Important declarations

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Associated Data

Data supplied by the author:
Zooplankton data is available at the UQ eSpace public repository: https://doi.org/10.14264/98ddbde

Required Statements

Reef manta rays forage on tidally driven, high density zooplankton patches in Hanifaru Bay, Maldives

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Manta rays forage for zooplankton in tropical and subtropical marine environments, which are generally nutrient-poor. Feeding often occurs at predictable locations where these large, mobile cartilaginous fishes congregate to exploit ephemeral productivity hotspots. Investigating the zooplankton dynamics that lead to such feeding aggregations remains a key question for understanding their movement ecology. The aim of this study is to investigate the feeding environment at the largest known aggregation for reef manta rays *Mobula alfredi* in the world. We sampled zooplankton throughout the tidal cycle, and recorded *M. alfredi* activity and behaviour, alongside environmental variables at Hanifaru Bay, Maldives. We constructed generalised linear models to investigate possible relationships between zooplankton dynamics, environmental parameters, and how they influenced *M. alfredi* abundance, behaviour, and foraging strategies. Zooplankton biomass changed rapidly throughout the tidal cycle, and *M. alfredi* feeding events were a significant predictor of high zooplankton biomass. *Mobula alfredi* switched from non-feeding to feeding behaviour at a prey density threshold of 53.7 mg m^{-3}; more than double the calculated density estimates needed to theoretically meet their metabolic requirements. The highest numbers of *M. alfredi* observed in Hanifaru Bay corresponded to when they were engaged in feeding behaviour. The community composition of zooplankton was different when *M. alfredi* were feeding (dominated by copepods and crustaceans) compared to when they were present but not feeding (more gelatinous species present than in feeding samples). The dominant zooplankton species recorded was *Undinula vulgaris*, a large-bodied calanoid copepod species which blooms in oceanic waters, suggesting offshore influences at the site. Here, we have characterised aspects of the

feeding environment for *M. alfredi* in Hanifaru Bay and identified some of the conditions that may result in large aggregations of these threatened planktivores, and this information can help inform management of this economically important marine protected area.

Reef manta rays forage on tidally driven, high density zooplankton patches in Hanifaru Bay, Maldives

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Abstract

Manta rays forage for zooplankton in tropical and subtropical marine environments, which are generally nutrient-poor. Feeding often occurs at predictable locations where these large, mobile cartilaginous fishes congregate to exploit ephemeral productivity hotspots. Investigating the zooplankton dynamics that lead to such feeding aggregations remains a key question for understanding their movement ecology. The aim of this study is to investigate the feeding environment at the largest known aggregation for reef manta rays *Mobula alfredi* in the world. We sampled zooplankton throughout the tidal cycle, and recorded *M. alfredi* activity and behaviour, alongside environmental variables at Hanifaru Bay, Maldives. We constructed generalised linear models to investigate possible relationships between zooplankton dynamics, environmental parameters, and how they influenced *M. alfredi* abundance, behaviour, and foraging strategies. Zooplankton biomass changed rapidly throughout the tidal cycle, and *M. alfredi* feeding events were a significant predictor of high zooplankton biomass. *Mobula alfredi* switched from non-feeding to feeding behaviour at a prey density threshold of 53.7 mg m^{-3}; more than double the calculated density estimates needed to theoretically meet their metabolic

40   requirements. The highest numbers of *M. alfredi* observed in Hanifaru Bay corresponded to
41   when they were engaged in feeding behaviour. The community composition of zooplankton was
42   different when *M. alfredi* were feeding (dominated by copepods and crustaceans) compared to
43   when they were present but not feeding (more gelatinous species present than in feeding
44   samples). The dominant zooplankton species recorded was *Undinula vulgaris*, a large-bodied
45   calanoid copepod species which blooms in oceanic waters, suggesting offshore influences at the
46   site. Here, we have characterised aspects of the feeding environment for *M. alfredi* in Hanifaru
47   Bay and identified some of the conditions that may result in large aggregations of these
48   threatened planktivores, and this information can help inform management of this economically
49   important marine protected area.
50
51

Introduction

53   Manta rays are large planktivores that inhabit tropical and subtropical waters globally, which are
54   generally oligotrophic (Marshall et al. 2009). Therefore, to meet their metabolic needs, manta
55   rays need to locate pulses of zooplankton productivity. Similar to other tropical planktivores,
56   such as leatherback turtles *Dermochelys coriacea* (Hays et al. 2006) and whale sharks *Rhincodon*
57   *typus* (Rohner et al. 2015), manta rays aggregate where and when conditions result in elevated
58   local productivity (Dewar et al. 2008, Anderson et al. 2011, Jaine et al. 2012). However, these
59   productivity 'hotspots' are ephemeral in nature and often difficult to locate and characterise
60   (Harris et al. 2020, Harris et al. 2021), which makes the direct study of planktivore feeding
61   ecology challenging.
62   There are a variety of approaches used to study a species' diet, including stomach contents
63   analysis, biochemical analyses, and direct observation. Two studies have recently explored the
64   diet of manta ray species based on stomach contents: one on oceanic manta rays *Mobula*
65   *birostris* taken in a fishery in the Philippines (Rohner et al. 2017), and one on a historic stomach
66   sample from a reef manta ray *M. alfredi* collected from eastern Australia (Bennett et al. 2017).
67   Traditionally, lethal approaches for dietary analysis, such as stomach contents analysis, are
68   inappropriate for vulnerable marine fishes (Cortés 1997), and only offer a 'snapshot' of a
69   species' diet (Rohner et al. 2013). Instead, biochemical approaches, including stable isotope and
70   fatty acid analysis, are non-lethal methods that provide an integrated signal that represents the
71   long-term diet and trophic position of species. Biochemical analysis has inferred that *M. birostris*
72   off Ecuador derive much of their calorific intake by feeding at depth (Burgess et al. 2016), as do
73   *M. alfredi* off eastern Australia (Couturier et al. 2013), and *M. alfredi* in the Seychelles target
74   pelagic zooplankton sources (Peel et al. 2019). Nevertheless, biochemical analyses lack
75   resolution, such as identifying and quantifying preferred prey species, whereas direct observation
76   of animal feeding allows simultaneous sampling of the feeding environment.
77
78   Currently, the only detailed direct observation of the diet in manta rays is from an aggregation
79   site off eastern Australia, where *M. alfredi* was observed feeding near the surface (Armstrong et

80   al. 2016). The study found *M. alfredi* feeding events were significantly associated with greater
81   zooplankton biomass, but were not influenced by zooplankton size or species composition.
82   Further, feeding activity and zooplankton density was tidally driven at this site. Similarly, the
83   occurrence of *M. alfredi* in Komodo National Park in Indonesia was heavily influenced by tide,
84   and was considered likely to be related to feeding activity (Dewar et al. 2008). An *in situ* prey
85   density threshold of 11.2 mg m^{-3} was determined for *M. alfredi* foraging in eastern Australia
86   (Armstrong et al. 2016). However, a theoretical estimate of the density threshold to meet their
87   metabolic requirements (25.2 mg m^{-3}) suggests they require additional energy from alternate
88   food sources, such as foraging at depth (Armstrong et al. 2016).
89

