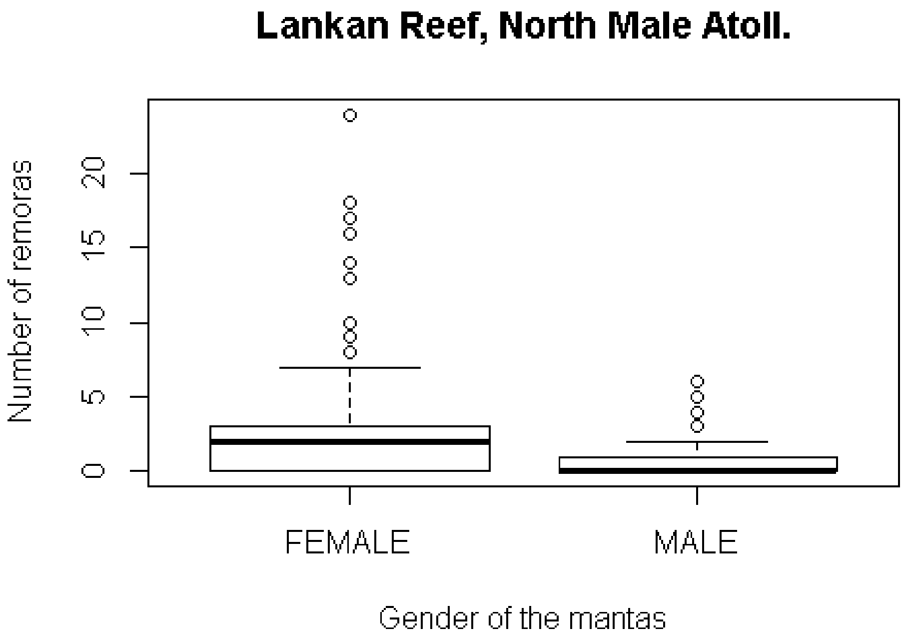


Figure 9 Median number of remoras associated with each mature female and male manta ray that frequently (>20 times) visited Lankan Beyru reef. Male n=320, female n=1388. The lower and upper boundaries of the boxplots denote 25th and 75th percentiles respectively, and the bars denote 95 percentiles.

To further limit the effect of confounding variables, mantas of only size class equal to ‘3’ were pulled out of the data. This ensured that the mantas were of similar size and the females were not pregnant, since only size class = 4 females can get pregnant. The number of remoras associated with size class 3 females was significantly higher than the number of remoras associated with size class 3 males (Exact rate ratio test, p <0.001, 95% CI [0.387, 0.651]).

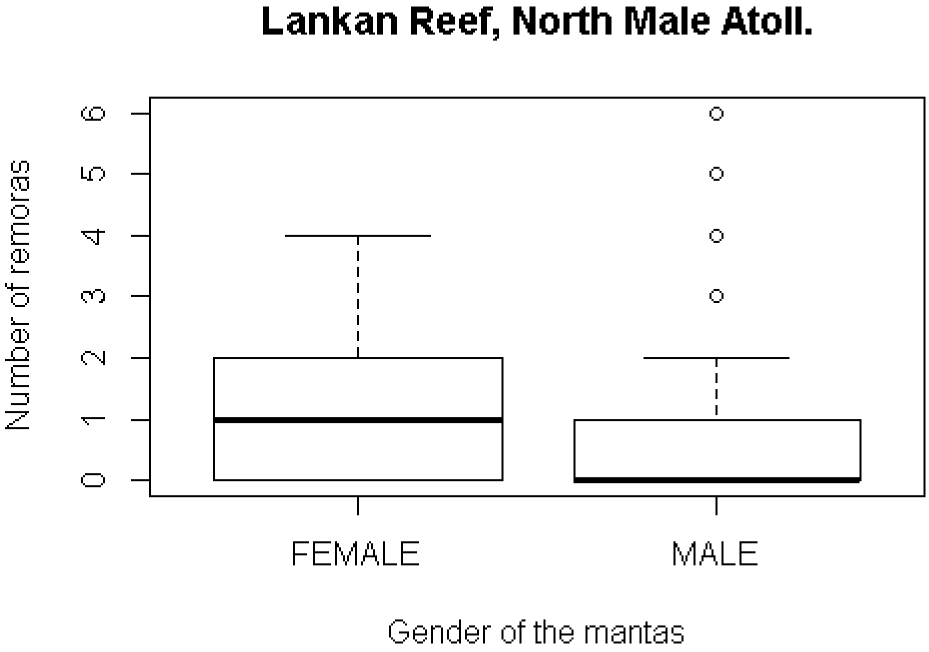


Figure 10 Median number of remoras associated with each size class '3' female and male manta ray that frequently (>20 times) visit Lankan Beyru reef. Male n=320, female n=63. The lower and upper boundaries of the boxplots denote 25th and 75th percentiles respectively, and the bars denote 95 percentiles.

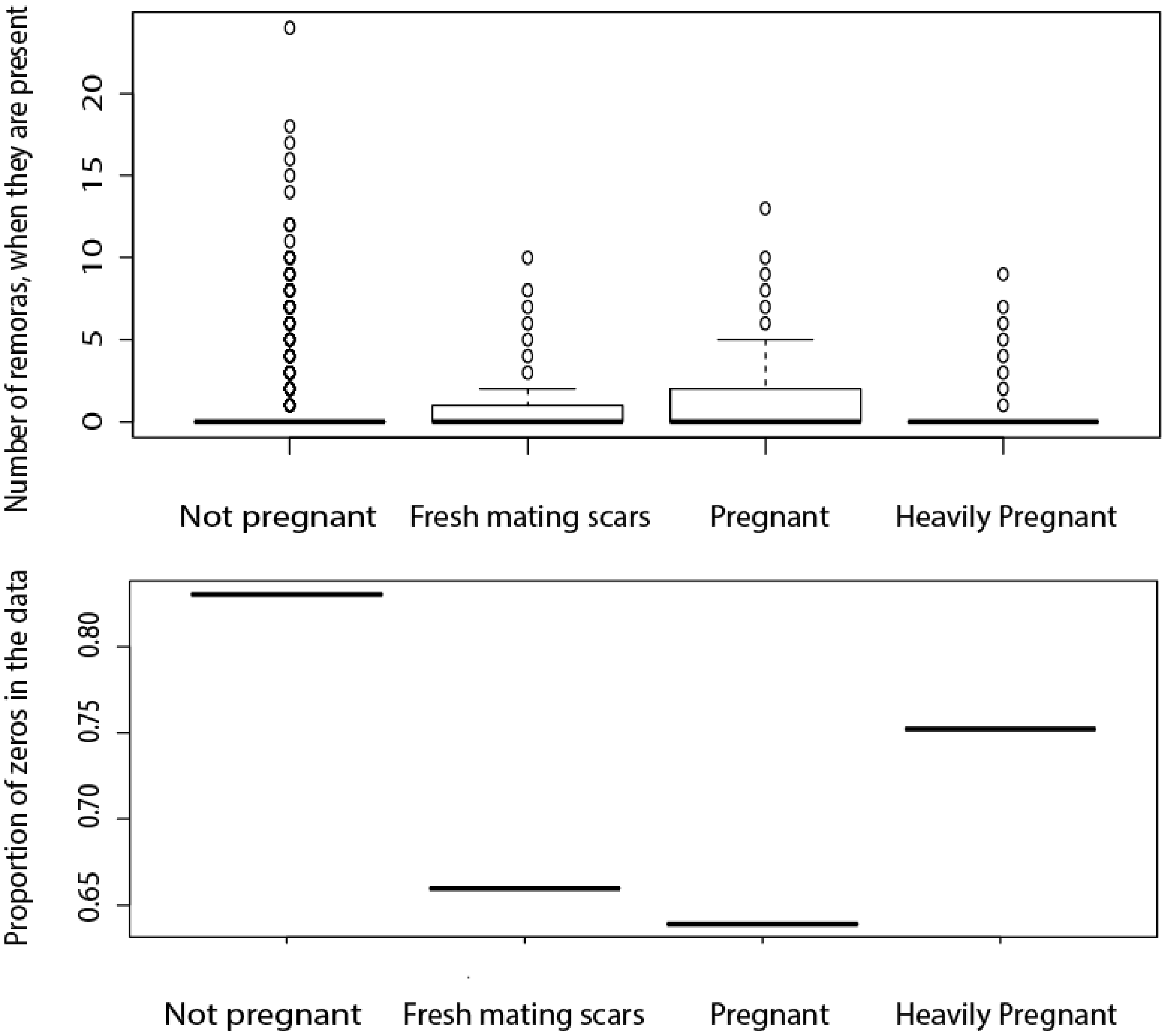
3.2.3 Pregnancy

The influence of four categories of “pregnancy”: not pregnant, fresh mating scars, pregnant and heavily pregnant, on the numbers of remoras were investigated. Average number of associated remoras per manta ray sighting differed between the four categories. The highest number of remoras was observed among pregnant females. Females with fresh mating scars appeared to have a higher number of remoras associated with them when compared to non-pregnant females. Heavily pregnant females appeared to carry fewer remoras than pregnant females.

