British Indian Ocean Territory

Vava II Expedition

March 13th-29th 2019

Expedition Report

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Contents

Executive summary

Between the 13th and 29th of March 2019 the M/Y Vava II supported a total team of 12 scientists from four institutions to successfully deliver the equivalent of 126 days of scientific research in the British Indian Ocean Territory. The expedition team are grateful for the tireless efforts and enthusiastic support of Vava II's crew and the Bertarelli family in producing such an enjoyable and productive expedition.

The trip enabled the team to conduct core activities in support of the Bertarelli Programme in Marine Science such as the annual maintenance of the acoustic array and the collection of instruments set out on the reef during the earlier Reef 1 expedition. It also afforded a rare opportunity to explore new habitats and pilot new methodologies that may have opportunities for future research in the BIOT MPA.

Overall weather conditions were excellent for most activities; dry, with little to no wind and flat calm. However, the air and water temperatures were both extremely high and this deterred larger marine animals from the surface making it challenging to catch animals for tagging. The team reported a few observations of coral bleaching but the Reef 2 team that followed a few weeks later saw greater evidence of the effects of these high temperatures.

Key results in summary:

- Counts made of all seabirds encountered on eight islands
- 42 nesting red footed boobies tagged
- Sea turtle nesting tracks and body pits counted on 50 islands
- Three hawksbill turtles satellite tagged in Turtle Cove on Diego Garcia
- All acoustic receivers in the array serviced for another year
- 66 animals from five marine species (manta, grey reef, silvertip sharks, and dogtooth and yellowfin tuna) electronically satellite tagged
- A tagged silvertip shark re-caught for the first time showing growth of 32 cm in three years
- 882,000 new detections downloaded from the deep-water acoustic receivers
- New seagrass sites recorded on the Great Chagos Bank
- Moresby mangrove mapped and surveyed for the first time since 2010 when it was discovered
- Helicopter surveys of vegetation and beach morphology conducted on 44 islands
- Plastics surveys conducted on seven islands in three atolls including sand cores taken for analysis of microplastics
- More than 300 specimens of terrestrial invertebrate collected from surveys of six islands, four of which had no previous records at all, with at least two new species records for BIOT
- Survey of sea cucumbers in Salomon lagoon showed increase in numbers from previous counts in 2009 and 2013
- First ever sound recordings of reefs in Peros Banhos and Salomon atolls made

A catalogue of all data collected on all BPMS expeditions is held at this location or available on request from rachel.jones@zsl.org.

1. Tracking sharks and tuna

Team: Dr. Taylor Chapple (Stanford), Robbie Schallert (Stanford), Dr. David Curnick (ZSL) Dr. David Jacoby (ZSL), Neil Sherman (BF).

1.1 Introduction

The team had two key objectives: 1) to service the receivers that form the acoustic array – downloading the data, cleaning the units, replacing batteries for another year of operations, and 2) to attempt to tag a new cohort of marine animals to continue the tracking research. These data contribute to a study started in 2015 that is building a picture of how reef and semi-pelagic sharks and other key species such as yellowfin tuna use the physical environment within the BIOT MPA.

1.2 Results and discussions

The first objective was met in rapid order due in large part to the help of the crew of the Vava II and to the capability of the tenders.

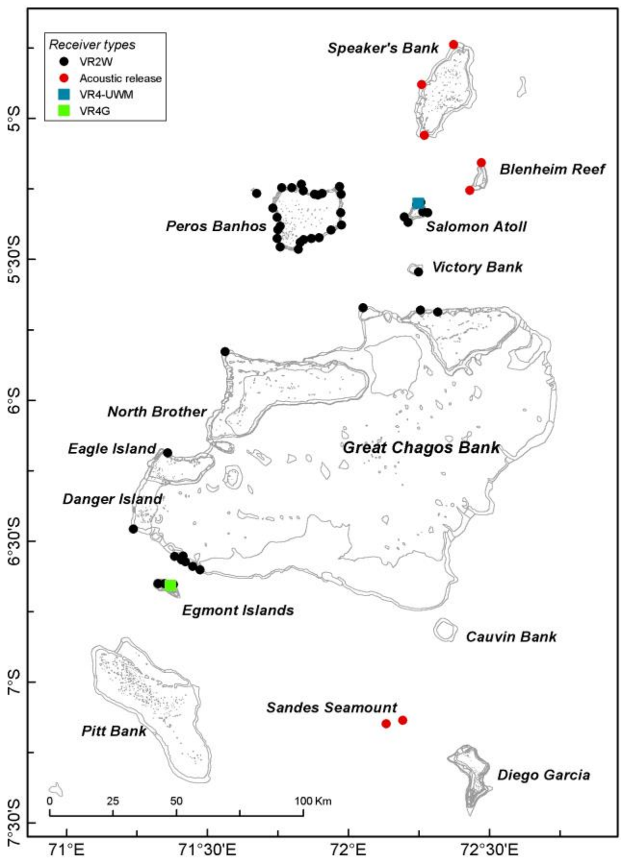

Fig 1. Map of acoustic receiver locations as of June 2019

Data recovered and downloaded from the deep-water acoustic receivers on the seamounts to the north-west of DG has added an additional 882,000 detections to the existing dataset of 1.25 million.

Weather conditions were challenging for fishing of large predators, extremely high surface waters deterred our target species such as yellowfin tuna from approaching the bait despite hundreds of hours spent on the water. The helicopter was used for spotting bait balls from the air and was successful at guiding the fishing teams into promising areas, but beyond the very early hours of morning fish activity was low. Despite these conditions, the team manged to electronically tag 66 animals from 5 species; manta ray, grey reef shark, silvertip shark, dogtooth tuna and yellowfin tuna as well as taking tissue sample for isotope analysis. Floy tags were also deployed (linked to the IOTC programme), with the process of floy tagging a yellowfin tuna demonstrated in this film.

An individual silvertip shark was caught and examined on the 24th Mar 2019 and discovered to have been previously tagged by the same team on the 15th Apr 2016. In the intervening three years the recorded tail length had increased from 88 -120cm a total of 32 cm. In the years that BF funded scientists have been tagging sharks in BIOT this is the first one they have re-caught *in situ* (one was recovered from a Sri Lankan fish market in 2018 with an acoustic tag inside it labelled Stanford University).

The team continued collecting samples for the stable isotope survey – with another 222 samples taken from 22 species. These data will further reveal the feeding ecology of the animals around BIOT and the full dataset now contains 559 samples from 31 species. The DNA samples will reveal how connected populations in BIOT are with the wider WIO and will start to build a picture of regional shark populations.

Other observations of interest include the sightings of whale sharks (*Rhincodon typus*) on three occasions and a thresher shark (*Alopias* sp.).

2. Assessment of sea turtle nesting and foraging in BIOT

Team: Dr. Nicole Esteban (Swansea University)

2.1 Introduction

The turtle research was led by Dr. Nicole Esteban with assistance from various members of the science team and crew. Six key objectives included:

1. Nesting population estimation via track surveys (island foot patrols, helicopter surveys) and remote camera recordings
2. Trial to assess foraging populations of turtles using drones (Autel Robotics EVO)
3. Assessment of climate change on species with temperature dependent sex determination (monitoring via temperature loggers at nesting depth)
4. Tracking movements of immature turtles in Turtle Cove, Diego Garcia: attachment of satellite tags to hawksbills at a different time of year (previous attachments in June and November).

5. Validation of green turtle foraging habitat from satellite tracking locations (see section 5: seagrass)
6. Exploring the effect of beach plastics on sea turtle incubation conditions (see section 6: island habitats)

2.2 Results and discussion

2.2.1 Nesting population estimations

This expedition was the first opportunity to assess nesting turtle populations on remote islands since the Vava II expedition in 2016. Recent track and longer-term body pit surveys were therefore a priority activity. In order to increase accuracy in nesting estimation, nine camera traps were mounted on trees overlooking nesting beaches on five islands of Peros Banhos.

- Track (example shown in Fig 2a) and body counts for hawksbill and green turtles were estimated in Peros Banhos and Salomon atolls by foot (10 islands) and by air (40 islands). Aerial surveys were conducted at 100 m altitude and 65 km/h speed.
- A large increase in turtle nesting activities between 2016 and 2019 on Salomon atoll islands may be indicative of population recovery after 50 years of protection.
- Camera traps (Fig 2b-c) were set up on Vache Marine (n=4), Ile du Coin (n=2), Ile Poule (n=1), Moresby Island (n=1), Ile de Passe (n=1). Cameras were mounted on trees at 1.5-2m height overlooking key nesting beach areas and programmed to take photos on a daily basis at 15 minute intervals between 0700-0800 hrs. It is hoped that data can be downloaded and batteries checked every 4-6 months so that this becomes a continuous monitoring activity to increase our confidence in estimates of nesting tracks on remote atolls.

Fig 2. Turtle tracks and camera traps

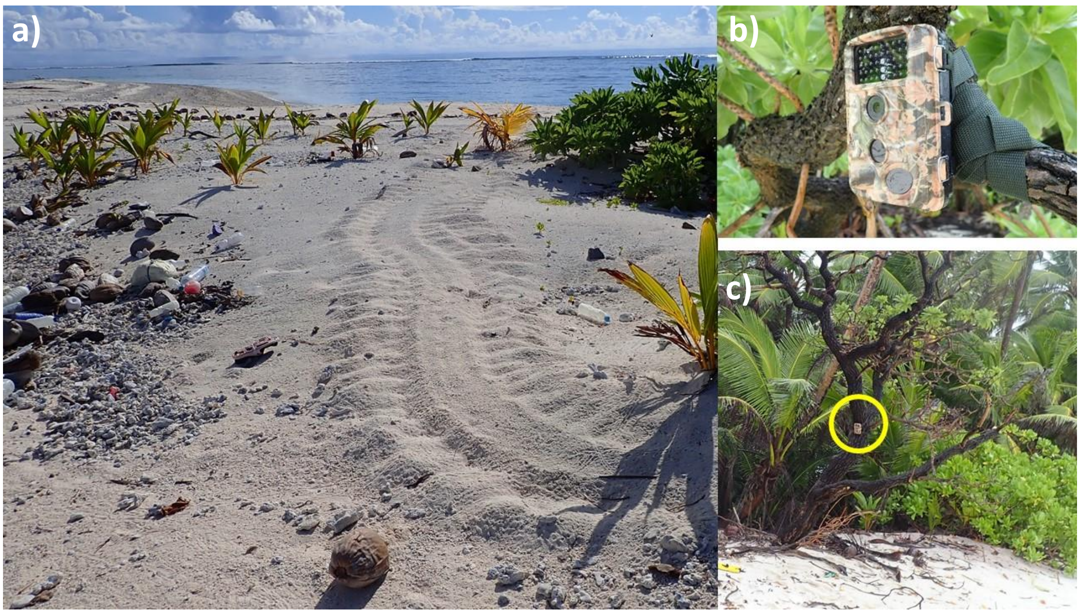

Fig 3. Snapshot from aerial survey by Autel Robotics EVO drone (inset) shows exceptional water clarity (during period of calm weather) to assist with observations of sea turtles for foraging population estimation.