90   Manta rays exhibit behavioural plasticity in relation to their feeding environment. In eastern
91   Australia (Jaine et al. 2012), Indonesia (Dewar et al. 2008), and the Chagos Archipelago (Harris
92   et al. 2021), surface feeding by manta rays is frequently observed during daylight hours, and
93   other large planktivores, such as basking sharks *Cetorhinus maximus* (Sims and Merrett 1997)
94   and *R. typus* (Prebble et al. 2016) also employ this strategy. In the Red Sea, *M. alfredi* swim in
95   various circular patterns when feeding on zooplankton in shallow water (Gadig and Neto 2014).
96   At Ningaloo Reef in Western Australia, *M. alfredi* is frequently observed to use a combination of
97   feeding modes, including surface feeding, somersaulting, and bottom feeding (AOA *pers. obs.*).
98   A unique strategy of "cyclone" feeding has been described at Hanifaru Bay in the Maldives,
99   where multiple individuals manipulate the water column to create a vortex that concentrates
100   zooplankton on which they then feed (Stevens 2016). Eight different feeding strategies have been
101   described at this site, and have been related to prey density using a subjective visual assessment
102   of the water column (Stevens et al. 2018). However, zooplankton density or composition has yet
103   to be quantified in relation to these strategies. Upwards of 250 individual manta rays aggregate in
104   Hanifaru Bay during peak feeding events, making it the largest known *M. alfredi* aggregation site
105   in the world (Harris et al. 2020). This is therefore an ideal location to test hypotheses regarding
106   habitat use, aggregative behaviour, feeding strategies, and zooplankton dynamics for this species.
107

108   Here, we investigate the food environment for *M. alfredi* at Hanifaru Bay. Manta rays are of
109   economic importance to both ecotourism and fisheries industries and have a conservative life
110   history, and so identifying the foraging requirements and habitat preferences of these threatened
111   rays should aid future conservation efforts (Stewart et al. 2018). We analyse the zooplankton
112   dynamics (biomass, size structure, and community composition) in relation to *M. alfredi*
113   presence, behaviour and feeding strategies, to improve our understanding of the feeding
114   dynamics of this large planktivorous species. This study aims to relate changes in zooplankton
115   biomass to *M. alfredi* behaviour; to establish a critical prey density threshold for feeding at this
116   site, and to determine whether prey density influences the type of feeding strategy *M. alfredi*
117   employ to exploit their prey. Further, we aim to investigate whether *M. alfredi* foraging
118   behaviour is influenced by changes in the zooplankton community composition, or size structure.
119

120

Materials & Methods

Study Site

The Maldives has a large resident population of *M. alfredi* which undertake biannual migrations linked to the changing monsoons (seasons) within the archipelago (Anderson et al. 2011; Fig 1A). During the Southwest Monsoon, or *Hulhangu* (April – November), *M. alfredi* frequent foraging aggregation sites on the eastern side of the nation's atolls (Harris et al. 2020). One site, Hanifaru Bay, is situated on the eastern edge of Baa Atoll, and attracts large feeding aggregations of this species annually (Stevens 2016, Harris et al. 2020). Hanifaru Bay is a small reef inlet (700 m long by 200 m wide) which forms part of a core marine protected area within the Baa Atoll UNESCO Biosphere Reserve (5°17'N, 73°15'E; Fig 1B). The shallow (maximum depth 22 m) inlet is periodically inundated with zooplankton-rich water. Motorised boat activity and SCUBA diving are prohibited in Hanifaru Bay due to the high numbers of manta rays and other megafauna that access the inlet (Murray et al. 2020).

Data collection

Fieldwork was conducted in the lead up to the new moon in August 2017, under Ministry of Fisheries Permit No. (OTHR)30-D/PRIV/2017/280, and Ministry of Environment Permit No's. EPA/2017/RP-01 & EPA/2016/PSR-M02. At this time of year, lunar and monsoonal currents combine to transport zooplankton from outside the atoll edge into Hanifaru Bay (Stevens 2016). The currents form a back eddy, trapping and concentrating plankton in this shallow reef inlet, resulting in *M. alfredi* foraging opportunities which peak during spring and high tides (Stevens 2016). Sampling was conducted during daylight hours and across the tidal cycle from 13 – 21 August 2017. Zooplankton was collected by two people using a 200 µm-mesh net towed by hand at the surface for a ~50 m transect between two coral features at the eastern end of Hanifaru Bay (Fig 2A). A flowmeter was fitted to the plankton net to allow calculation of the volume of water sampled. Flowmeter calibration was performed prior to the field trip in a swimming pool of known length to establish an accurate measurement of distance per flowmeter revolution. Samples were kept on ice and fixed with 10% buffered formalin solution at the end of each day.

Each net tow was accompanied by an in-water observer recording manta ray activity in vicinity of the tow. This included: (1) manta ray abundance; (2) behaviour (Feeding, Non-feeding – when manta rays were present but not feeding, and Absent – when manta rays were not present); and (3) most common feeding strategy employed (as described in Stevens 2016; Fig 2B).

Temperature and salinity data were collected at 1 s intervals from a CTD unit deployed at the site for the study duration (except for ~24 hrs from 17 – 18 August for battery exchange). Temperature and salinity ranges were relatively small throughout the study (28.6 – 29.2°C and 34.3 – 35.0 ppt respectively). These variables were excluded from the models as their inclusion resulted in missing values. Tide data were obtained from a local government representative.

160

161 *Sample processing*

162 Zooplankton samples were processed in the CSIRO Plankton Ecology Laboratory in Brisbane,

163 Australia. Flowmeter readings and the area of the net mouth were used to estimate the volume of

164 water filtered.

165

166 *Zooplankton biomass*

167 Zooplankton samples were split into two halves using a Folsom splitter ([Harris et al. 2000](#)). The

168 first half was used to determine dry mass, with each sample oven-dried at 70°C for 24 hrs prior

169 to weighing. Zooplankton dry mass (hereafter referred to as biomass) per unit volume of filter-

170 seawater for each tow was calculated by dividing the dry mass of the sample (mg) by the volume

171 of filtered water (m^3):

172

173 *Biomass (mg m^{-3}) = Dry mass (mg) / Volume of water filtered (m^3)*

174

175 *Zooplankton identification*

176 The second half of the sample was used to examine size structure and community composition

177 via a 2400 dpi ZooScan system and microscopy. The Hydroptic v3 ZooScan (EPSON Perfection

178 V700 Flatbed) is a high resolution, waterproof scanner that digitises particles for size and

179 biovolume measurements ([Gorsky et al. 2010](#)). An aliquot of each sample was prepared using a

180 Stemple pipette of known volume and placed on the scanning tray. To avoid overlap, particles

181 were manually separated using a cactus spine. Once separated, the sample was scanned and

182 particles were extracted into vignettes for categorisation into broad taxonomic groups (24

183 groups) using Plankton ID software (Version 1.2.6) and manual validation ([Gorsky et al. 2010](#)).