However the sample sizes for the four categories were very different (Figure 11).

To statistically examine whether pregnant females carry more remoras than non-pregnant females, a subset of the data with only mature female manta rates (size class 4) was created, since only females of this size class can get pregnant. ‘Heavily pregnant’ and ‘pregnant’ females were grouped together into one category and compared with the non-pregnant category. The 'fresh mating scars' category was discarded, since it could not be determined whether the mantas were or were not pregnant.

The results showed that number of remoras was significantly higher on pregnant females compared to non-pregnant mature females (Exact rate ratio test, $p < 0.001$, 95% CI [2.22, 2.56])



Total number of sightings in each category			
n=10790	n=141	n=831	n=109

Figure 11 Variation in the number of remoras associated with size class 4 female manta rays with different pregnancy status. The box plots display the median numbers of the remoras when they are present (ignoring the absence data), the middle figure shows the proportion of zeros in the data and the table underneath displays the number of sightings.

3.2.4 Size class

The influence of four categories of "size class"; 1, 2, 3 and 4, on the numbers of remoras were investigated. In an attempt to minimize the effect of confounding variables, a subset of non-pregnant females and males from a specific location was created and each size class was investigated for the number of remoras. Analysis revealed a pattern in which the number of the remoras increased along with an increase in size of the mantas. Both females and males that were at cleaning stations displayed this pattern. Males however do not attain size class 4 and no juveniles from size class 1 were observed at a cleaning station considered. This pattern was not observed at the main feeding stations (not plotted here).

Figure 12 Median number of remoras associated with each size class of males seen at t Sunlight faru reef. The lower and upper boundaries of the boxplots denote 25th and 75th percentiles respectively and the bars denote 95 percentiles

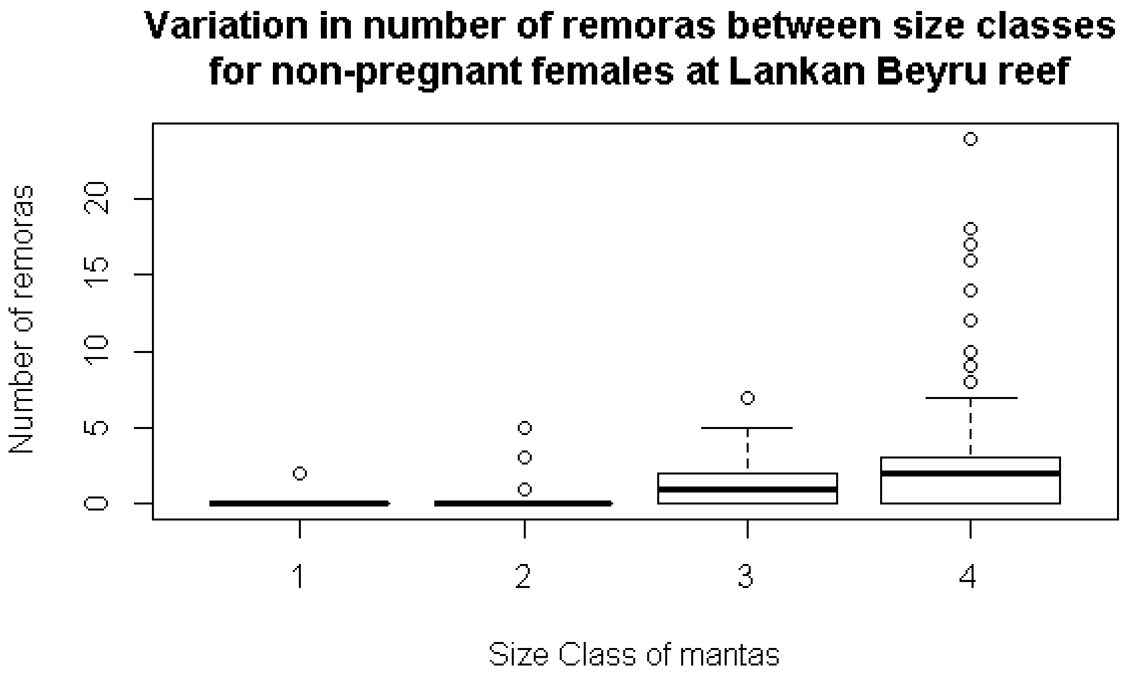


Figure 13 Median number of remoras associated with each size class of non-pregnant females seen at Lankan Beyru reef. The lower and upper boundaries of the boxplots denote 25th and 75th percentiles respectively and the bars denote 95 percentiles

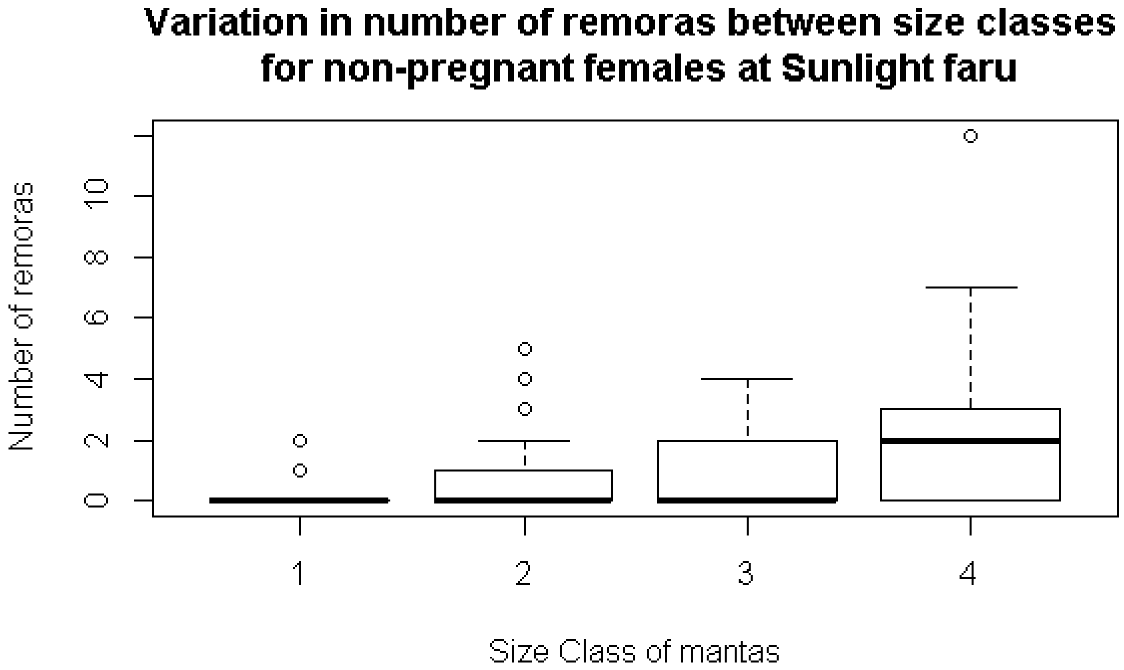


Figure 14 Median number of remoras associated with each size class of non-pregnant females seen at Sunlight faru reef. The lower and upper boundaries of the boxplots denote 25th and 75th percentiles respectively and the bars denote 95 percentiles

3.2.5 Location

The top ten atolls (those with highest number of recorded manta sightings) have been compared to see the variation in ratios of the remoras to mantas from different locations. Mantas seen in North – Male and Ari atolls had the highest ratio of remoras to mantas, much higher than the remaining atolls (Figure 15).