2.2.3 Sea turtle nest incubation conditions

Studies of incubation conditions in BIOT have been largely focussed on Diego Garcia with *ad hoc* sampling of sand temperature on remote atolls. During the 2016 Vava II expedition, we deployed temperature loggers in Egmont atoll and had not yet been able to excavate the loggers. After two hours of searching, all six temperature loggers were successfully located from turtle nesting depths (30 cm, 50 cm, 70 cm) in shaded and unshaded conditions in north-western Egmont atoll (Fig 4, inset map).

Fig 4. After three years, six temperature loggers were successfully located on Egmont atoll

2.2.4 Tracking immature sea turtles, Turtle Cove, Diego Garcia

The first satellite tracking of immature turtles took place in 2018 with attachments to hawksbill (n=18) and green (n=2) turtles in June and November. This expedition provided the opportunity to tag turtles foraging in Turtle Cove during a different season.

- On 19 March, three hawksbill turtles were captured at low water, weighed, measured and tagged with Fastloc-GPS satellite tags.
- Turtles ranged in size from 64-71 cm Curved Carapace Length (CCL) and 25-34 kg weight. Individual details are Vava (ID178882; 34 kg, 71 cm CCL, blue line); André the Giant (ID178884; 30 kg; 69 cm CCL; red line); Rishi (ID178881; 25 kg,; 64 cm CCL; orange line).
- Preliminary location analysis indicates that all turtles have remained close to Turtle Cove (Fig 5; Vava: blue; André the Giant: red; Rishi: orange).
- The team at Turtle Cove were able to capture four turtles with existing satellite tags in order to check fouling of tags. Tags were cleaned and repainted.

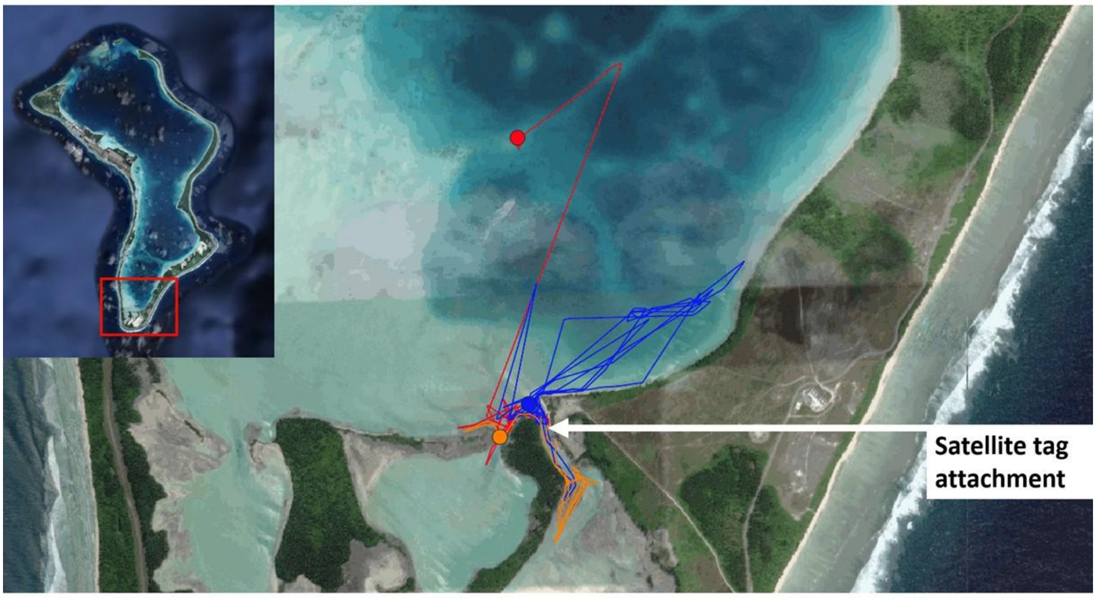

Fig 5. Tracks of hawksbill turtles show they remained close to Turtle Cove during the 72 hour period after satellite tag attachment in March 2019

3. Seabirds

Team: Peter Carr (ZSL)

3.1 Introduction

Through 14 – 21 March 2019 as part of the BPMS science trip to BIOT aboard VAVA II, 42 red-footed booby *Sula sula* were caught as part of the long-term ornithological research project in to how a top avian predator uses the BIOT Marine Protected Area (MPA) whilst breeding and, throughout the non-breeding period. The specific aim on this trip was to gather further morphometric data (wing length and mass) from one of the northern atolls (Peros Banhos), to establish if there is any variation in sexual dimorphism in red-footed boobies throughout the archipelago (morphometric data has already been gathered from Diego Garcia in the southeast and from Nelson's Island in the west).

3.2 Methodology

All birds were captured on five islands in the Peros Banhos atoll (Fig. 10). These were captured on the nest, ringed with a uniquely coded British Trust for Ornithology (BTO) metal ring, had their wing length and weight recorded and, finally, had a small number of breast feathers removed for DNA analysis. The DNA was used to define the sex of the bird. DNA analysis was conducted by ZSL staff in London.

Initially, Principle Component Analysis (PCA) was employed to ascertain which morphometrics (wing length, mass, culmen depth, culmen length and tarsus diameter – all data collected from the colony on Diego Garcia) were the most important for correctly sexing red-footed booby in the field (i.e. without the requirement for DNA analysis). Discriminant Function Analysis (DFA) was then used to define the biometric parameters required to be able to correctly sex all birds studied in the project. It was also used to statistically assess whether there are sexually dimorphic biometric differences in colonies on different atolls.

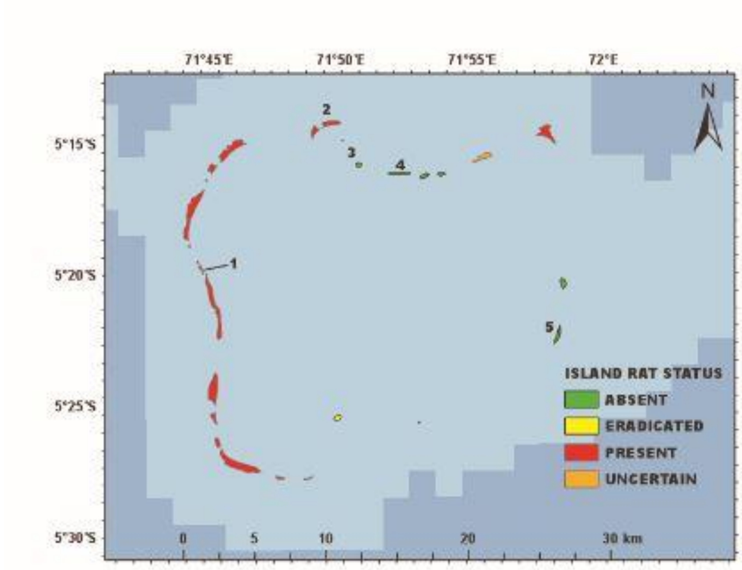

Fig 6. Peros Banhos atoll showing the islands where red-footed booby were caught, measured and released as part of the BPMS research project into how top avian predators use the BIOT MPA. 1 = Verte, 2 = Moresby, 3 = Parasol, 4 = Longue, 5 = Grand Coquillage.

3.3 Preliminary results

PCA demonstrated that mass was the principle component for determining the sex of red-footed booby in BIOT (0.992 of variance) with wing length being the second (together accounting for 0.998 of variance). DFA demonstrated there are significant differences in sexual morphometrics (mass and wing length - Figs 7 and 8). It further demonstrated there are no significant differences in the morphometrics of birds of the same sex between the three atolls studied to date (Figs 9 and 10).

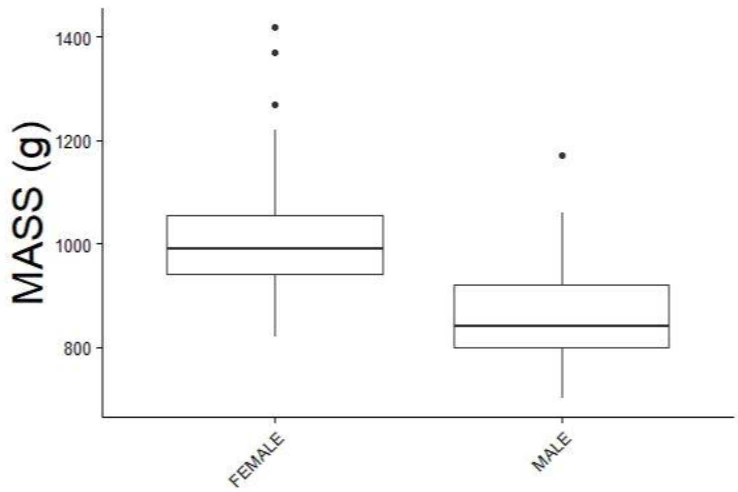

Fig 7. Mass of red-footed booby by sex from three atolls in the BIOT MPA. There is a significant difference between sexes (F=100.05, P<0.01)

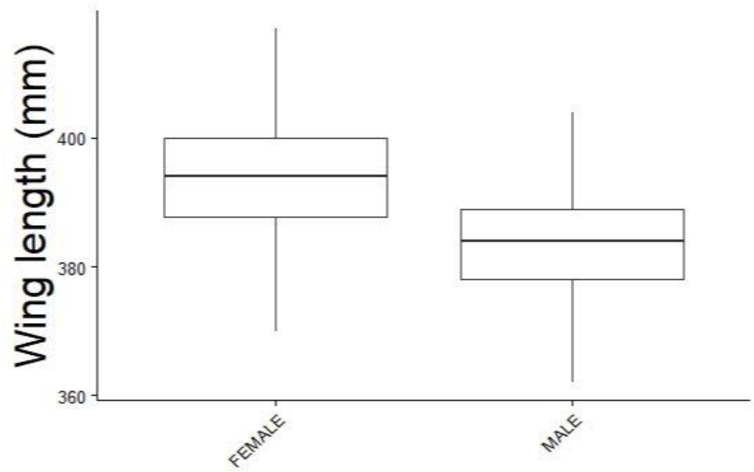

Fig 8. Wing length of red-footed booby by sex from three atolls in the BIOT MPA. There is a significant difference between sexes (F=58.3, P<0.01)