184 Objects classified as sand, fibre, detritus, bubbles and shadows were excluded from further

185 analysis (as per [Rohner et al. 2015](#)). For visualisation, taxa that comprised <5% of the total

186 abundance were grouped as "other", and these included cnidaria, polychaetes, echinoderm

187 larvae, bryozoan larvae, fish larvae, salps, and various classes of arthropods.

188

189 To investigate which species were responsible for the majority of the biomass at the site when

190 overall biomass values in the water were high, samples were analysed taxonomically via

191 microscopy. A subsample was prepared using a Stempel pipette, and organisms were identified

192 and counted in a Bogorov tray using a microscope. Dominant members were identified to genus

193 or species with assistance from trained plankton taxonomists at the CSIRO Plankton Ecology

194 Laboratory ([Eriksen et al. 2019](#)).

195

196 *Zooplankton size structure*

197 A size distribution of the sample particles, known as a Normalised Biomass Size Spectra, was

198 produced to analyse the size structure of the zooplankton community ([Vandromme et al. 2012](#)).

199 Spherical biovolume was calculated from the size measurements obtained from ZooScan. Each

200   particle was assigned to one of 50 logarithmic size categories based on its spherical biovolume.
201   The sum of the spherical biovolume of the particles in each size class (mm^3) was standardised by
202   the fraction of sample scanned and the volume of water filtered (m^3), and normalised by dividing
203   this value by the width of the size class measured in biovolume (mm^3). Both axes of the
204   Normalised Biomass Size Spectra use a logarithmic scale.
205

206   *Drivers of zooplankton biomass and manta numbers*
207   To investigate potential drivers of zooplankton abundance and *M. alfredi* visits to Hanifaru Bay,
208   we constructed generalised linear models (GLMs) using R (R Core Team 2019). Separate
209   analyses were conducted for two response variables: (i) Zooplankton biomass (mg m^{-3}), with a
210   Gamma error structure and log-link function; and (ii) Manta ray abundance (number of *M.*
211   *alfredi* observed during zooplankton sampling), with a negative binomial error structure and log-
212   link function (Poisson error structure was overdispersed). We visually inspected diagnostic plots
213   to assess assumptions of homogeneity of variance and normality. Predictors in both models were
214   Tide (hours from high tide) and Behaviour (Feeding, Non-feeding and Absent). To account for
215   the circular nature of Tide (~12-hr cycle), the variable was transformed using a truncated Fourier
216   series (a harmonic function of sines and cosines). This ensures that the cyclical nature of this
217   predictor is captured, while guaranteeing that the response values predicted at the extremes of the
218   predictor range are the same (i.e., the same prediction for Zooplankton biomass or Count at times
219   of 0 and 24 hours). For the Manta ray abundance model, the Behaviour predictor was reduced to
220   two categories – namely Feeding and Non-feeding, and Zooplankton biomass (mg m^{-3}) was
221   included as a predictor. Models were plotted on the response scale using the package "visreg" in
222   R (Breheny and Burchett 2017).
223

224   *Critical thresholds for feeding behaviour and strategy*
225   We assessed whether there might be a critical threshold for *M. alfredi* feeding – i.e., a level
226   above which the likelihood of feeding increases dramatically. We thus used a GLM with a
227   binomial error structure to analyse manta ray behavioural response (Non-Feeding = 0, Feeding =
228   1) in relation to zooplankton biomass (mg m^{-3}) as a predictor. The critical density threshold was
229   taken as the zooplankton biomass at which the proportion of feeding was 0.5.
230

231   Feeding samples were categorised into either Solo feeding (Straight, Surface and Somersault) or
232   Group feeding (Piggy-back and Chain) based on the most common strategy observed in the
233   manta rays (Stevens 2016). A GLM with a binomial error structure was used to analyse manta
234   ray feeding strategy response (Solo = 0, Group = 1) in relation to zooplankton biomass (mg m^{-3})
235   as a predictor. The critical density threshold was taken as the zooplankton biomass at which the
236   proportion of Group feeding was 0.5.
237

238   *Zooplankton community analysis*

239   To determine how different the zooplankton communities were for the *M. alfredi* behaviours
240   (Feeding or Non-Feeding), non-metric multidimensional scaling was used based on abundance
241   counts of the different taxonomic groups from the Zooscan analysis. The Bray Curtis distance
242   measure was used because it is unaffected by joint absences of taxonomic groups in samples. To
243   account for abundance of certain taxa, data were transformed using a root transformation. To test
244   for differences in community composition between *M. alfredi* behaviours (Feeding and Non-
245   Feeding), we performed an adonis analysis, a multivariate analysis of variance. Both the adonis
246   and non-metric multidimensional scaling were conducted using the "vegan" package in R
247   (Oksanen et al. 2007).

248

249

250   **Results**
251   A total of 77 zooplankton samples were collected (Feeding = 33, Non-feeding = 22, and Absent
252   = 22) over a period of nine days. Zooplankton biomass ranged between 0.7- and 643.1 mg m^{-3}
253   (mean = 90.7, SD = 130.9).

254

255   GLM analyses showed that Zooplankton biomass in Hanifaru Bay was significantly related to
256   Tide and Behaviour (Fig 3). Zooplankton biomass was greatest just following high tide (t = -
257   3.83, p = 0.0003, Fig 3A), and *M. alfredi* were more commonly observed feeding when
258   zooplankton biomass was higher (t = -2.83, p = 0.006, Fig 3B).

259

260   Manta ray behaviour was significantly related to zooplankton biomass (z = 3.08, p = 0.002), with
261   a prey density threshold of 53.7 mg m^{-3} calculated for feeding *M. alfredi* (Fig 4).

262

263   Manta ray abundance was significantly predicted by Behaviour (z = -5.55, p = 0.000000003; Fig
264   5), with more *M. alfredi* present when they were feeding in Hanifaru Bay. Tide and Biomass did
265   not predict manta ray abundance.

266

267   There was no significant difference in zooplankton biomass among different feeding strategies
268   during the study (ANOVA: F = 1.02, df = 4,28, p = 0.41). In addition, there was no significant
269   difference in zooplankton biomass, when samples were pooled into Solo feeding strategies and
270   Group feeding strategies (z = 0.98, p = 0.33). However, only groups were observed feeding when
271   biomass concentrations exceeded 200 mg m^{-3}.

272

273   *Zooplankton community composition*
274   There were differences in the zooplankton community composition between Feeding and Non-
275   feeding samples when analysed using non-metric multidimensional scaling on the Zooscan
276   taxonomic counts (Fig 6A). The 95% confidence ellipses for Feeding and Non-feeding were not
277   overlapping, implying that they were significantly different zooplankton community
278   compositions, and this was confirmed by the adonis analysis (F = 9.42, df = 1,53, p = 0.001).