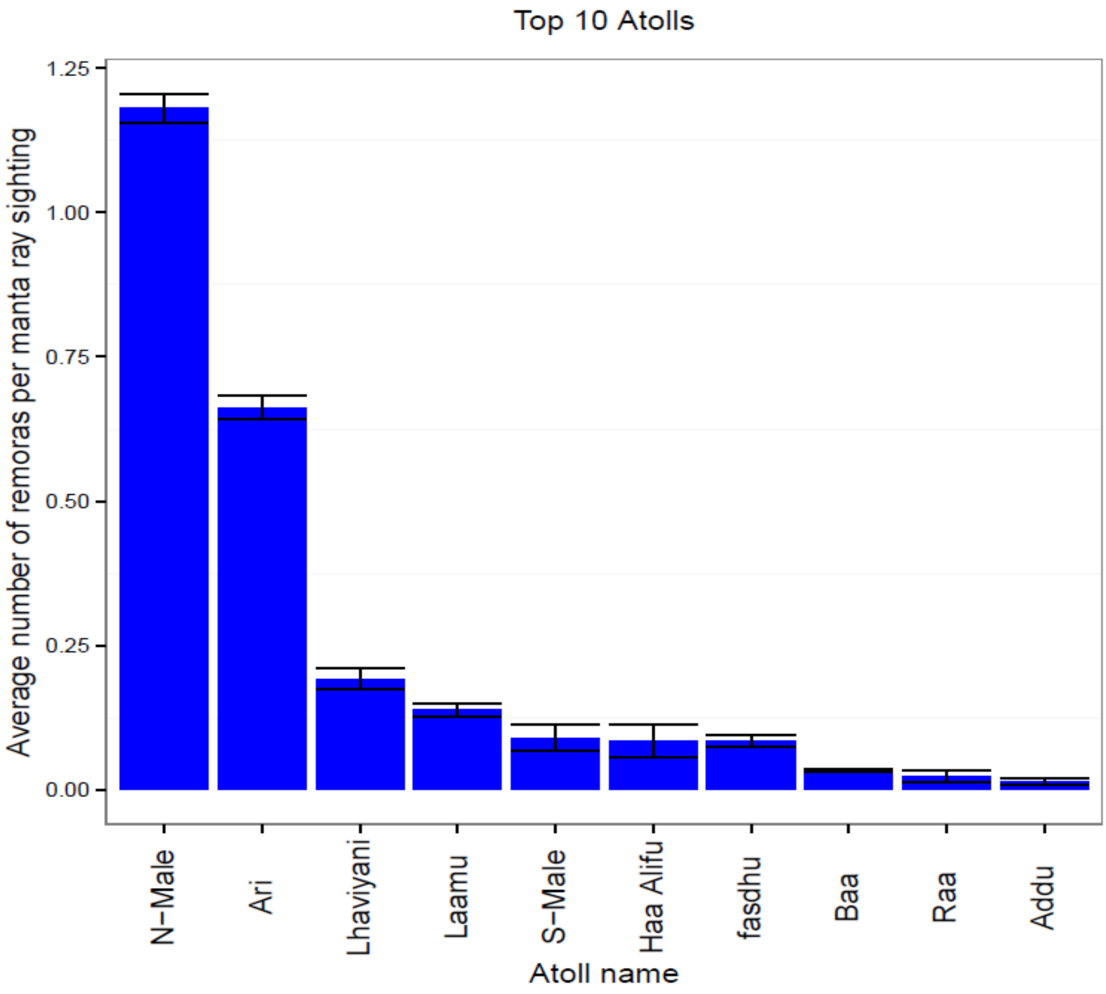


Figure 15 Average number of associated remoras per manta ray sighting from ten most frequently sampled atolls (averages from the entire >10 year sampling period). Error bars show standard error.

The ratios of remoras to mantas from top cleaning stations (those with the highest number of manta sightings, where the mantas were engaged primarily in cleaning) are shown in figure 16. Mantas seen at the Lankan Beyru cleaning station had the highest ratio of remoras to mantas, followed by Sunlight faru and Moofushi Bojamhadi. Even the stations located within the same atoll showed considerable variation in the average number of remoras (Lankan Beuru, Sunlight Fary and Rasafari North are all part of the Ari atoll).

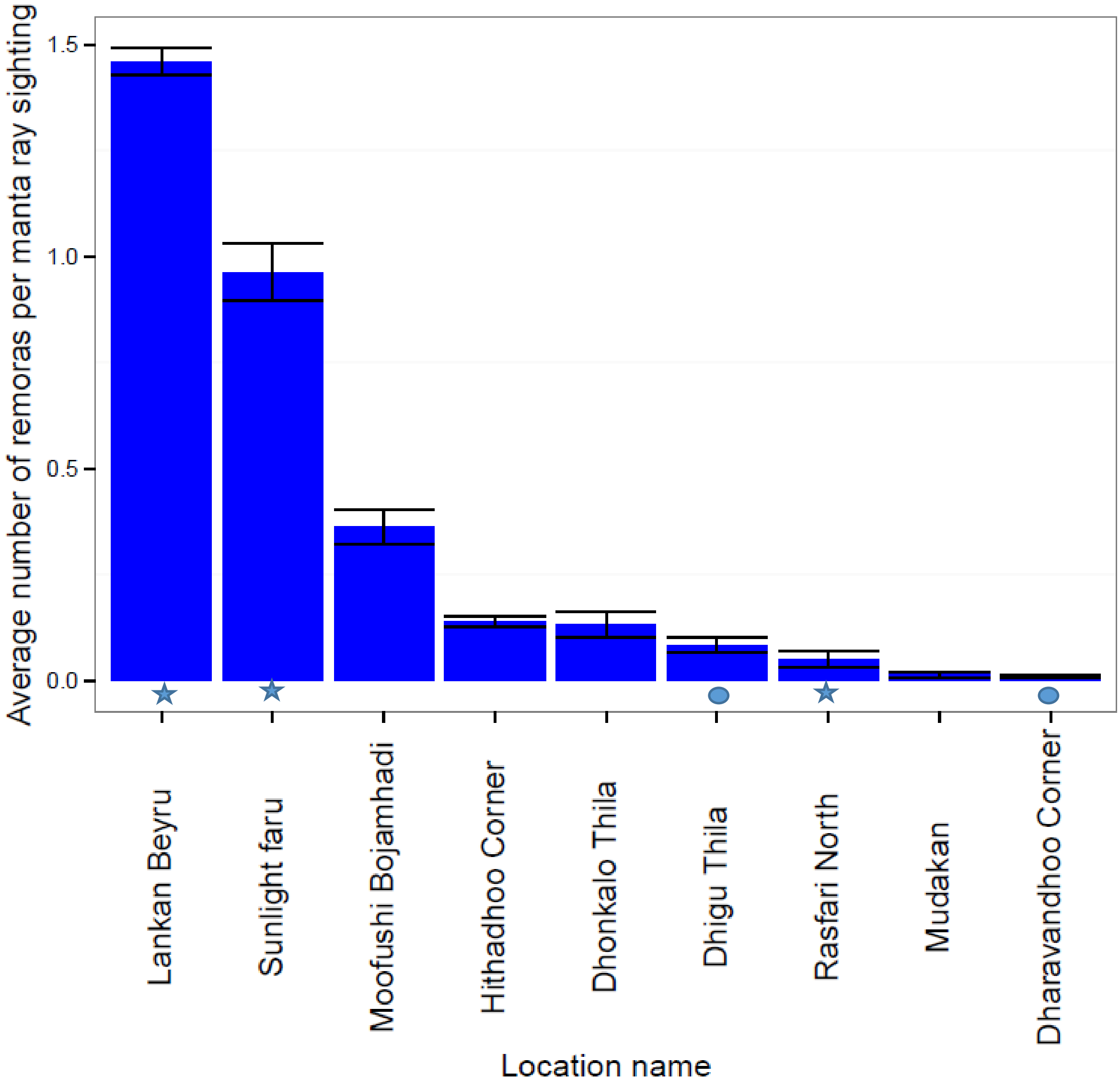


Figure 16 Average number of associated remoras per manta ray sighting from most frequently visited cleaning stations (averages from the entire >10 year sampling period). Stars denote stations located within the North Male atoll while circles denote stations located within the Baa atoll. Error bars show standard error.