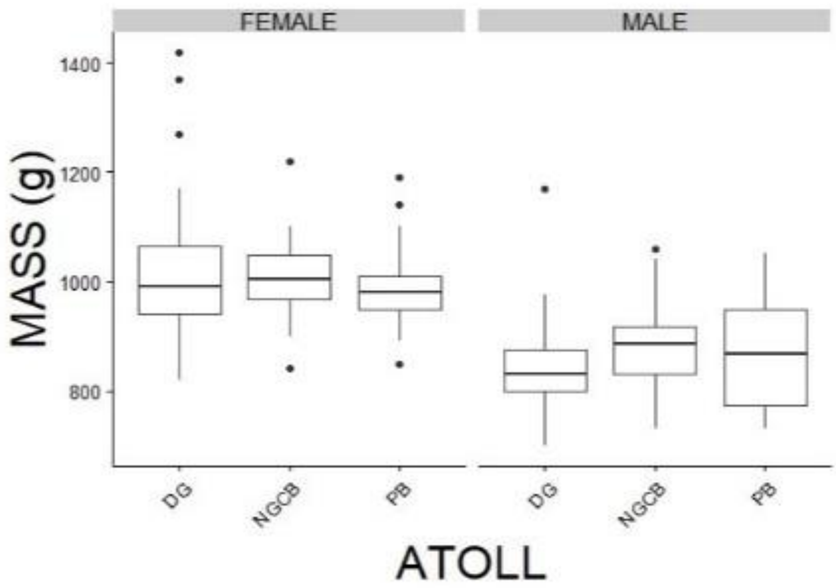

Fig 9. Mass of red-footed booby by sex from three atolls in the BIOT MPA. There is no significant difference in mass by sex between atolls. DG = Diego Garcia, NGCB = northern Great Chagos Bank, PB = Peros Banhos.

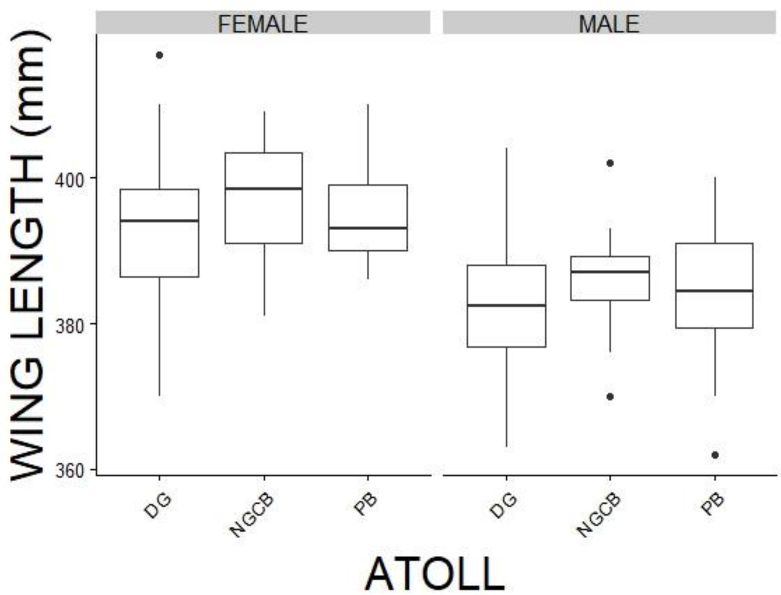

Fig 10. Wing length of red-footed booby by sex from three atolls in the BIOT MPA. There is no significant difference in wing length by sex between atolls. DG = Diego Garcia, NGCB = northern Great Chagos Bank, PB = Peros Banhos.

DFA was further used to determine the biometric parameters for each sex (Fig 11). From the analysis the morphometric parameters of wing length and mass were calculated (Tables 1 and 2).

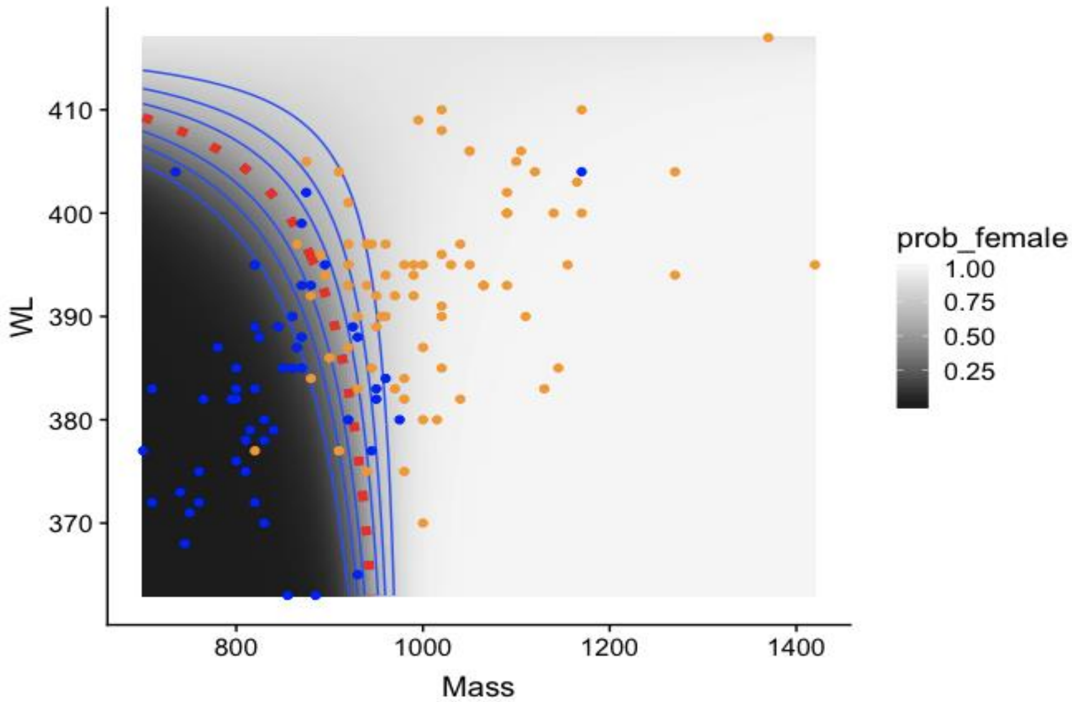

Fig 11. Sexual dimorphism in red-footed booby by mass and wing length (WL). Blue dots = males, Orange dots = females, Red squares female threshold. Dark to light shade = increased probability of being female.

Table 1. Sexually dimorphic mass parameters of male and female red-footed booby in the BIOT Marine Protected Area.

SEX	MEAN MASS (g)	STANDARD ERROR	LOWER CONFIDENCE LEVEL	UPPER CONFIDENCE LEVEL
FEMALE	1015	12.6	990	1040
MALE	859	12.1	835	883

Table 2. Sexually dimorphic wing length parameters of male and female red-footed booby in the BIOT Marine Protected Area.

SEX	MEAN WING LENGTH (mm)	STANDARD ERROR	LOWER CONFIDENCE LEVEL	UPPER CONFIDENCE LEVEL
FEMALE	395	1.13	393	397
MALE	384	1.07	382	386

Tables 1 and 2 highlight that a female red-footed booby is 95% likely to have a mass > 990g and a wing length > 393mm whereas a male is likely to have a mass < 883g and a wing length < 386mm. We note that mass and wing-length co-vary (see Fig. 6) with some lighter males showing wing-lengths above 386mm. Using both criteria may provide a more accurate way to identify sex.

3.4 Discussion

To date, 396 red-footed booby have been uniquely marked for research purposes in BIOT as part of the BPMS Chagos Seabird Ecology (ChaSE) project. Of these, 175 have been sexed using DNA analysis, 93 being female and 82 being male. Using the results that are available following the March 2019 Vava II expedition, the remaining 221 unsexed birds can now be categorised.

One of the key components of the ChaSE project is understanding how a top predator uses the MPA for feeding and foraging, both when breeding and throughout the non-breeding season. In several species of pelagic seabirds, it has been proven that the differing sexes use the marine environment in different ways, especially when breeding. Sexually dimorphic species such as red-footed booby often fill separate ecological niches. For analysis of tracking data investigating the use of the MPA for feeding and foraging by this species, a central tenet of the seabird research programme, it was imperative to be able to sexually segregate the research birds.

3.5 Value of research to BIOTA

The research undertaken as part of the March 2019 Vava II expedition has enabled the ChaSE programme to accrue more data on their principal research species, red-footed booby. These data came from Peros Banhos, a northern atoll and has “balanced” the spread of information on breeding colonies from throughout the archipelago, i.e. from Diego Garcia in the south, Danger Island in the west, Nelson’s Island in the central northeast and now, Peros Banhos in the far northwest.

Having data from DNA sexed birds from across the archipelago has facilitated a robust analysis of how to use empirical data (mass and wing length) to sex all the research birds. This in turn allows a more thorough analysis of how a species at the top of the food chain in the MPA uses the area for feeding and foraging. This analysis should identify marine hotspots of biodiversity, where the target species is interacting as an obligate associate with underwater predators such as tuna. These areas in turn could be indicative of where poaching activities are centred and, may warrant IUCN marine Important Bird and Biodiversity Area status.

4. Coral reef research

4.1 ARMS and Environmental DNA collection

Team: Dr Catherine Head & Margaux Steyaert (Institute of Zoology, ZSL & University of Oxford)

Objective 1: Recover nine ARMS and process the motile and sessile fauna inhabiting these ARMS for biodiversity assessments.
Objective 2: Water sampling to establish plankton community structure using Environmental DNA methods

Objective 1: Recover nine ARMS and process the motile and sessile fauna inhabiting these ARMS for biodiversity assessments.