279 Crustaceans (such as copepods) were more associated with Feeding samples, compared to
280 gelatinous taxa (such as chaetognaths and eggs), which were more associated with Non-feeding
281 samples.
282
283 Calanoid copepods comprised 66.3% of Feeding samples compared to 46.7% of Non-feeding
284 samples (Fig 6C and 6D respectively). Chaetognaths were 5.3% of Feeding samples, and 11.9%
285 of Non-feeding samples. Fish eggs were less than 2% of Feeding samples, and 13.0% of Non-
286 feeding samples. Based on microscopy, juvenile and adult *Undinula vulgaris* were the dominant
287 calanoid copepod species in both Feeding and Non-feeding samples (25.0% and 30.7%
288 respectively, Fig 6B).
289
290 *Zooplankton size structure*
291 Analysis of the size structure of zooplankton from Hanifaru Bay revealed that the biovolume of
292 zooplankton increased in the majority of size categories when *M. alfredi* were feeding (Fig 7).
293 The biovolume of zooplankton was significantly higher across particle size categories during *M.*
294 *alfredi* Feeding events than Non-feeding events (Mean total standardised biovolume: Feeding =
295 288.4 and Non-feeding = 172.1; t = -2.66, df = 51.38, p = 0.01). Feeding and Non-feeding
296 samples had similar biovolumes of small and large particles, but Feeding had significantly more
297 moderate-sized particles (from $10^{-1.2}$ to $10^{0.5}$ mm^3).
298
299
300 **Discussion**
301 *Summary*
302 Zooplankton concentrations influence the number of *M. alfredi* present and their observed
303 behaviour in Hanifaru Bay. Rapid changes in zooplankton are observed across the tidal cycle,
304 and *M. alfredi* feed when biomass reaches a critical density which is higher than predicted to
305 meet their theoretical metabolic requirements. *Mobula alfredi* forage when the zooplankton
306 community is dominated by calanoid copepods, and are less likely to feed when there are greater
307 numbers of gelatinous taxa (such as chaetognaths or eggs). Taxonomic analysis reveals large-
308 bodied copepods, *Undinula vulgaris*, dominate the zooplankton environment at Hanifaru Bay,
309 suggesting oceanic incursions may play an important role in bringing zooplankton to this small
310 reef inlet.
311
312 *Tidal influence on zooplankton density and manta ray foraging*
313 Manta rays feed when zooplankton biomass is high, which is typically observed on the high to
314 ebbing tide at Hanifaru Bay. Oceanographic investigations in Hanifaru Bay suggest tidal currents
315 draw zooplankton into the shallow reef systems of the atoll, where they become trapped inside
316 due to a back-eddy mechanism created by the unique shape of the reef system and the
317 combination of the lunar and monsoon currents (Hosegood *pers comms*). Tides are known to
318 influence the distribution and abundance of zooplankton around island inlets in the Great Barrier

319   Reef (Alldredge and Hamner 1980), and have been shown to influence manta ray feeding
320   behaviour at aggregation sites in Indonesia (Dewar et al. 2008), eastern Australia (Armstrong et
321   al. 2016), and the Chagos Archipelago (Harris et al. 2021). Therefore, short-term *in situ*
322   observations of zooplankton concentrations in relation to tidal cycles and manta ray behaviour
323   can help inform when *M. alfredi* are likely to be observed in Hanifaru Bay.
324
325   *Animal movements and productivity hotspots*
326   Large planktivores seeking to exploit ephemeral food sources in surface waters are likely to
327   respond to currents and water movements that concentrate zooplankton. *Cetorhinus maximus*
328   forages along thermal fronts (Sims and Quayle 1998), *R. typus* targets regions of upwelling
329   (Ryan et al. 2017), and surface foraging in *M. alfredi* is often tidally driven (Dewar et al. 2008,
330   Armstrong et al. 2016). In conjunction with responding to physical oceanographic cues, animals
331   that seek patchily distributed prey sources are also likely to congregate in areas where they have
332   previously encountered energetically rewarding prey abundances, resulting in larger numbers of
333   animals in reliable foraging regions. For example, *M. alfredi* predictably switch to the down-
334   current side of the atolls in the Maldives in response to monsoonal winds and primary
335   productivity (Harris et al. 2020). Area-restricted search theory predicts that animals will remain
336   localised in areas where they have a higher probability of encountering prey (Bailey et al. 2019),
337   and this perhaps explains why some *M. alfredi* individuals remain in Hanifaru Bay when not
338   feeding. This location also has two cleaning stations used by *M. alfredi* (Stevens 2016), and it is
339   hypothesised manta rays will frequent cleaning stations in close proximity to foraging
340   opportunities (Armstrong et al. 2021). Manta rays in Palmyra Atoll used area-restricted searching
341   when adjacent to ledges or channels with high plankton concentrations, but their movements
342   were more random at larger spatial scales (Papastamatiou et al. 2012). Area-restricted searching
343   has also been observed in two dolphin species (*Tursiops truncatus* and *Delphinus delphis*) in
344   areas of high prey availability, and where they have had previous successful foraging experience,
345   suggesting memory plays a role in their movement ecology (Bailey et al. 2019). For *M. alfredi*,
346   their apparent preference for returning to the same cleaning stations over time (Armstrong et al.
347   2021), suggests they may form a cognitive map of shallow reef environments, and this is likely
348   the case for known productivity hotspots as well.
349
350   *High critical feeding threshold for manta rays at Hanifaru Bay*
351   The critical prey density threshold for *M. alfredi* feeding in Hanifaru Bay (53.7 mg m^{-3}) is more
352   than four times higher than that in east Australia where *M. alfredi* feeds (11.2 mg m^{-3}; Armstrong
353   et al. 2016), and in east Africa where *R. typus* feeds (12.4 mg m-3; Rohner et al. 2015). It is also
354   double the theoretical prey density threshold calculated to meet the metabolic requirements for
355   *M. alfredi* (25.2 mg m^{-3}; Armstrong et al. 2016), which may explain why this site hosts such a
356   large feeding aggregation of this species. However, these large planktivorous elasmobranchs are
357   assumed to feed in the mesopelagic layer (Couturier et al. 2013, Burgess et al. 2016), so an
358   understanding of the prey densities available at these depths is required to gauge the relative

359   importance of aggregations sites such as Hanifaru Bay in meeting these species' energetic
360   requirements. Sampling zooplankton at depth remains a logistical challenge for researchers, but
361   with technological advances, such as satellite tags equipped with accelerometer data loggers, and
362   unmanned video submersibles (Stewart et al. 2018), these inferences can be better investigated.
363

364   *Manta ray feeding strategies*
365   In the current study, plasticity in *M. alfredi* feeding strategies in response to changes in prey
366   biomass in Hanifaru Bay is not supported. This contrasts with work previously conducted in
367   Hanifaru Bay that found manta rays were significantly more likely to employ group feeding
368   strategies as prey density increased (Stevens 2016). The previous work was based on a
369   qualitative visual index for prey density, with data obtained over a long time period (> 5 years)
370   and included aggregations upwards of 150 animals. However, we did see that when zooplankton
371   biomass values were very high, over 200 mg m^{-3}, that only Group feeding strategies were used,
372   and no Solo feeding was seen. But in either scenario, it is uncertain whether the observations are
373   due to true cooperative feeding strategies, or simply that coordinate movements reduce collisions
374   with other manta rays (Stevens 2016). It is likely our relatively short sampling duration failed to
375   detect this phenomenon, and our results suffer from small sample size. More work needs to be
376   done to quantitatively assess whether the presence of higher zooplankton biomass is positively
377   correlated with group feeding events.
378