The ratios of remoras to mantas at top feeding stations (those locations with the highest number of manta sightings, where the mantas are engaged primarily in feeding) are shown in figure 17. Mantas seen at Fesdu Falhu followed by Dhigurah falhu and Maavaru falhu feeding stations had the highest ratio of remoras to mantas. All of these stations were located within the North Male atoll. Much lower ratios of remoras to mantas were observed at stations located in Baa atoll, with Hanifaru Bay, the most frequently sampled station, showing the lowest average number of remoras per manta ray sighting.

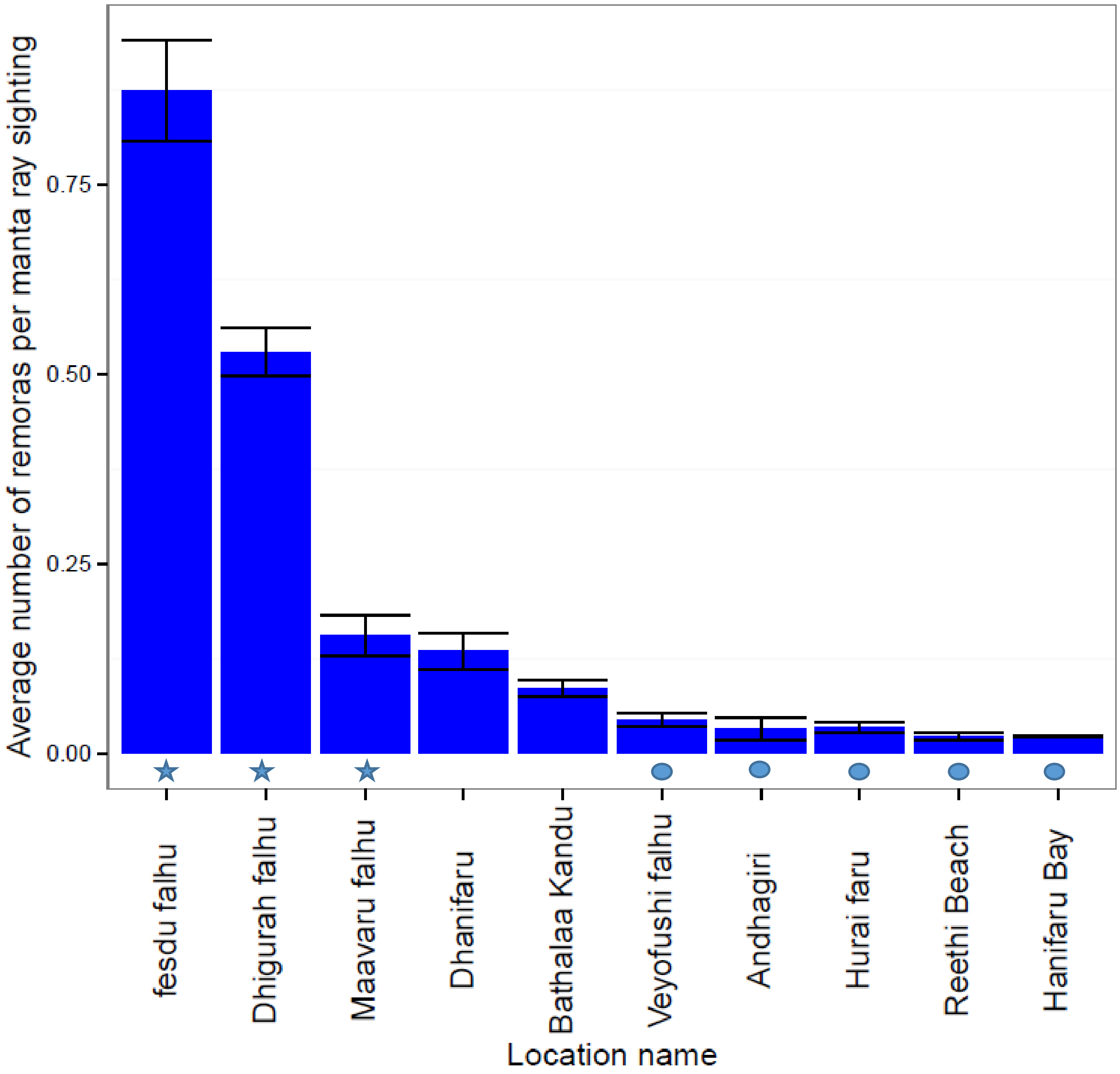


Figure 17 Average number of associated remoras per manta ray sighting from the most frequently visited feeding stations (averages from the entire >10 year sampling period). Stars denote stations located within the North Male atoll while circles denote stations located within the Baa atoll. Error bars show standard error.

3.2.6 Temporal variation

An individual manta ray which was sighted repeatedly in the same atoll and on the same reef over a course of two weeks had been pulled out of the database. The number of the remoras associated with this individual was highly variable and changed from day to day, ranging from two to six remoras (figure 18).

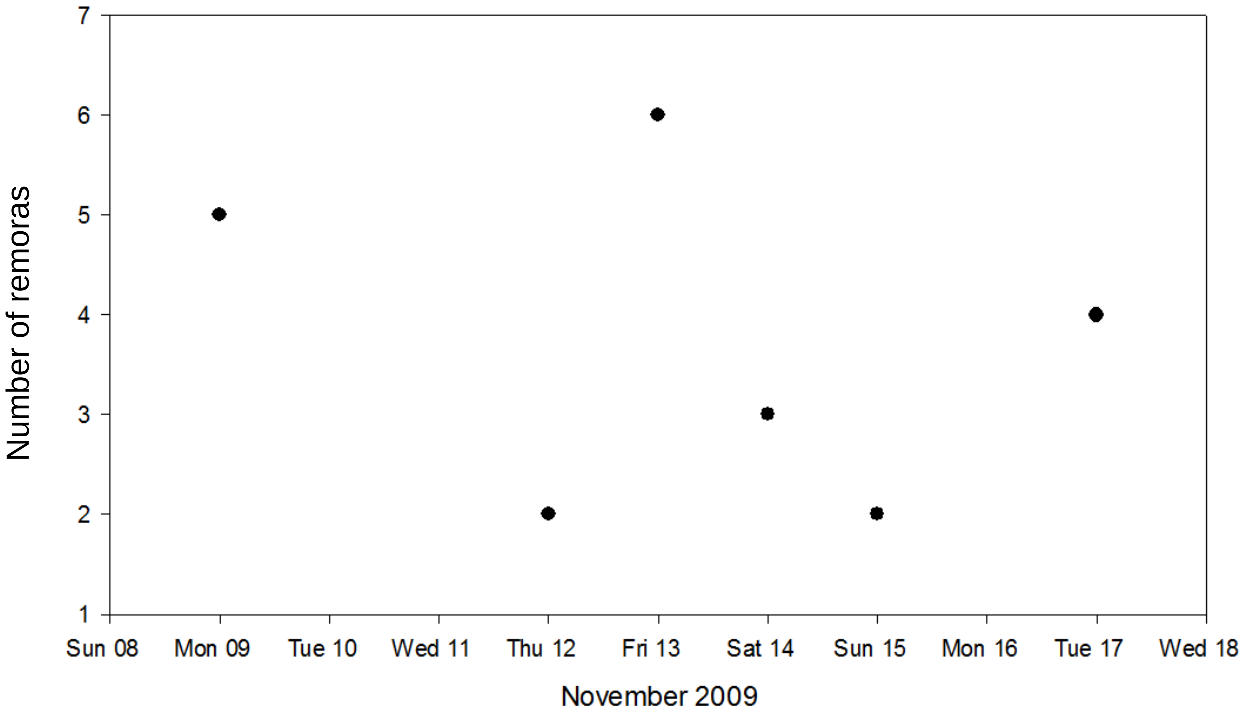
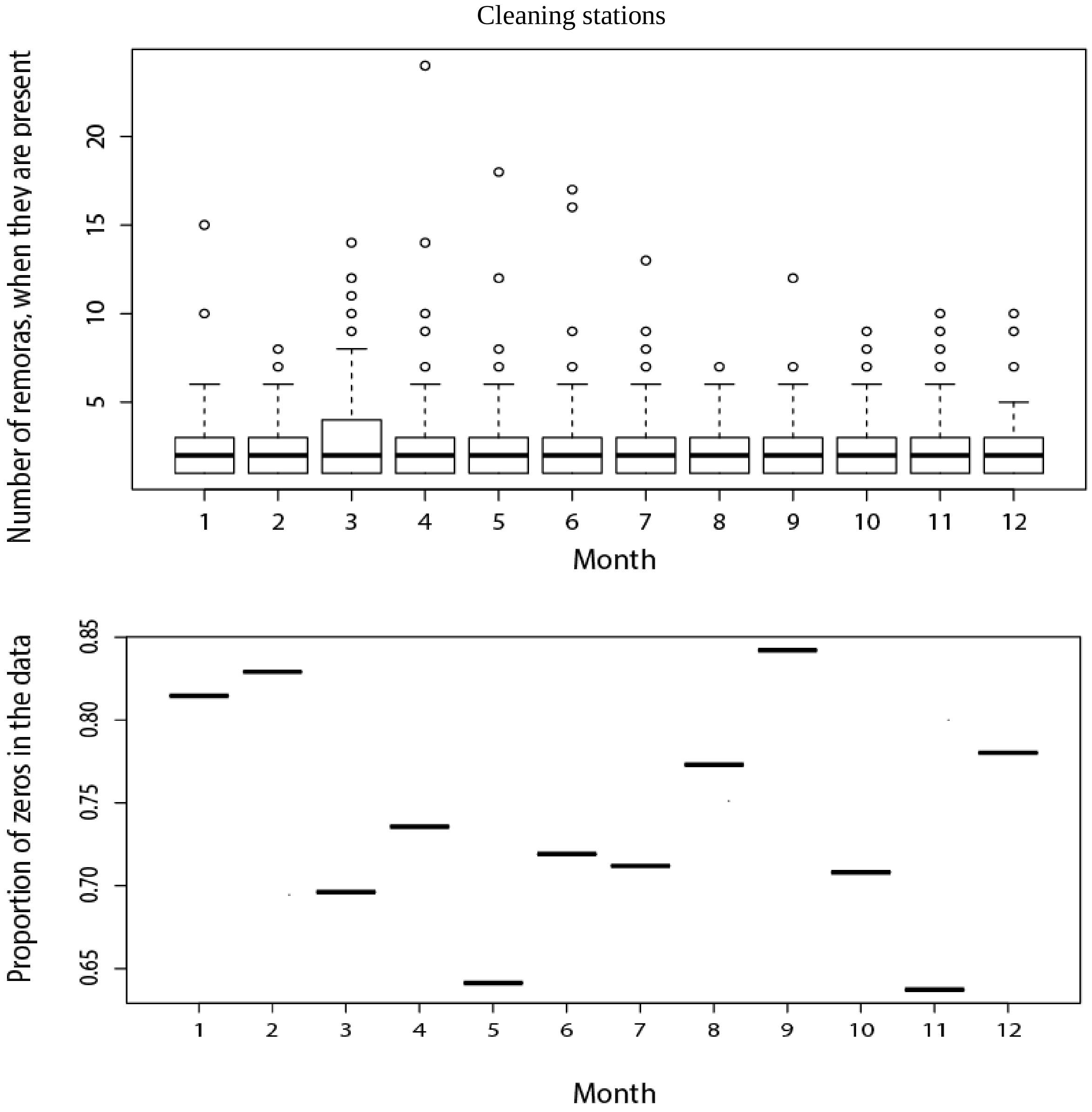