4.1.1 Introduction

Tropical coral reefs are some of the most biologically diverse and complex ecosystems on the planet, with a large portion of this biodiversity found amongst the small, hidden and inconspicuous organisms living in and amongst coral, commonly referred to as the 'cryptofauna'. These organisms, including both motile (e.g. crabs, shrimps, brittlestars) and sessile (e.g. sponges) invertebrates, are critical to the health and resilience of reefs despite being poorly studied. Autonomous Reef Monitoring Structures, otherwise known as ARMS devices, are novel and highly standardised tools used to study and monitor marine biodiversity. Composed of nine stacked PVC plates, these small devices aim to provide, to some extent, enough structural complexity to resemble coral reefs. ARMS devices are typically attached onto the reef matrix and provide substrata for the recruitment and colonisation of various sessile and motile invertebrates. These devices enable us to collect, quantify and analyse the biodiversity of cryptic invertebrates living amongst corals. Due to their highly standardized nature, ARMS have been used to survey and compare the biodiversity of coral reef cryptofauna across the world's oceans (Al-Rshaidat et al., 2016; Pearman et al., 2016; Ransome et al., 2017; Pearman et al., 2018).

Thirty ARMS were deployed at four locations around Peros Banhos and Salomon atolls in March 2018. This year we collected in the first nine ARMS devices from three of these sites and processed them aboard the Grampian Frontier and Vava II vessels. Our aim is to collect three devices at each location each year, over a period of three years. These devices, along with cutting edge genetic techniques, enable us to study the change in invertebrate community structure over time. Furthermore, as part of an ongoing collaboration with Rob Dunbar's team at Stanford, an array of high precision instruments recording environmental factors (temperature, pressure/wave loggers, salinity, depth, oxygen, current, light) have been deployed at ARMS sites. These instruments, alongside the ARMS devices, will help to elucidate what drives local invertebrate communities in BIOT. Overall, this project will enable us to better understand how ocean physics and changes in environmental factors shapes cryptobenthic diversity in tropical coral reefs.

4.1.2 Methodology

Nine ARMS were collected in sets of threes from Moresby (Peros Banos), Ile du Coin (Peros Banos) and Ile Anglaise (Salomon) on scuba in March 2019 and processed according to the protocol by Leray & Knowlton (2015). Three of these were collected whilst on board the Vava II vessel. In brief, ARMS plates were first disassembled, labelled and set aside from the main plate structure. Following this, high definition photos were taken of both top and bottom sides of each plate, along with individual sessile organisms of interest (see Figure 12). Tissue subsamples were also taken from such individual sessile organisms and set aside for downstream genetic analysis (barcoding). Attached organisms were then scraped off of each plate and homogenised in bulk using a blender, then set aside in DNA preservation buffer for downstream genetic analysis (metabarcoding).

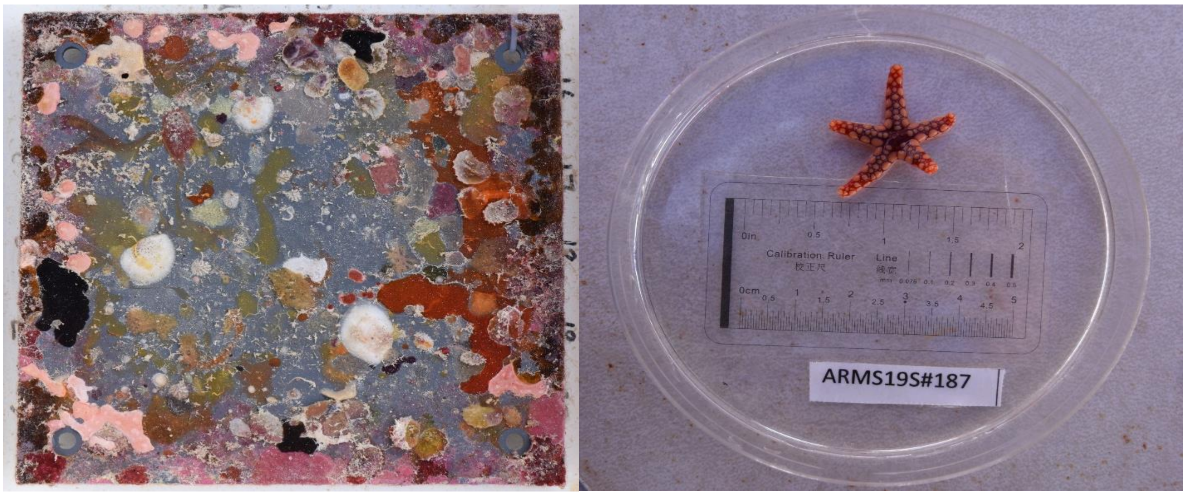

Fig 12. Close up photograph of the bottom of one ARMS PVC plate colonised by sessile species (*left*) and a photograph of one motile sea star species prior to preservation in ethanol for downstream genetic analysis (*right*).

Following the disassembly of the ARMS device, free-living motile organisms remain in the discarded water in a large bin. This water is sieved and motile organisms are grouped into three main size fractions (2mm and above, between 0.5mm and 2mm, between 0.1 and 0.5mm). Individual large animals (>2mm) are photographed and set aside for downstream genetic analysis (barcoding)(see Figure 12). The remaining two smaller fractions are then preserved in bulk in ethanol, frozen and set aside for downstream analysis (metabarcoding). For each ARMS location, triplicate 20m benthic video transects were carried out on scuba in order to visualise and identify the surrounding reef matrix (see Figure 13).

Fig 13. Dr Catherine Head laying out a 20m transect line for a video benthic survey.

The following genetic analysis will now be carried out at the Institute of Zoology at the Zoological Society of London (ZSL). The next step is to extract DNA from both motile and sessile samples to identify species present on each of these ARMS. Following this, barcode DNA from each sample will be amplified and sequenced. Sequence data will then be analysed using a customised bioinformatics pipeline. From these data we will be able to quantify and identify the number of species collected, which will be vital when comparing biodiversity values across locations and over time in BIOT. Plate photos will be analysed using imagery software in order to calculate the percentage cover of sessile organisms and also the colonisation success of certain taxonomic groups. Photos of individual organisms will be used to measure body size and serve as an inventory for the potential description of new species (or morphospecies) using taxonomic expertise.

A total of 332 large individual motile organisms (112 aboard the Vava II, 220 aboard the Grampian Frontier) and 224 samples of sessile organisms (aboard the Vava II, aboard the Grampian Frontier) across the 9 ARMS. A total of 63 samples were collected for the small bulk motile fractions (21 aboard the Vava II, 42 aboard the Grampian Frontier) and 54 for the bulk sessile fractions (18 aboard the Vava II, 36 aboard the Grampian Frontier). As this is the first year of sample collections for this project, we expect to have results later this year.

Objective 2: Water sampling to establish plankton community structure using Environmental DNA methods

4.1.3 Introduction

Planktonic organisms comprise the base and lower levels of the marine food-chain, which supports everything else, including commercial fish species. In addition, during stress events, such as increased sea surface temperature, zooplankton become an even more important food source when corals expel their photosynthetic symbionts and rely on external food sources. Unlike other fauna, plankton community structure in BIOT has never been assessed, partly perhaps because of the very labour-intensive nature of traditional collection and identification methods. Now revolutionary new molecular techniques, called meta-barcoding, enable us to identify Environmental DNA (eDNA) from a representative non-invasive environmental sample, and compare these DNA sequences against a reference database of known organisms' DNA sequences to identify the diversity and community structure in that environment. We began collecting eDNA water samples on the 2016 expedition. To investigate the changes in community structure through time, using a snap-shot from each year, we collected further samples from 29 sites across the Archipelago on this year's expeditions aboard the Grampain Frontier and Vava II (Fig. 14). To maintain a time series dataset, we will continue to collect eDNA water samples in each year of the project to monitor change. We also aim to identify what physical environmental variables are driving this community structure and any associated change. This is made possible by collaborating with our project partners, Rob Dunbar and his team at Stanford University, who have begun collecting data on the abiotic environmental parameters e.g. currents, pH, O_2 levels.

4.1.4 Methodology

Triplicate 2-Liter water samples were collected from the water directly surrounding the reef (i.e. approx. 1m from the reef framework) at depths of approximately 6-7m, using platypus bottles whilst diving (dependent on dive plan) at 29 sites across the Archipelago (Fig. 14).

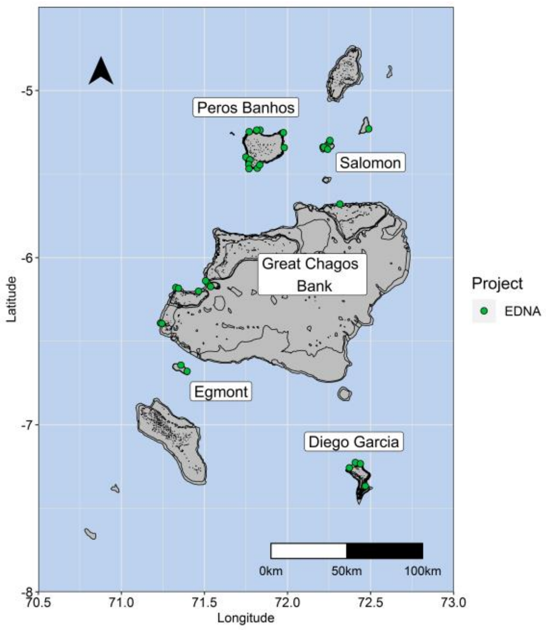

Fig 14. Locations of e-DNA sampling

Water samples were filtered back in the lab container using 47 mm diameter membrane filters and a vacuum pump system to pull the water through the filters (Fig 15). All biological material larger than 0.7µm in size becomes trapped on the filters. We then preserved the filters by wrapping them in sterile foil and storing them in the ship's freezer before transporting the filters back to Oxford on ice.

Molecular analysis of eDNA samples will be undertaken on an Illumina MiSeq platform using genetic markers 18S, and COI to identify phytoplankton and invertebrates, respectively. Well-developed specific primers and protocols for DNA extraction, PCR, and bioinformatics will be used. The resulting sequences will be assigned to Operational Taxonomic Units (OTUs) using a custom script that enables analysis of gene markers (https://github.com/jimmyodonnell/banzai). OTUs will be matched against a reference database (e.g. GenBank, BOLD) to assign species taxonomy where possible, and biodiversity indices will be calculated using both OTUs and species IDs. Further eDNA samples will be collected on next year's expedition to continue the data series. Plankton tows will also be carried out to validate eDNA results against traditional methods.

Fig 15. Environmental DNA processing set up on the Vava II.