379   *Zooplankton composition and size*
380   Differences in the composition of the zooplankton community were observed between *M. alfredi*
381   feeding and non-feeding events, and *M. alfredi* was observed feeding when the overall
382   biovolume of zooplankton was greater. Calanoid copepods dominate the zooplankton community
383   for manta rays at Hanifaru Bay, with *U. vulgaris* the most prominent species. *Undinula vulgaris*
384   is a key species in tropical areas due to its large size and tendency to swarm in high numbers,
385   making it a good food resource for planktivorous fishes (Alvarez-Cadena et al. 1998). This
386   species has been observed at numerous large planktivore feeding aggregation sites, including
387   those visited by *M. alfredi* in eastern Australia (Couturier et al. 2013, Armstrong et al. 2016), *R.*
388   *typus* in the Gulf of Tadjoura, Djibouti (Boldrocchi et al. 2018), both *M. birostris* and *R. typus* in
389   the Gulf of California (Notarbartolo-di-Sciara 1988, Lavaniegos et al. 2012), and both *M. alfredi*
390   and *R. typus* in the Philippines (Canencia and Metillo 2013, Yap-Dejeto et al. 2018). *Undinula*
391   *vulgaris* is considered an indicator of the influence of neritic-oceanic waters in reef
392   environments, and its local distribution can suggest oceanic water sources (Alvarez-Cadena et al.
393   1998). Further investigation into the ecology of *U. vulgaris* in tropical environments may aid our
394   understanding of how vital swarms of this species are for supporting large tropical planktivores,
395   and whether their distribution and abundance is likely to be impacted by a rapidly changing
396   climate.
397
398

Conclusions

Identifying important foraging opportunities for vulnerable species such as manta rays remains a goal for implementing effective conservation strategies for the species. Here, we conducted the first analysis of the food environment for *M. alfredi* at Hanifaru Bay, and highlighted the importance of tidal regimes and high zooplankton density in driving *M. alfredi* aggregations at this site. Conducting high resolution investigations into the dietary basis of aggregations can help inform drivers of species movements and habitat preferences. This can be challenging in remote locations where resources are sparse, and where fieldwork is logistically difficult (i.e., hand-towing for zooplankton is seldom recorded in methods), which may provide an explanation as to why most feeding studies for marine megafauna have only superficially investigated zooplankton dynamics. This study failed to record the zooplankton dynamics during a mass feeding aggregation at this site, and so our findings are suggestive of what can be observed at this location, but they may not provide the whole picture. Longer term sampling, and more targeted methodologies that allow for sampling of zooplankton throughout the water column, will help elucidate what leads to mass feeding aggregations and the role of different feeding strategies for *M. alfredi* at this site. Here, we have determined the importance of zooplankton dynamics in driving the aggregative behaviour of *M. alfredi* at their largest aggregation site at Hanifaru Bay, and this information can help inform management of this ecologically and economically important marine protected area and core zone within a UNESCO Biosphere Reserve.

Acknowledgements

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551

Figure 1

Figure 1. Study site in Hanifaru Bay in Baa Atoll, The Maldives.

A) Map of The Maldives, black star indicates location of Hanifaru Bay in Baa Atoll; and B) Satellite image of Hanifaru Island and Lagoon, with key study site of Hanifaru Bay (Credit: Copernicus Sentinel data 2020, accessed via USGS EarthExplorer and processed by AJA).
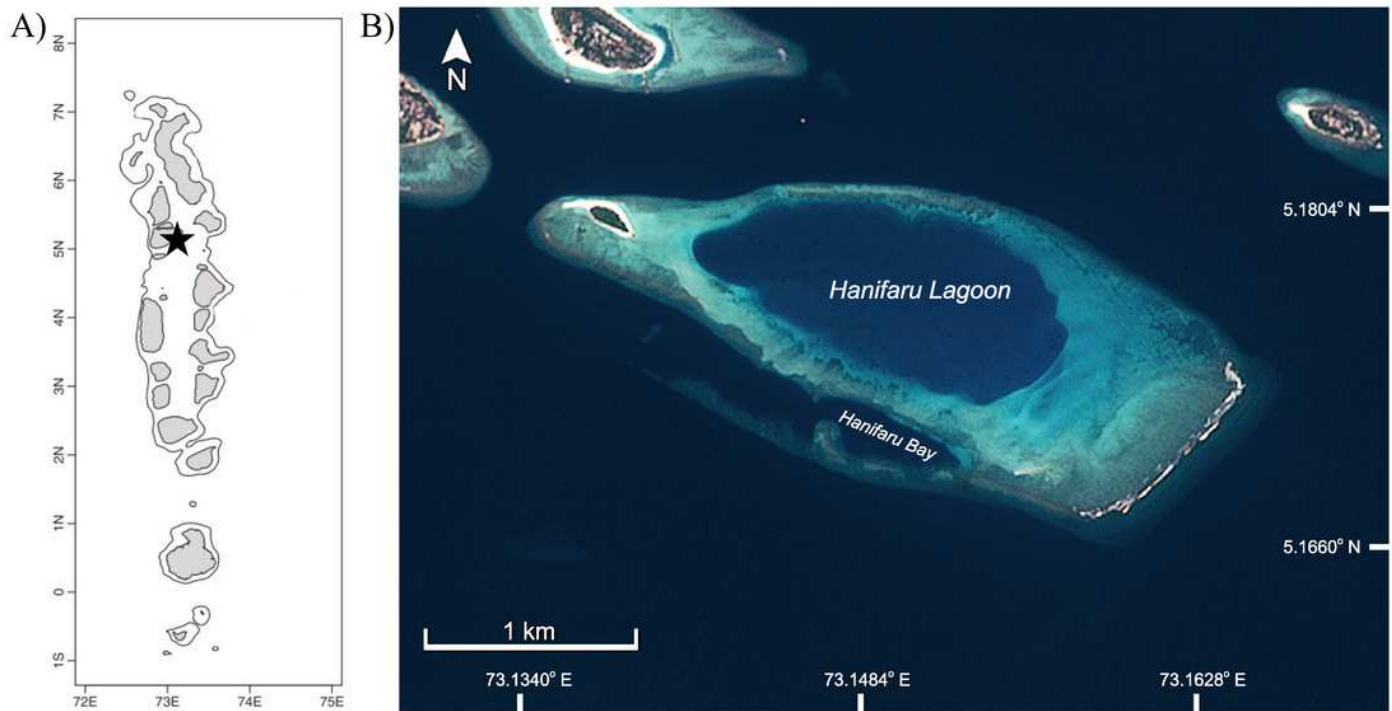
Figure 2

Figure 2. Zooplankton sampling and reef manta ray *Mobula alfredi* feeding strategies.