Figure 18 The number of *Echeneis naucrates* remoras associated with a single individual of a reef manta ray, repeatedly sighted at the same reef over the course of several days at Lankan Beyru reef at North Male atoll.

Intra-annual variation

To investigate the impact of seasonality on the average number of associated remoras, cleaning stations and feeding stations have been analysed separately. This has been done to account for potential bias – an artefact of sampling more at the cleaning stations during first years of the Maldivian Manta Ray Project.

Although some apparent variation occurs in both cases, a clearer difference between the dry (December to April) and wet (May to November) seasons occurs in case of the feeding stations (figure 20).



Total number of sightings in each category

n=480	n=690	n=708	n=492	n=396	n=477	n=910	n=798	n=1165	n=1737	n=1845	n=496

Figure 19 Seasonal variation in the number of remoras associated with manta rays present at cleaning stations. The box plots display the variation where the remoras are actually present (ignoring the absence data), the middle figure shows the proportion of zeros in the data, the table underneath displays the number of sightings.

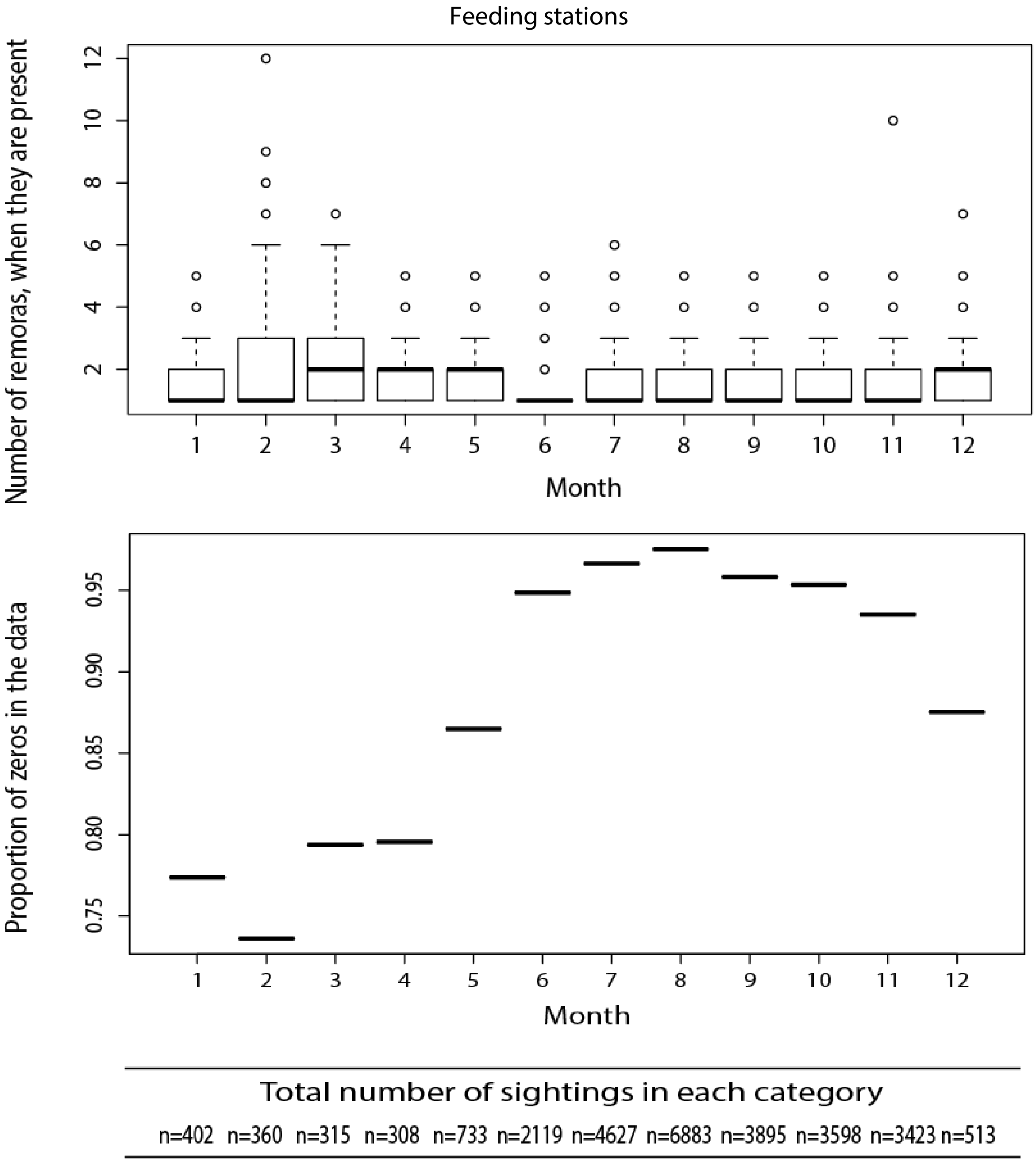


Figure 20 Seasonal variation in the number of remoras associated with manta rays present at feeding stations. The box plots display the variation where the remoras are actually present (ignoring the absence data), the middle figure shows the proportion of zeros

Inter-annual variation

The inter-annual variation in remora numbers has been investigated for the most commonly visited cleaning station, the Lankan Beyru reef. The average number of remoras per manta ray sighting varied between the years but did not show an obvious pattern. The lowest average number of remoras was in 2015 (0.0749 ± 0.0206, for a sample size of n=227 sightings in that year). During the years from 2004 to 2009 the average number of remoras per manta ray sighting was over 1 with a peak in 2008 (2.45 ± 0.110, for a sample size of n=427 sightings in that year).