4.1.5 Value of this biodiversity research to BIOT

Investigating local cryptofauna and plankton diversity patterns and the interaction between environmental factors and these invertebrate communities is vital when trying to understand how coral reef systems function and thrive. BIOT provides a unique opportunity to study protected coral reef systems where human disturbances such as fishing and pollution are removed. It has already been demonstrated that decapod diversity in BIOT waters is exceptionally high and harbours many rare species (Head et al., 2018). It is likely other groups of cryptic reef invertebrates reflect similar record-breaking diversity patterns in BIOT.

Over 1,600 identical ARMS devices are currently deployed worldwide across a range of marine ecosystems. This substantial and evolving dataset of global marine benthic diversity, to which we are contributing, allows for large scale comparisons. The ability to compare our results with other coral reef studies around the world can enable us to demonstrate the benefits of highly protected marine reserves such as BIOT. Once completed our project will serve as a vital baseline for global comparison of tropical reef diversity.

4.2 Reef Physics, Environmental Monitoring, and Benthic Fluxes

Team: Professor Rob Dunbar and David Mucciarone (School of Earth, Energy, and Environmental Science, Stanford University)

Objective 1: Understand how historical, physical, and biogeochemical drivers influence reef biodiversity, biomass, and productivity over time and how these drivers impact the provisioning of resources to the pelagic ecosystem.

Objective 2: Understand how net primary and carbonate productivity vary among Chagos reef systems, and how this correlates with community composition and diversity. Identify possible hotspots for productivity and export as targets for conservation.

Vava II Activity 1: Retrieve benthic flux monitoring systems deployed at Oxford University long-term recruitment measurement sites (ARMS sites).
Vava II Activity 2: Recover and Deploy additional instruments at multiple island sites to develop multi-year records of temperature, salinity, wave energy, and ocean circulation on the reefs and within the lagoons of the BIOT.

4.2.1 Introduction

The Stanford University/Oxford University science teams worked aboard Vava II from March 21-29, 2019 to conduct research under BPMS Project 9: “Understanding the conservation value of managing coral reef biodiversity for the wider BIOT MPA”. We transferred from the Grampian Frontier in Diego Garcia following a deployment cruise that operated from March 2-18, 2019. Our strategy for using both vessels was to deploy high-resolution benthic monitoring systems as soon as possible from the Grampian Frontier and recover them as late as possible from Vava II. This allowed for deployment intervals of 16 days (Ile du Coin), 21 days (Ile Moresby), and 14 days (Ile Anglaise), spanning multiple semi-diurnal and diurnal tidal cycles, portions of the Spring-Neap transition, and several wind events. These systems measure current velocities, oxygen concentrations, light intensity, wave heights, pH, temperature, and salinity and allow us to calculate rates of net community primary production/respiration as well as net reef precipitation/dissolution of calcium carbonate under different conditions. Longer deployments allow us to measure the sensitivity of the reef as a producer of food and builder of substrate under to different environment conditions. We co-locate these benthic flux systems where the Oxford University team is assessing reef biodiversity. Eventually, we will understand how different organisms contribute to maintenance of the reef and what conditions are most favourable. We are also establishing early baselines against which we can assess the impacts of global warming and ocean acidification.

4.2.2 Methodology

Forty-three SBE-56 high precision sea temperature loggers were recovered during the expedition (Figs 16 and 17). About half were deployed in April 2018 and were still logging upon recovery in March 2019. The remaining T loggers were deployed for short duration experiments examining stratification within the coral reef water column. Many forereef records show short-lived (minutes to hours) and sudden declines in temperature at 10 meters (Figure 18). These short-lived cooling events can be large, as much as 7°C and can achieve temperatures as low as 21 to 22°C. They are not apparent in lagoons, so we suspect they result from interactions of internal waves with the island pedestals. Corals that live along many BIOT forereefs are exposed to very different thermal regimes than corals in the lagoons and we will assess both differences in genetic makeup as well as thermal tolerance within species but across sites (2020 expedition).

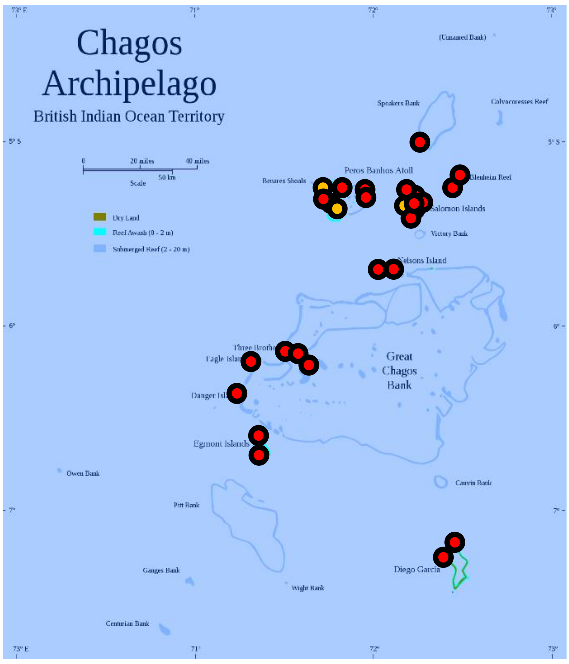

Fig 16. Red dots show locations of instruments installed on forereefs and in lagoons for operation during 2019-2020. Orange dots show locations of Benthic Flux experiments wherein up to 20 different instruments were installed to log at high frequency for 2-3 weeks during March 2019. Altogether 39 instruments (logging T, S, O_2, currents, light, pressure) were deployed for the year. Another 43 instruments (logging T, S, currents, O_2, CO_2, pH, pressure, and light) were deployed for the benthic flux experiments.

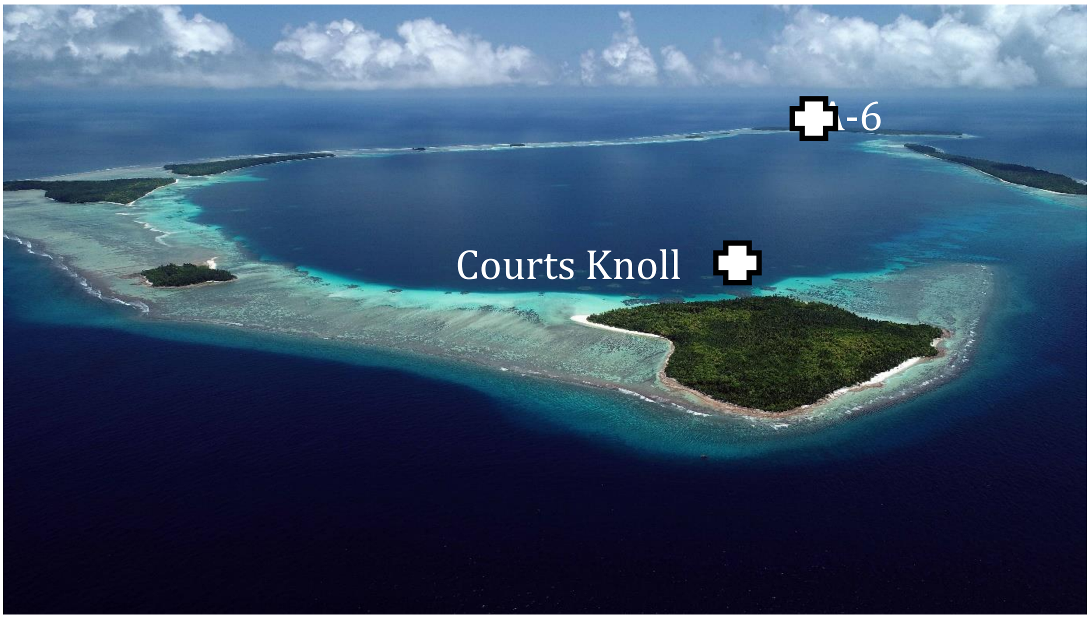

Fig 17. Aerial View of Salomon Atoll captured by drone during March, 2019. Arrows and labels show locations of temperature loggers with data shown in Figure 3.

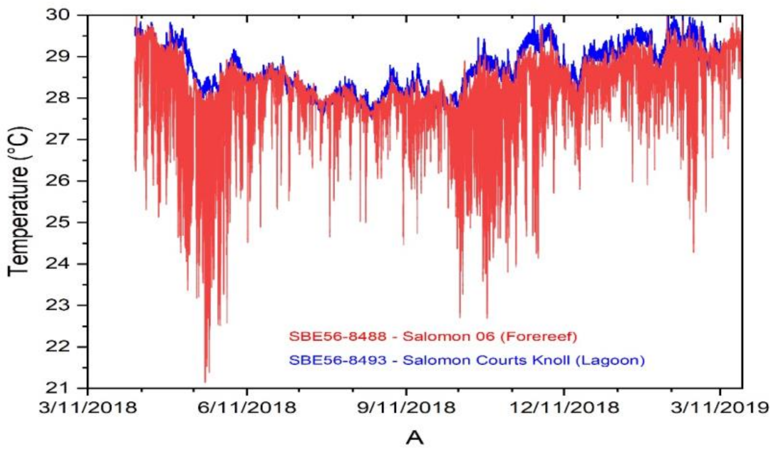

Fig 18. Time series of sea temperature at 10 meters from two sites at Salomon Atoll, one in the lagoon and one on the forereef outside the lagoon (red: Salomon Forereef site 6; blue: Salomon Lagoon, Courts Knoll). Temperatures were slightly warmer in the lagoon and varied from 28-30°C. Forereef temperatures range from 21-30°C, a large range for a coral reef.

Figs 18, 19 and 20 show the Benthic Flux experimental setup. We used new techniques to measure the rates of two key processes, Net Community Production/ Respiration (NCP/R) and Net Community Calcification/Dissolution (NCC/D). NCP/R captures the balance between primary production of fixed C (cell mass and food) and respiration whereas NCC/D elucidates the balance of calcification and dissolution on a reef. Sustained measurements of both parameters are important for reef studies, as this determines reef condition. Is a reef tract gaining mass from precipitating carbonate minerals versus losing mass through dissolution? Is the reef acting as a net exporter of food to surrounding marine environments? The ratio of NCP/R to NCC/D is thought to be a direct indicator of coral reef health. When reefs degrade, community dominance usually shifts from stony corals to macroalgae causing the ratio of NCP/R to NCC/D to rise. Previous work suggests that the maintenance of NCP/R to NCC/D ratios within a healthy range requires substantial reef biodiversity, hence our interest in coupling biogeochemistry with analyses of reef biodiversity. The biogeochemical characterization of reef metabolic state is complementary with the other reef-based project work funded by the BPMS. We are making all of our physical and biogeochemical data available to all PIs and are collaborating with Professor Chris Perry and his research team exploring other methods of determining reef carbonate mass balance. Fig 21 shows a high resolution 14-day time series of pH recorded at the Ile Anglaise “Super-BEAMS” benthic flux experimental site. This the first time this spectrophotometric instrument has been used on a coral reef and comparison with a DuraFET-based logging instrument shows good agreement.