A) Zooplankton samples were collected by two snorkellers surface swimming a 200 μm-mesh net with flowmeter for ~50 m; and B) Ethogram of feeding strategies: i) Straight, ii) Surface, iii) Chain, iv) Piggy-back, v) Somersault, vi) Cyclone, vii) Sideways, and viii) Bottom (Illustration credit: Marc Dando).
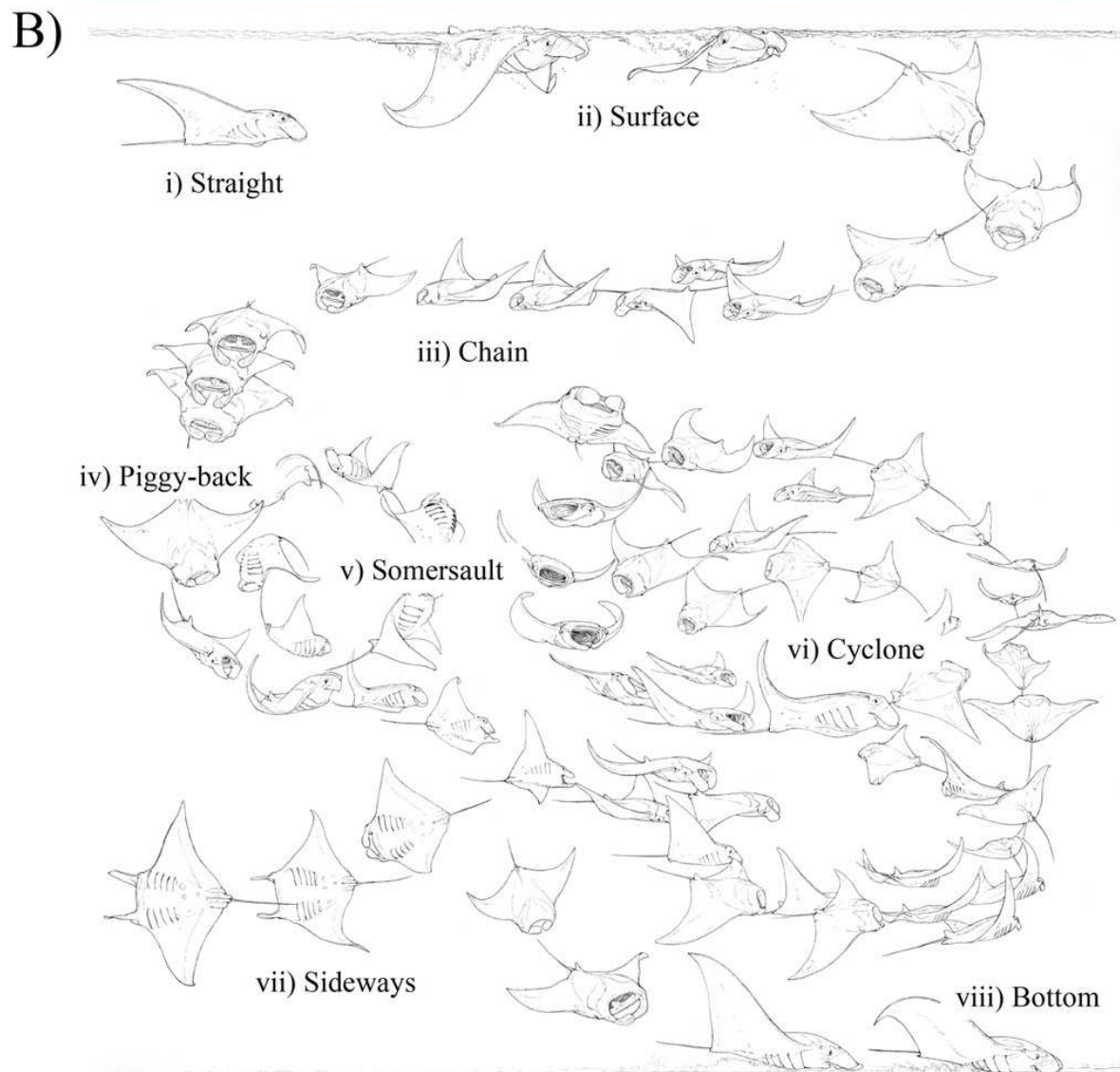
A)

B)

i) Straight

ii) Surface

iii) Chain

iv) Piggy-back

v) Somersault

vi) Cyclone

vii) Sideways

viii) Bottom

Figure 3

Figure 3. Predictors of Zooplankton biomass in Hanifaru Bay, Maldives.

Significant predictors of Zooplankton biomass included A) Tide (hours from high tide), and B) Manta ray behaviour (Feeding, Non-feeding and Absent). Biomass is on the response scale, with 95% confidence intervals.
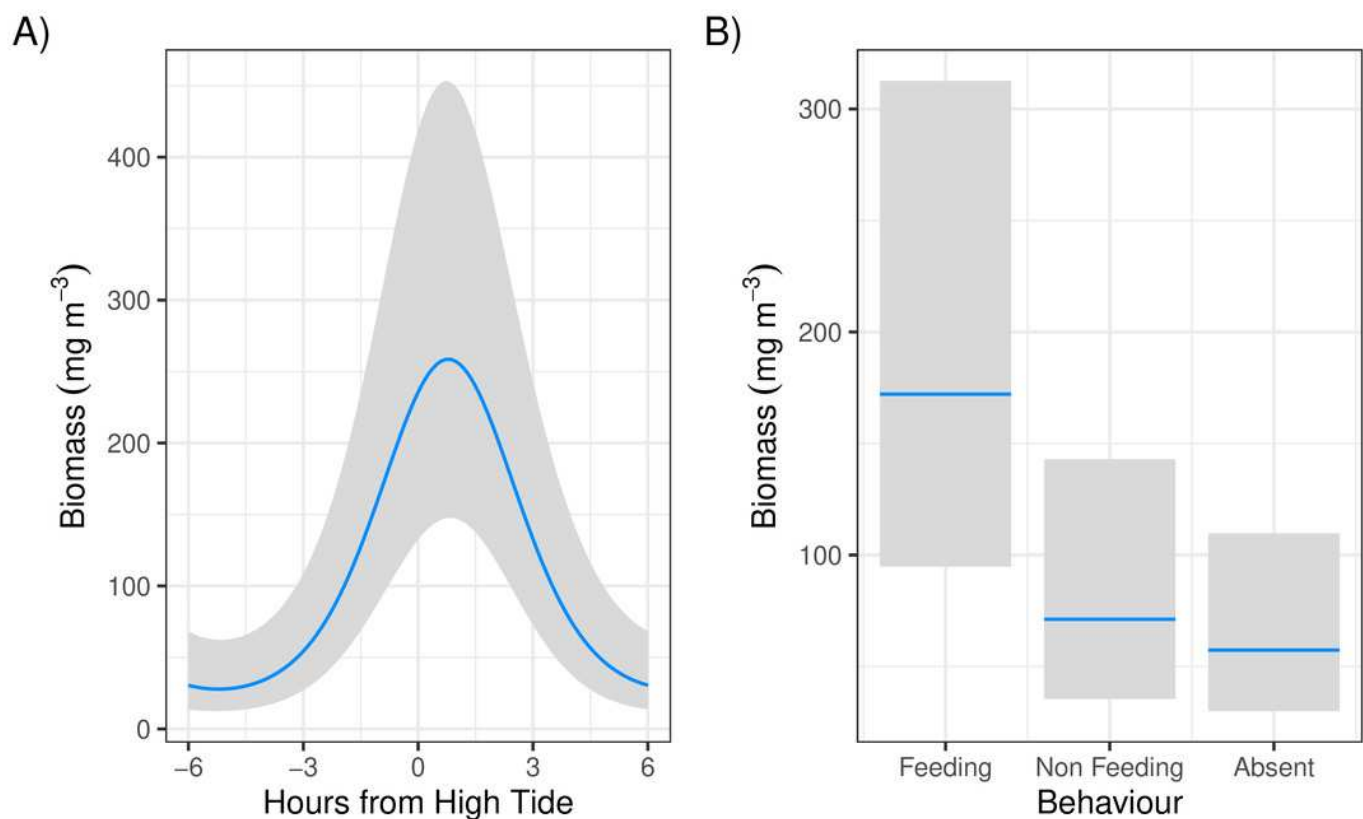
Figure 4