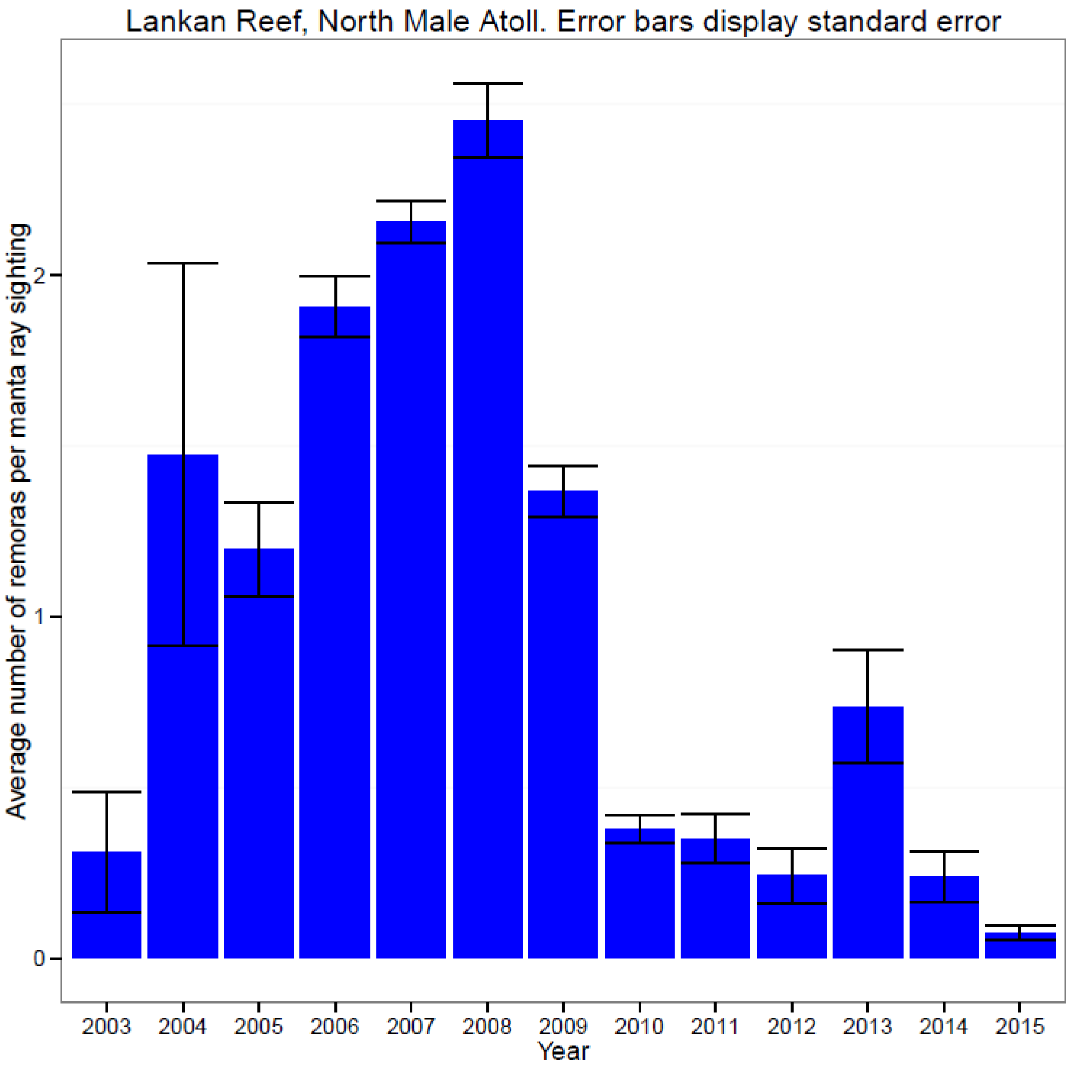


Figure 21 Average number of associated remoras per manta ray sighting from Lankan Reef cleaning station in North Male atoll, for each year from 2003 to 2015. Error bars show standard error.

3.3 Results from the modelling

The influence of the factors such as sex, maturity, behaviour, pregnancy status and location on the number of remoras associated with the mantas was further explored with zero-inflated model. The model output containing negative binomial regression coefficients for each of the variables along with standard errors, z-scores, and p-values for the coefficients is shown in Appendix 1.

All of the predictors in both the count and inflation portions of the model were statistically significant. This model fit the data significantly better than the null model, i.e., the intercept-only model. To show that this is the case, the current model was compared to a null model without predictors using a chi-squared test on the difference of log likelihoods, which was shown to be significant. The Vuong test suggested that the zero-inflated negative binomial model is a significant improvement over a standard negative binomial model. (Vuong Non-Nested Hypothesis Test-Statistic: 11.2197, **p**<0.001)

According to the model, for those mantas that did carry the remoras, manta's sex, maturity, behaviour (presence at cleaning or feeding stations), pregnancy status, as well as location of sighting (Baa atoll or another atoll) are all significant factors in predicting the number of the remoras. According to the model, for those that have the propensity to carry remoras, female manta rays have 82% higher number of remoras than males, assuming that other variables are constant. The model also predicts that the count of remoras on pregnant mantas increases by a factor of 1.28 when compared to non-pregnant mantas, if all other variables are kept constant. Other factors can be interpreted in a similar fashion. The presence of mantas at Baa atoll was a highly significant factor in accounting for the excess zeros in the data (**p**<001).

3.4 Additional photographic evidence.

The following is a choice of photographic evidence that might help further reveal the nature of the echeneid-host association between manta rays and remoras.

Figure 22 A reef manta ray with two remoras which are clearly not directly attached, but simply swim in close proximity to their host.

Figure 22 Remoras associated with mantas are thought to feed of the offal material

Figure 23 Remoras often invade mouths and gill chambers of manta rays, but they are sporadically found even in the cloacal openings of their hosts.

Figure 25 During the MMRP project the remoras have been observed to move between the manta rays, supporting the hypothesis of a loose association between remoras and their hosts.

Figure 26 Remoras have been found to cause injuries and abrasions to the manta's skin where the remora clings on. This is especially common for the oceanic manta ray – true remora association as pictured above.

Figure 27 An entourage of true remoras clinging on to the underside of pectoral fin of an oceanic manta ray, which could have an impact on drag, thus affecting the swimming efficiency of the host. Source: portal youtube.com .Copyright GoPro 2014.

Figure 28 Several true remoras (R. remora) invading the cloacal opening of an oceanic manta ray. Source: portal youtube.com .Copyright GoPro 2014.

4. DISCUSSION

4.1. Limitations of the dataset and the approach taken

There is a considerable difficulty associated with working on uncontrolled, zero-inflated and over dispersed data such as this study was based on. There was a degree of non-independence in the variables chosen to predict the number of remoras that associate with mantas, adding further complications. These factors made it impossible to construct truly unambiguous tests. However, there were strong ecological signals present (for instance the proportion of zeros in the data differed significantly between the locations and with factors such as primary behaviour or pregnancy status of mantas) and the data had an obvious non-random structure. Using logical arguments, prior knowledge and carefully chosen subsets of data, this study aimed to handle the independent variables in such a way as to allow for the best explanation of the observed variation. It is important therefore to stress that the findings of this study reflect possible explanations of the variation in numbers of the remoras, but are not necessarily caused by the actual factors themselves. For instance, the fact that pregnant females carried more remoras than non-pregnant females might not necessarily be related to the pregnancy itself, but the fact that pregnant females utilize different habitat, one that can also be preferable to the remoras. Thus even though the factor 'pregnancy' might explain a lot of the variance, the pregnancy itself is not necessarily causing it.