Instruments deployed in 2018 for recovery during 2019 as well as those deployed during the March 2019 Grampian Frontier and Vava II cruises operated with a 95% success rate, a good result for moored instrumentation in energetic reef environments. Initial results indicate net autotrophy at all sites, suggesting that food is available for export offshore and to depth in at least some BIOT reef systems. Examining the time series data from 2018-2019 we observed several additional instances of cold-water intrusions on BIOT forereefs. We first reported these events at the 2018 London BPMS symposium and now consider them to be widespread and likely to impact thermal resilience/tolerance of BIOT corals.

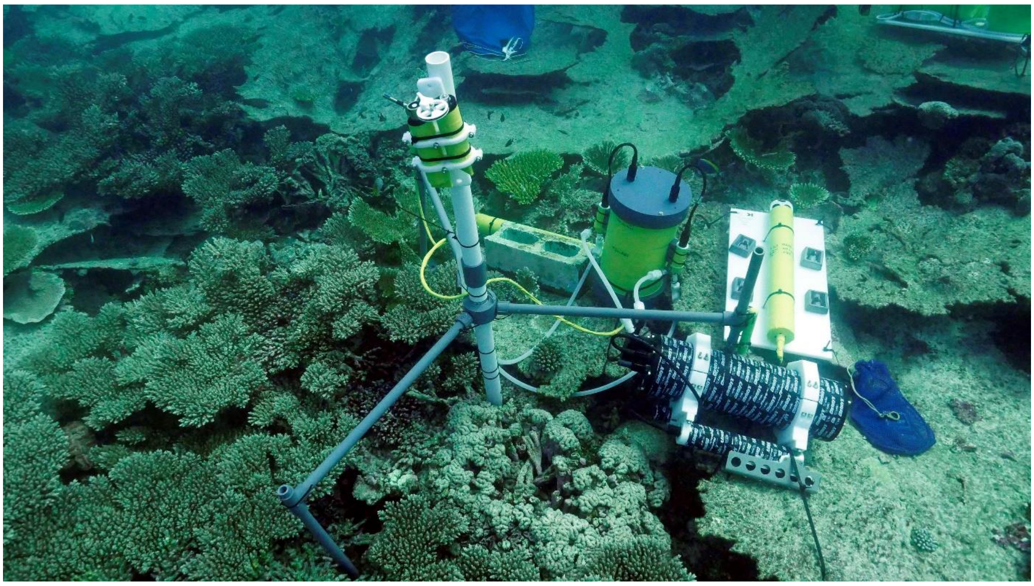

Fig 18. (above). Benthic flux measurement system deployed at Ile du Coin from March 4-21, 2019. The system includes a 2Mhz HR Acoustic Doppler Profiler (ADP) as well as a SeaBird Electronics SeaPhOx unit, measuring O_2 concentrations, pH, salinity, and temperature in a seawater stream pumped from different depths above the substrate. Combined with knowledge of the flow regime, near-bottom biogeochemical gradients are used to calculate net carbonate and primary production. We also track water depth (tides and waves) as well as incoming solar intensity.

Fig 19. (left). We used Acoustic Doppler Velocimeters, such as this Vector instrument, to measure rapid small-scale changes in 3D velocity. This helps us understand turbulence and benthic boundary layer conditions.

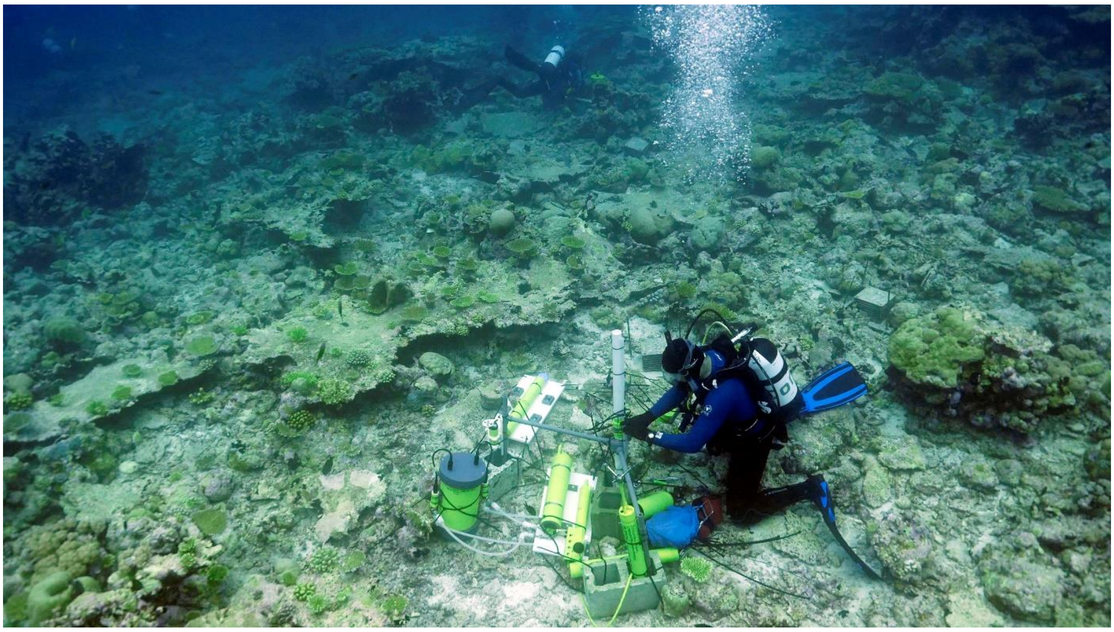

Fig 20. (above). Benthic flux measurement system deployed at Ile Anglaise from March 10-24, 2019. The basic setup was similar to that shown in Figure 4 but included an additional 6 instruments with new technology pH loggers, a direct pCO_2 logger, multiple oxygen loggers, a thermistor chain, and several kinds of high precision current meters. An Acoustic Doppler Current Profiler (ADCP) was deployed for a year (2019-2020) and can be seen at the very top of the above photograph.

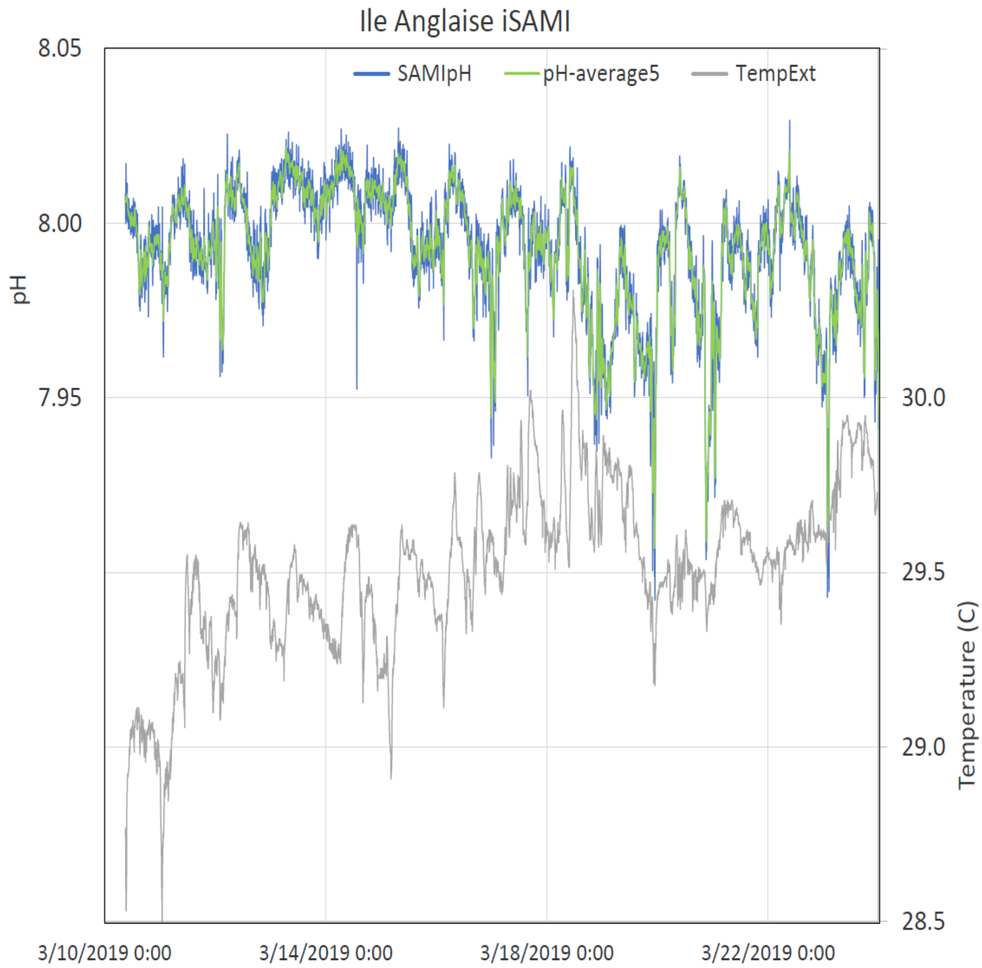

Fig 21. (left). pH and temperature recorded by a new design pH logger that exhibits both high precision and low drift during long ocean deployments. Diurnal variability is seen in pH as the community exhibits large changes in both primary production and carbonate formation, in this case forced by the day/night cycle.

4.2 Sound recordings

4.3.1 Introduction

The team were collecting data in collaboration with the University of Exeter (Steve Simpson, Tim Gordon) to feed into their global sound database and to incorporate acoustic monitoring into existing protocols for monitoring reef health on a global scale.