Figure 4. Critical prey density foraging threshold.

Logistic regression of reef manta ray *Mobula alfredi* behaviour (Feeding = 1, Non-feeding = 0) in relation to zooplankton biomass (mg m^{-3}). The black dashed line represents the critical prey density threshold of zooplankton biomass required to trigger manta ray feeding from *in situ* sampling (53.7 mg m^{-3}), and the red dashed line represents the theoretical prey density threshold calculated to meet the metabolic requirements of foraging *M. alfredi* (25.2 mg m^{-3}; Armstrong et al. 2016).
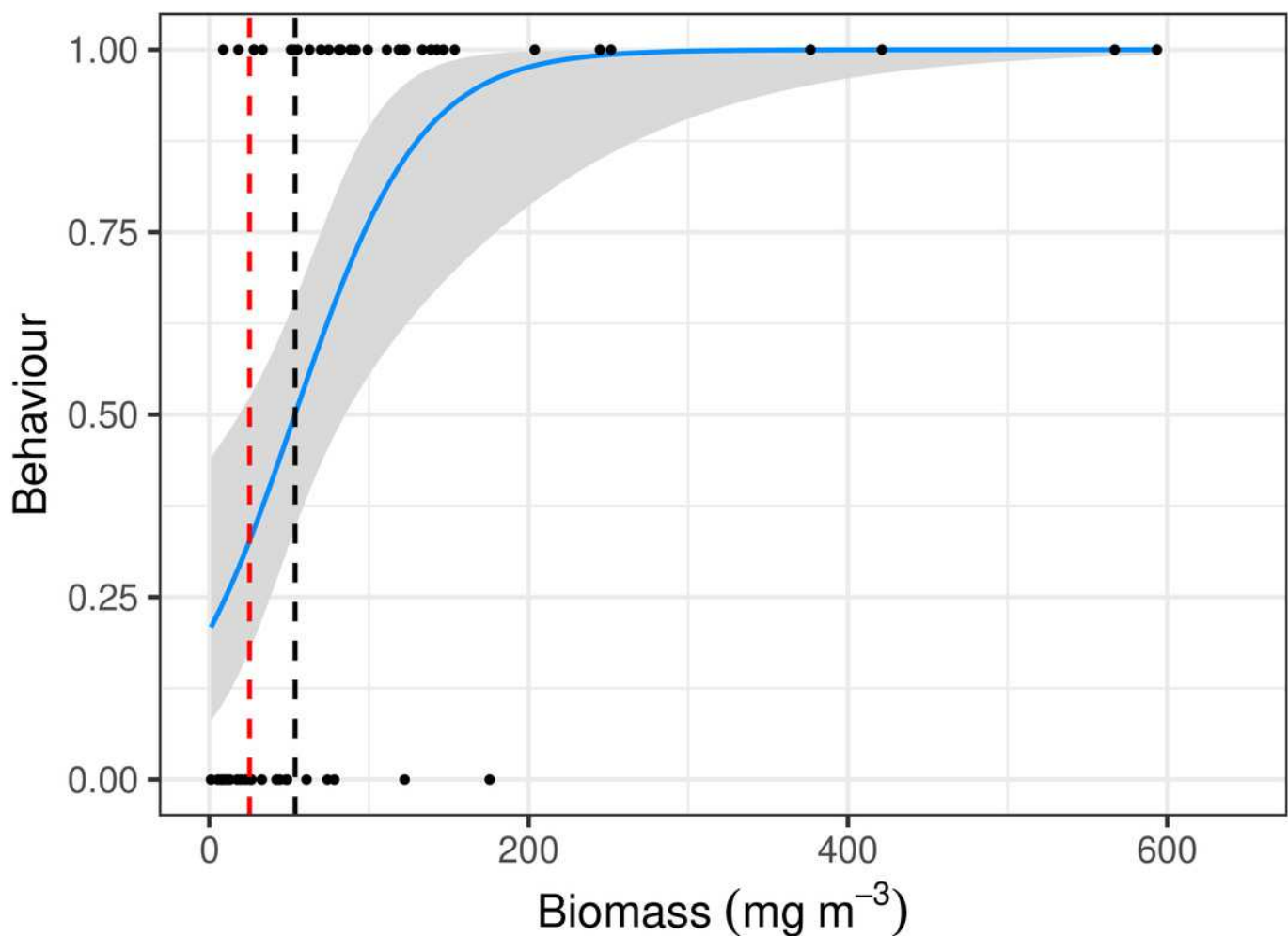
Figure 5

Figure 5. Predictors of reef manta ray *Mobula alfredi* abundance in Hanifaru Bay, Maldives.

Feeding Behaviour was a significant predictor of greater manta ray numbers in Hanifaru Bay.