Different species of remoras associate with different species of mantas

The sharksucker (*E. naucrates)* was the most commonly associated remora seen with the reef manta ray *(M. alfredi)*, while the true remora (*R. remora)* was the most commonly associated fish seen with the oceanic manta ray (*M. birostris)*. This difference in associated species is likely due to different habitat usage by the two species of mantas (Guy Stevens 2016, pers. comm.). While occasional remoras were spotted on the oceanic mantas that ventured into the shallow reefs and likely picked *E. naucrates* there, the true *R. remora* fish have not been seen associated with the reef manta rays. *R. remora* is pelagic and likely associates with the mantas for a prolonged period of time, actually clinging on to its host and following it everywhere. Although in this dataset there were no more than two true remoras recorded on an individual oceanic manta ray, literature reports that one oceanic manta was observed with seven large remoras attached to its body (Coles, 1916). Additional search for manta videos on youtube.com portal confirmed that there can be several remoras attached to oceanic rays (Figures 27 and 28).

The dataset involving oceanic manta rays was not large enough to allow analysis of potential factors contributing to the variance in number of associated remoras. The following discussion pertains mostly to the association between remoras and the reef manta ray.

4.2 Comments on the factors influencing the number of remoras

Primary behaviour

There was sufficient evidence to reject the null hypothesis 1, since reef manta rays that at the time of observation were engaged in cleaning behaviour had significantly more remoras associated with them than those that were engaged in feeding, Cleaning stations consist of specific locations along the reef where individuals solicit host cleaner fish that feed on parasites and other unwanted materials on their skin (Losey Jr, 1972). Since these areas are often shallow water lagoons and hence provide favourable conditions for coral growth, they are also a likely habitat for the remoras. It is possible that the longer the mantas stay on cleaning stations, the more remoras they acquire. Although the highest number of remoras were seen for mantas engaged in courtship, it is possibly due top confounding variables and the fact that courtship often happens at cleaning stations and involves mature animals.

Gender

Females of both the reef manta ray and oceanic manta ray appeared to carry more remoras than did the males, and the statistical tests allowed for rejecting the second null hypothesis. Instead, an alternative hypothesis has been accepted: (H_1: Factor ‘Sex of the manta’ influences the number of remoras that associate with the manta.) Female mantas are known to spend more time on cleaning stations than males, although the reason for this is not yet clear (Manta Trust, 2016). This difference in length of time spent on shallow reefs could explain why there is a difference in remora ratios between the two genders.

Pregnancy

The variance in number of remoras was much higher for female mantas than for males, suggesting that some of the traits associated with being female make it more likely for mantas to acquire remoras. Pregnant manta rays had more remoras associated with them than non-pregnant females of the same size class. Although heavily pregnant females anecdotally have even more remoras, the analysed dataset did not reveal such trend, possibly due to a limited number of sightings of heavily pregnant individuals. It is theoretically possible that the remoras associate with pregnant females in hope for a free meal during the parturition, however it seems unlikely that that would be the main cause of this association (it would be a long time to wait for a single event meal). It seems more plausible that pregnant females exhibit somewhat different behaviour and for instance spend time at specific locations or cease to undertake deep dives. In theory, the longer the time the manta rays spend in shallow reef environments where there are remoras, the higher the chance of picking up the remoras. Perhaps the difference in ratios of the remoras between pregnant and non-pregnant females is due to different habitat utilization of those two groups. For instance, pregnant females could spend more time at shallow waters due to thermal regulation, or might cease to undertake deep dives that could put their offspring at risk. Suitable pupping grounds such as shallow reefs may be more favourable to pregnant females (AD Marshall & Bennett, 2010). There might be an overlap in the conditions favoured by the remoras and pregnant females, which could help explain the differences in remora numbers.

Location

The ratios of remoras to manta rays varied with location, with certain sites exhibiting higher average numbesr of remoras per manta ray sighting than other sites. Null hypothesis 5 was rejected and an alternative hypothesis (H_1: Factor 'location' influences the number of the remoras) was accepted. Atolls like North Male and Ari had particularly high numbers of remoras, while Baa, Raa and Addu atolls had very low ratios. At the remote Addu atoll, right in the very south of the Maldives, there were almost no sightings of remoras associated with mantas. There is a small population of about 100 mantas living within this atoll and yet very rarely are any remoras seen associated with them. The remoras have however been seen in this atoll (Guy Stevens 2016, pers. comm.), so rather than it being due to the absence of the remoras, there ought to be another reason. Future studies could investigate this. Sometimes the number of remoras varied largely between different stations, even within a single atoll (as in case of the Lankan Beuru, Sunlight faru and Rasfari North cleaning stations, all located within the North Male atoll).

Short time scale temporal variation

While the individuals of *E. naucrates* follow the reef manta rays and obviously take advantage of the association, they seem to be rather loosely associated with their hosts. Observations of frequently sighted manta ray individuals over time revealed that this association is of a rather tentative nature, with numbers of remoras changing from day to day. This is consistent with other observations in the field, where the remoras appear to be loosely associated with the mantas. They follow them on most occasions, but often do not attach permanently. In some cases the remoras have even been reported to change hosts. Loose association of remoras has also been mentioned in the literature on the association between sirenians and remoras: ‘Sharksuckers are often only loosely attached with manatees’ (Williams et al., 2003).

Seasonal and inter-annual variation

Although there seemed to be a seasonal pattern, when the variation in the number of remoras was investigated at feeding stations, close examination of the raw data revealed that this was associated with a sampling artefact. Due to the changing weather between the seasons, the Baa atoll has been sampled much more often during the wet (May to November) season. This particular atoll had a very low ratio of remoras to mantas and its extensive sampling largely contributed to the number of excessive zeros in the data. When seasonal influence has been investigated at the Ari atoll, which was sampled more equally throughout the year, the seasonal pattern was no longer apparent. Uneven, seasonal sampling bias is a common issue with fisheries data and quantifying effort is essential to meaningfully compare the data (Pope & Willis, 1996). There was a difference in sampling effort between the two seasons in the Maldives, and the apparent signal corresponding to change between the dry and wet season was not due to the underlying ecological reasons, but purely a change in sampling effort. There was not enough evidence to reject null hypothesis 7.

The ratios of the remoras to manta rays varied between the years. Prior to 2009 the ratios of the remoras recorded at Lankan Reef were mostly higher than in subsequent years.

A brief critique of the model

The zero inflated model used to predict the number of remoras based on factors such as the gender, size, pregnancy status and behaviour of the mantas proved to be of a limited value, mostly due to the observed inter-dependency between the variables. It was useful however in determining where the excess zeros could have come from. To a certain extent it also showed what factors can explain the variance in the number of the remoras, and to what degree. The tabulated results should however be interpreted with caution, since for instance factor ‘pregnancy’ depends on factor ‘sex’ and factor ‘maturity’.

4.3 Further speculations regarding the nature of the association

Unwanted hitchhikers?

The remoras’ benefits of being associated with a manta ray are clear, along with free food and shelter, the remoras are also likely to save on energy expenditure, due to the hydrodynamics around manta’s body. Pilot fish are often associated with manta rays and they are not attached to the elasmobranchs, yet ride the pressure wave created by the larger animals. In such a way they can conserve energy while swimming close to their hosts. Even if the remoras is not directly attached to the manta, it can potentially still conserve energy by finding a hydrodynamically advantageous area and remaining in it. Remoras can cause hydrodynamic drag, presumably hampering the host’s swimming performance. This effect would be exacerbated if the attached fish was large, or occurred in pairs or higher numbers (Sazima & Grossman, 2006). This likely has a larger effect in case of the oceanic manta ray and true remoras association, where the animals remain attached to their hosts for most of the time and the remoras themselves are often large and stubby. Under some scenarios, such as an escape from a shark attack or involvement in courtship, which occurs under faster than average swimming speeds (Yano et al., 1999), this potentially lessened swimming performance could have a crucial impact on the importance.

Parasite pickers or just parasites?

Marshall (2008) reports lack of heavy ectoparasitic loads or gill slit infections among mantas from Mexico, however some individuals had small patches of caligid copepods around their mouths. In Mozambique, the remoras have not been observed cleaning manta rays in any respect (Marshall, 2008). Juvenile mantas from another study did not have visible ectoparasite loads (Marshall & Bennett, 2010). Cressey & Lachner (1970) also report that mantas are rarely seen infected by parasites, but Manta Trust disagrees (Guy Stevens, 2016, pers. comm). Stomach content analysis would be required to confirm whether the remoras on mantas feed on parasites.