Healthy coral reefs are inherently noisy places: communities of soniferous invertebrates and fish combine to produce 'soundscapes' that can be heard for miles around. A range of invertebrate and fish species use reef sound to guide their detection, orientation and settlement to suitable habitat after a pelagic larval stage (Simpson *et al.*, 2005). However, recent work on the Great Barrier Reef shows that degradation reduces the intensity and changes the acoustic characteristics of reef soundscapes (Gordon *et al.*, 2018). Between 2012 and 2016, two severe cyclones and unprecedented bleaching caused profound damage to the northern Great Barrier Reef. Over this period of time, the intensity of reef soundscapes dropped by 75%. Further work found that degraded soundscapes had lost their attractiveness to reef fishes (Gordon *et al.*, 2018). Field experiments using underwater loudspeakers to broadcast healthy or degraded soundscapes around patch reefs; degradedsounding reefs attracted 40% fewer fishes than their healthy-sounding counterparts. Reduced attractiveness of degraded soundscapes has concerning implications for reef recovery prospects. Healthy fish populations help reefs recover from degradation, as grazing of macroalgae facilitates coral regrowth (Graham *et al.* 2015). Fish populations are sustained by larval recruitment, so if young fish can no longer hear their way home, this will exacerbate degradation (Fig. 2).

4.3.2 Methodology
Two methods were trialled for the first time in BIOT during the Vava II expedition; the first was a 24 hour deployment of a Soundtrap hydrophone at locations in the lagoons of both Peros Banhos and Salomon atolls and the second was a one hour deployment of a 10 GoPro cameras at 100m intervals along a transect in Peros Banhos and Salomon atolls. The sound files are now with the team at Exeter University for analysis.

4.4 Sea cucumber survey:

Sea cucumbers were historically a target for illegal fishing in BIOT, resulting in a marked decline in population abundance of *Stichopus chloronotus, Holothuria atra* and *Holothuria nobilis* between 2002 and 2010 (Price et al., 2010). A count of the sea cucumber population in Salomon lagoon survey was done in 2009 and 2013 and this count using the same methodology was repeated in 2019 as part of the Vava II expedition.

The method used two observers seated in the bow of a RIB each observing a transect of benthos approximately 2m wide from the centre line of the bow of the boat – making an entire transect 4m wide. A track of >20km was followed around the outer edge of the lagoon, tracking as close to the shore as depth would allow (Fig 22). The entire area surveyed was therefore 8km^2 and the survey took more than six hours to complete.

The total count was 5,340 sea cucumbers which compare to 2,146 in 2009 and 1,661 in 2013 using the same method. A key observation in the heterogeneity of the distribution with long stretches of the transect recording no sea cumbers at all and a long stretch parallel with Ile Anglaise towards the end of the transect recording the majority of the observations.

Fig. 22 Waypoints recording the route taken around the Salomon atoll lagoon for the sea cucumber survey

These figures may show continued recovery from poaching events in the mid 2000s and this would be an interesting survey to repeat more frequently.

5. Seagrasses on the Great Chagos Bank

5.1 Introduction

20% of satellite tagged green turtles nesting on Diego Garcia have migrated to the south-eastern Great Chagos Bank (GCB), the only foraging habitat for green turtles identified in BIOT to date (see Esteban et al. 2018). Foraging ground locations of green turtles satellite tracked to the south-eastern Great Chagos Bank in 2016-2018 were analysed to select separate home range areas for survey to assess the seagrass ecosystem health, productivity, environmental conditions and importance for associated fauna (Fig 23). These surveys were also conducted at a shoal south of Danger Island.

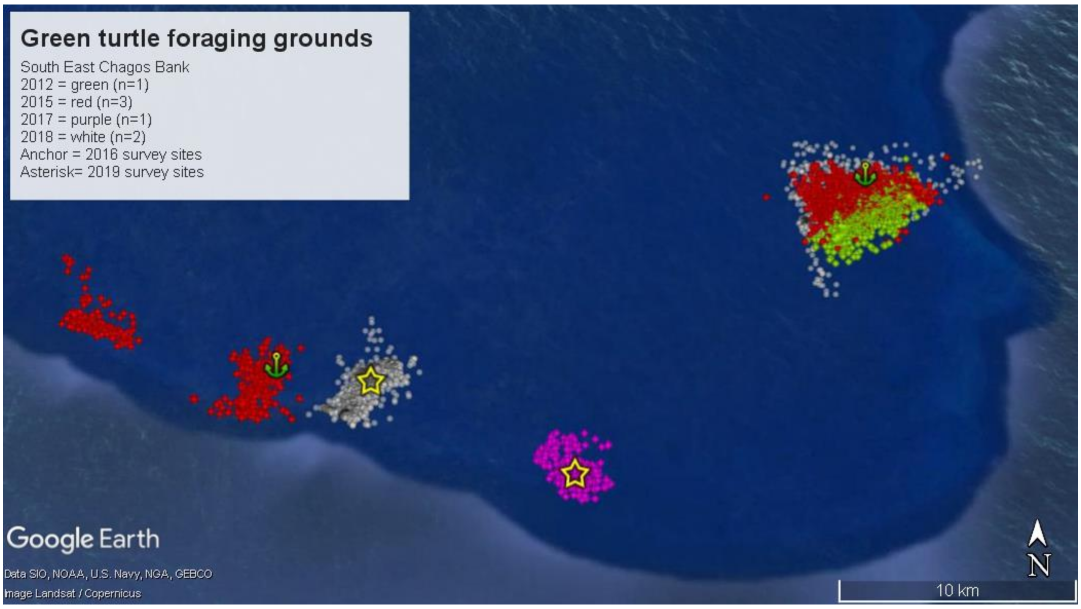

Fig 23. Overview of foraging locations by green turtles in south-eastern Great Chagos Bank showing two areas surveyed in 2016 (anchor symbol) and area surveyed in 2019 (star symbol; purple circles).

5.2 Methodology

Surveys took place on 20 March (SE GCB; NE & RJ) and 18 March (Danger Island; NE & HK) to assess seagrass cover, morphometrics, light conditions, associated fauna and take samples. Unfortunately, despite our best efforts and due to extreme swell and current conditions, it was not possible to conduct the planned turnover rate experiment at Danger Island or in south-eastern GCB. Whilst at the sites, dropdown video was deployed to validate foraging (diurnal) and resting (nocturnal) sites for tracked turtles and assess importance of connectivity between seagrass and reef ecosystems. We also used Baited Remote Underwater Video (BRUV) to assess importance of seagrass on the GCB for fish diversity.

5.3 Key results included:

- All daytime locations of green turtles satellite tracked on GCB are at sites with seagrass *Thalassodendron ciliatum* (depth range: 23-30 m). These sites are new confirmed locations for seagrass meadows in BIOT. Midpoint location of seagrass site: S 06°42.9775' E 072°11.247'E.
- Deployed BRUVS and light loggers on the southeastern GCB seagrass beds (depth range: 23-28 m), and SCUBA dived to video survey 3 x 60m transects and collect seagrass samples for further analysis.
- Extended records of distribution of seagrass meadows near Danger Island by >1 km with depth range of 6.5-9.5 m and covering an area >0.4 km2. Midpoint location of site: S 06°27.428' E 071°14.515'. Deployed BRUVS and light loggers at two sites (conditions unsuitable for dive surveys).
- Observation of adult green turtle surfacing at 7 km distance from the nearest beach on Danger Island confirms that this is likely to be a green turtle foraging ground.
- Visited two atolls in the Maldives (Laamu on 6 March; Meemu on 7 March) on route down to BIOT to survey and validate foraging grounds of green turtles satellite tracked from Diego Garcia in 2012 and 2018. The benthic habitat was dominated by macroalgae (*Halimeda* sp; *Caulerpa* sp).

Fig 24. Seagrass sampling in southeastern Great Chagos Bank. (a) Apical meristem of *Thalassodendron ciliatum* shows the vertical and horizontal rhizomes providing a dense canopy that is used by large shoals of fish.

6. Island habitats

6.1 Island vegetation surveys

Helicopter flights were organised by NE between 15-29 March using the same methodology (speed, altitude, camera settings) as 2016 to systematically map island vegetation. Photos were taken of islands in permitted areas visited during the expedition (i.e., Peros Banhos, Salomon and Egmont atolls, parts of Great Chagos Bank). Fig 25. demonstrates the value of these periodic aerial surveys for assessment of vegetation and sediment morphological change of islands across the Archipelago. Image files are available from NE.

Fig 25. Aerial photos taken from helicopter can be used to assess island accretion and map vegetation, for example in Peros Banhos (a) The existing offshore sand bars at the tip of each island have extended to join Ile Passe and Moresby Island since 2016; (b) Vegetation has now grown on the former sand bar between Ile Gabrielle and Ile Monpatre so that they can be considered as one island.

6.2 Assessment of plastics debris on beaches across BIOT

The objective of this assessment was to commence quantification of plastic debris on beaches of uninhabited islands across the Archipelago. Plastic accumulation was assessed on or close to the sea turtle nesting line (defined as a transect line connected body pits closest to the sea). Data will be analysed as part of a study to assess the effects of plastics on sea turtle nesting activities. Three surveys were conducted by members of the science team (NE, HK, RJ, PC) with assistance from crew. Macroplastics were assessed using (1) a photo quadrat method (20 photos of a 0.25 m2 quadrat in a 100 m2 plot between the most recent high water level and littoral vegetation boundary with the beach), (2) recording all marine debris along a 100 m transect along the beach (between high water line and impenetrable vegetation) on the Marine Debris Tracker app (University of Georgia, using the National Oceanographic & Atmospheric Agency (NOAA) beach debris list) and (3) sampling 10 cm diameter cores from the surface to 60 cm depth to assess microplastics.

Achievements include:

- Surveys of macroplastics on seven islands and three atolls (Egmont, Peros Banhos, Salomon) documenting 918 items of plastic. This is the first contribution of data from BIOT to Marine Debris Tracker's global, open access database.
- Sand core sampling to assess the effect of microplastics on sea turtle nesting across three atolls, Egmont, Peros Benhos, Salmon (5 samples at 20% intervals along turtle nesting beaches of one important nesting island on each atoll).

Fig 26. (a-c) Sand core sampling took place at Ile Parasol (Peros Banhos), Ile Boddam (Salomon) and Lubine complex (Egmont Islands). (d) Photo quadrats were taken at seven islands on the same three atolls.

Over 80% was found to fall into three categories: polystyrene pieces, flips flops and single-use plastic bottles. Plastic bottles had labels originating from 17 countries from Japan to Tanzania with the largest contributor being Indonesia, almost 3000km to the west.