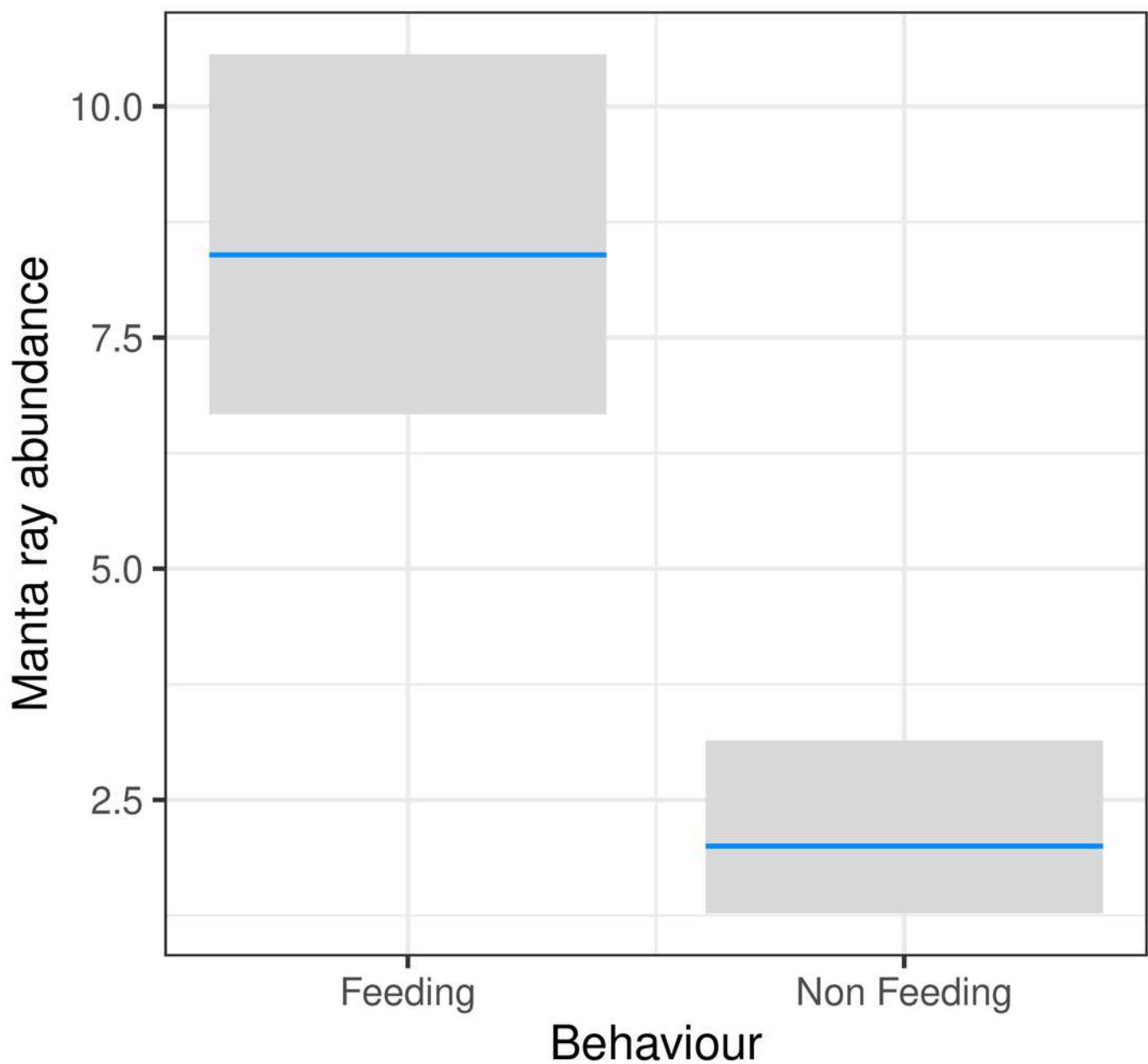Manta ray abundance is on the response scale, with 95% confidence intervals.

Figure 6

Figure 6. Zooplankton composition and reef manta ray *Mobula alfredi* behaviour.

A) Non-metric multidimensional scaling analysis of zooplankton community composition. Ellipses represent 95% confidence intervals and broad taxonomic groups are labelled as per their association with manta ray behaviours. B) *Undinula vulgaris* specimens (Credit: Julian Uribe-Palomino). Percentages of zooplankton community composition in Hanifaru Bay in relation to manta ray behaviour: C) Feeding; and D) Non-feeding. 'Other' comprises taxonomic groups that contributed less than 5% to the total community composition.
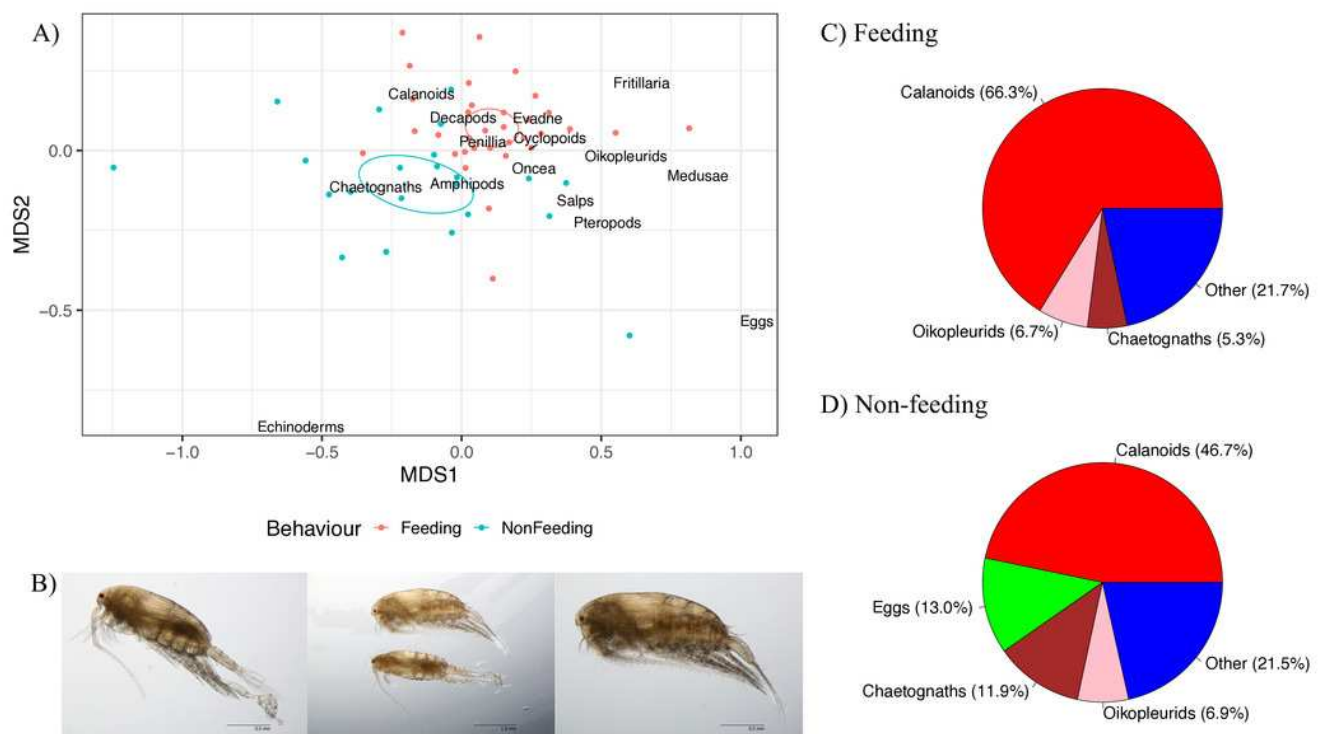
Figure 7

Figure 7. Zooplankton size structure analysis.

Normalised Biovolume Size Spectra of the zooplankton community when reef manta rays *Mobula alfredi* are Feeding (n = 33) and Non-feeding (n = 22). Dashed lines represent standard error.