Figure 26 shows clearly an irritation on the skin of an oceanic manta, caused by the sucking disk of the remora. Remoras seem to favour particular spots for attachment, likely due to the local hydrodynamics. Repeated attachment to those sites can cause skin abrasions and even wounds that could potentially become infected. Marshall (2008) in her study of mantas in Mozambique also noted that attachment sites for remora fish causes skin irritations on mantas. If the remoras were really bothering the mantas, the hosts would likely try to remove them. Homma et al., (1999) observed that in order to remove the remoras, mantas press their bellies against the rocks. Ritter (2012) observed an interesting behaviour in which a blacktip shark, *Carcharhinus limbatus*, used its pectoral fin in a scoop-like manner to specifically target the removal of a sharksucker, *E.naucrates*, from the flank area.

Potential for increased mating opportunities?

Silva-jr & Sazima (2003) suggested that in case of the spinner dolphin – whalesucker association, attachment fidelity to the same individual could increase the remora’s chance to mate. Associating with hosts allows for increased reproduction potential of the remoras, through finding mates when the hosts aggregate, especially if the hosts are social animals (Silva-jr & Sazima, 2003). Though manta rays are often observed swimming alone, *M. birostris* has also been documented to exhibit schooling behaviour, with as many as 50 manta rays seen swimming together at one time (Bigelow and Schroeder 1953; Homma 1999). It is also possible, that the remoras which are often found associated with the oceanic manta rays in pairs, might actually be reproductive pairs, but that would require further research.

Difference in physiological limits?

It has been suggested that mantas feed on the deep-scattering layer and thus undertake deep dives in search of plankton. Guy Stevens believes that the mantas found in Hanifaru Bay have been feeding on such deep layers (Guy Stevens, 2016, pers.comm). Remoras might have different physiological limits and tolerances to such factors as pressure and temperature, and thus could dissociate from the mantas which undertake deep dives. Speculating even further, it is possible that pregnant females cease to undertake deep dives and instead remain in shallow lagoons, a habitat more suitable for thermoregulation. However since the dataset in this study does not contain any depth data, this hypothesis cannot be tested. Future studies should investigate the depth factor.

Breaching behaviour

A possible mechanism for the removal of the remoras is through breaching, just like it has been proposed in case of sharks and dolphins. Ritter & Brunnschweiler (2003) studying blacktip sharks, *Carcharhinus limbatus*, and *Echeneis naucrates* remoras, reported that irritation caused by remora attachment might induce jumping behaviour. Hester et al., (1963) suggested that aerial manoeuvres executed by spinner dolphins (*Stenella longirostris*) were aiding the removal of remoras. Mantas and mobulas are sometimes seen leaping out of the water. The jumping behaviour of manta rays has been suggested to relate to sexual displays, parturition (giving birth) or the removal of parasites (Clark, 1969). Many popular articles cite breaching of mantas as behaviour that could be linked to the removal of remoras. However during a five-year long study by Marshall, (2008) breaching was rarely seen to occur independent of reproductive or mass feeding activities. While removing parasites or remoras may be a by-product of breaching, the author claims that breaching serves primarily as a form of intraspecific communication, rather than the purposeful removal of remoras.

Further limitations of the study

The results most likely underestimate the actual numbers of remoras associated with manta rays (some individuals could be missed or were temporarily away from their host), it is however very unlikely that the reported values are ever overestimations of the actual values.

The quality of the photos varied due to the angle, visibility and distance from observer to target animal, sometimes making the identification and counting of the remoras challenging. Lack of photos depicting ventral sides of the mantas represent further challenges. In the case of the oceanic manta rays, photos of both ventral and dorsal sides are highly desirable in order to obtain reliable counts that do not underestimate the true vales. In case of the reef manta rays, to which remoras seem to prefer to attach to the ventral side and very rarely are seen on the dorsal side, this seems to be less of a problem.

The dataset for the oceanic manta rays was much smaller and thus any findings based on it should be treated with care and supported by future study on a bigger dataset.

Finally, the remoras were only visually identified, with no direct measurements being taken or genetic samples collected, thus misidentification of the species could have occurred.

4.4 Summary of the major findings and conclusions

- There is a clear structure in the data and strong ecological signals present. This allows for some inferences to be made, but those cannot be demonstrated definitively with the kind of data utilised.
- The following factors seem to influence the presence of the remoras:
    - Different species of remoras associate with the two different species of mantas. Oceanic mantas carry true remoras (*Remora remora*) while reef mantas predominately carry sharksucker remoras (*Echeneis naucrates*).
    - Female manta rays carry more remoras than males
    - Pregnant manta rays carry more remoras than same size, non-pregnant females.
    - The number of remoras associated with reef manta rays can vary from day to day, showing the loose nature of this association.
    - The ratios of remoras to manta rays vary between locations (such as different atolls within the Maldives archipelago), with some atolls having very low ratios (for instance the Baa atoll), while other exhibiting high ratios (*e.g.* North Male atoll).
- The study used retrospective data with a strong degree of interdependency between variables. It is difficult to unambiguously extract answers from this kind of data.
- In an ideal scenario the research question would be proposed prior to collecting the data and thus the data collection could be carried in such a way that would minimize sampling bias.

4.5 Suggestions for future research

This study investigated the general patterns of association between mantas and remoras. In order to better understand the relationship between mantas and remoras, studies investigating the stomach content of the remoras, as in the study by Cressey & Lachner (1970), but focusing on remoras found on the mantas, would be necessary. Close examination of parasites living on the bodies of mantas and their comparison to parasites found in stomach contents of remoras associated with the specific mantas, could help reveal to what degree the remora-manta relationship is beneficial to the hosts. Furthermore, following the example of a study of sharks by Mucientes et al. (2008), the ectoparasitic load between the mantas that carry remoras and those which do not have any associated remoras could be compared.

Future studies could also investigate how long individual remoras remain attached to the same host. Studies of echeneid-host association from Fernando de Noronha revealed that such an association can last for a period of three months, with the longest reliable record of 87 days for an association between the whalesucker *Remora australis* and spinner dolphin *Stenella longirostris* (Silva-jr & Sazima, 2003). The challenge would lie in being able to reliably identify both the hosts and the remoras, so perhaps utilizing some tagging methodology like the one for mark and recapture would be necessary. Using camera equipment that can be attached directly to the mantas themselves, such as the CritterCams, recently deployed on mantas by the Scripps Institute of Oceanography, could be useful in studying the remoras. Observations from such cameras could reveal how long remoras remain attached to their host, and the proportion of time they spend free swimming around their hosts.

Comprehensive genetic studies on the remoras collected from mantas could help reveal whether they are indeed members of a single species, since the remoras seem to show some variation in morphology, especially the colouration.

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6. APPENDIX

Table 3 Output table from the model**.** Call: Zeroinfl (formula = remoras ~ female, maturity, cleaning, pregnant | Not Baa Atoll,, dist = "negbin", link = "logit") Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Theta = 2.5101 Number of iterations in BFGS optimization: 17 Log-likelihood: -1.581e+04 on Df. 9.)

(A) Count model coefficients (negbin with log link):					
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Significance**
(Intercept)	-1.53608	0.08395	-18.299	< 2e-16	***
X4 Female	0.82001	0.03619	22.658	< 2e-16	***
X4 Maturity	0.62554	0.0405	15.444	< 2e-16	***
X4 Not Baa Atoll	0.60179	0.07679	7.837	4.63E-15	***
X4 Cleaning	0.27737	0.04233	6.553	5.64E-11	***
X4 Pregnant	0.24821	0.05526	4.492	7.06E-06	***
Log(theta)	0.92032	0.10366	8.879	< 2e-16	***
(B.) Zero-inflation model coefficients (binomial with logit link):					
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Significance**
(Intercept)	2.82077	0.07042	40.05	<2e-16	***
Not Baa Atoll	-2.76839	0.07527	-36.78	<2e-16	***

Pearson residuals:

Min	1Q	Median	3Q	Max
-0.6202	-0.1609	-0.1288	-0.1212	32.6928

Table 4 Output table from the above model specifying the coefficients and confidence intervals.

	CO	**2.50%**	**97.50%**
count_(Intercept)	0.21522262	0.182572	0.253713
count_X4female	2.27052904	2.115051	2.437436
count_X4maturity	1.86925551	1.726597	2.023701
count_X4notbaa	1.82539161	1.570324	2.12189
count_X4cleaning	1.31966111	1.214598	1.433812
count_X4pregnant	1.28173534	1.150172	1.428348
zero_(Intercept)	16.78970336	14.62511	19.27467
zero_X2	0.06276319	0.054154	0.072741