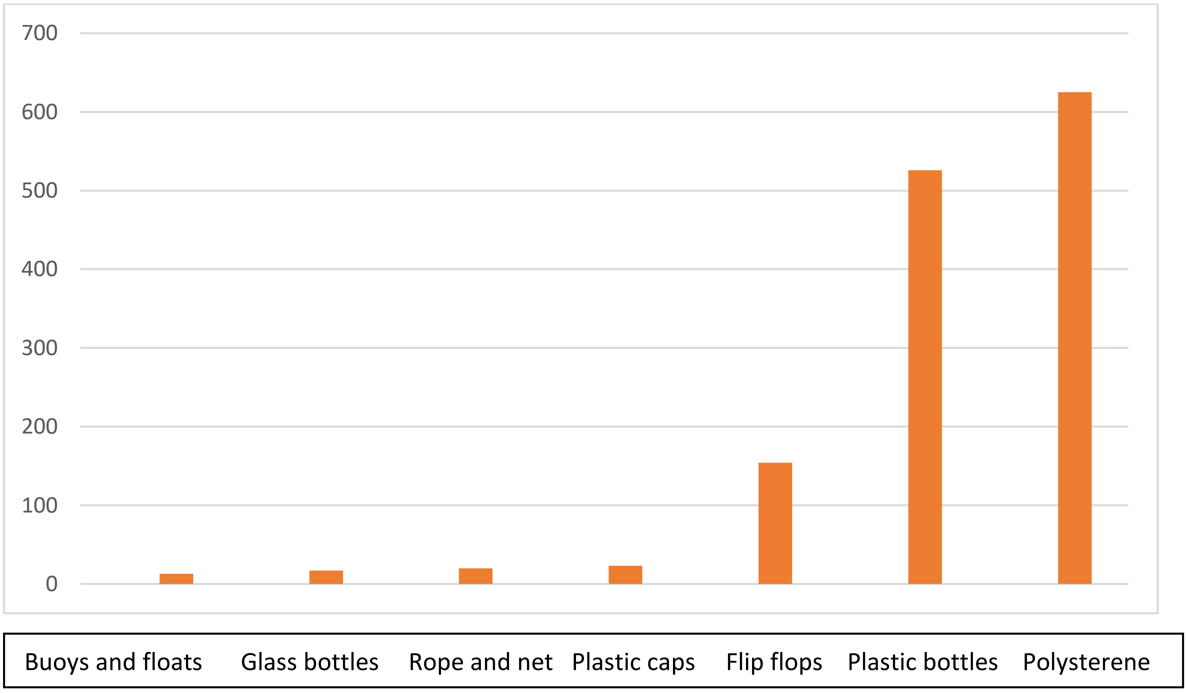

Figure 27. Preliminary results from 100m transects – most common items recorded

6.3 Mangrove swamp on Moresby Island

There are only two islands in BIOT with mangroves, Moresby and Eagle Island with the latter being very degraded. The Moresby mangroves were only discovered in 2010 during island surveys by the Royal Botanic Gardens Kew, comprising two species of mangrove – *Lumnitzera racemosa* and *Pemphis acidula*. As part of the Vava II expedition, a team dedicated a day to reassess the status and health of the mangrove system on Moresby. The assessment was completed using three methodologies: aerial survey, GPS mapping and mangrove community structure.

6.3.1 Aerial survey

An aerial survey (using drone Autel Robotics EVO) was conducted by NE following methodology provided by Kew. Transect coordinates and video survey files will be analysed by Kew and used to help prepare vegetation maps.

6.3.2 GPS mapping

A physical map of the Moresby Island mangrove swamp was documented by walking the perimeter using a GPS track (figure 28) and using a series of waypoints to mark locations of possible ingress points of water that is required to maintain the viability and health of the mangroves. This map and mangrove area will be compared with previous survey, last conducted in 2010, as part of the collaboration with Kew.

Twelve inundation points (fig 28) were marked on the seaward boundary but there did not appear to have been any very recent flooding. Large amounts of plastic debris had been observed in the swamp on previous visits (Pete Carr *pers. comm*.) but this was not seen on this occasion, which suggests an inundation big enough to carry this waste back out to sea.

Fig 28. Example of inundation point documented by waypoints during the mangrove survey

6.3.3 Mangrove community structure.

The MCS survey follows a method adopted from English et al. (1997) where 100m^2 plots are surveyed at representative points through a mangrove forest. The general status of the mangrove ecosystem was assessed as to species composition, density and health. A total of three 10m X 10m plots were surveyed at the northern, southern and middle points of the forest on the seaward side, with GPS coordinates taken at each corner of the plot. The number of trees (girth at breast height GBH >4cm), saplings (GBH < 4 cm, height > 1 m) and (seedlings: GBH < 4 cm, height < 1m) were recorded at each plot as well as the reproductive (fruiting, flowering) and health status.

In terms of species composition, the Moresby Island mangrove swamp is dominated by *Lumnitzera racemosa* with small patches of *Pemphis acidula*. Only one *P. acidula* tree (healthy, but not fruiting) was recorded in Plot 2.

Fruiting and flowering were recorded in *L. racemosa* at all plots, showing the trees are reproductively active across the island (fig 29). Saplings were recorded at all sites, but seedlings only recorded in plot 2 (fig 30). In addition, dead trees were also recorded at all plots and in notably high numbers in plot 2 (fig 29, fig 31). It would be worth completing these surveys annually to monitor the health status of the forest more closely.

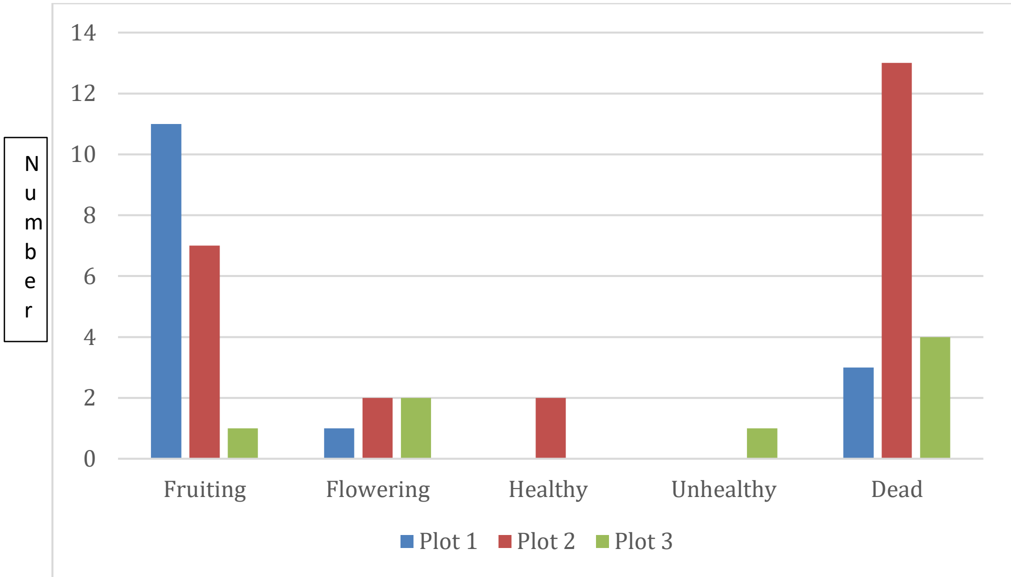

Fig 29. Health and reproductive status of mangrove (*Lumnitzera racemosa*) on Moresby Island. Healthy/non healthy records apply to specimens that were not flowering or fruiting.

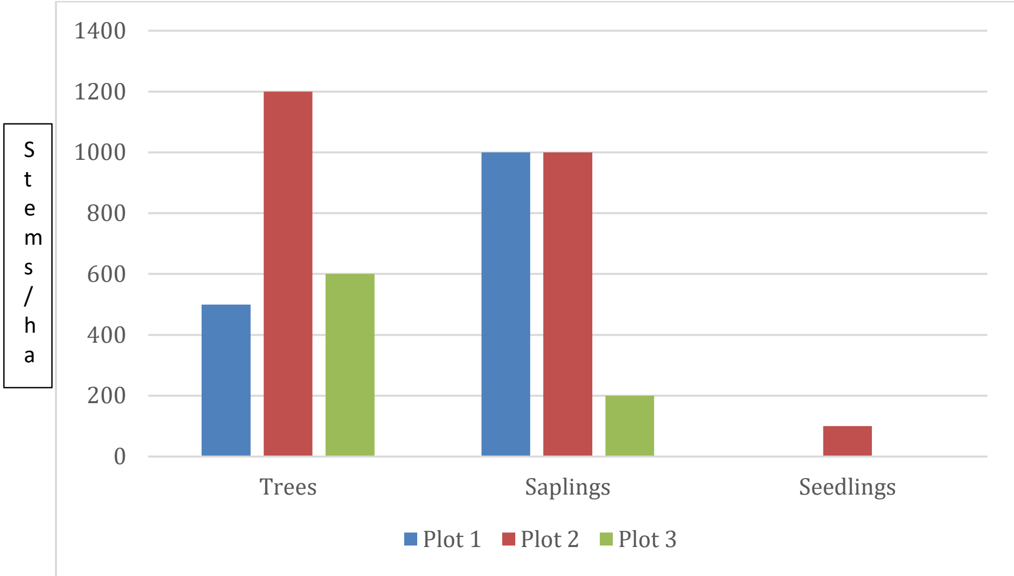

Fig 30. Population structure of mangrove (*Lumnitzera racemosa*) on Moresby Island (stems/ha).

a) Example of healthy mangroves

b) Example of dead mangroves

Fig 31. Examples of the healthy and dead patches of mangroves observed on Moresby Island

6.3.4 Water quality parameters

The mangrove water level was quite restricted (Fig. 32) with areas that had clearly been inundated previously but were now dry. Where standing water remained, it had a maximum depth of approximately 40cm and there were large areas of dry mud bordering each body of water where the level had receded.

Fig 32. Water quality testing in the mangrove on Moresby

Water quality readings were taken from stationary water in locations across the mangrove forest using a high-grade water quality meter (loaned from ZSL's Aquarium). Water quality tests showed extremes of temperature and pH (again suggesting extensive evaporation and no recent flooding of seawater) and the absence of mosquitos was noticeable – the conditions being too extreme for them to reproduce in the water.

Site	pH	O2 mg/l	Temp ºC	Conductivity ppt
Plot 1	6.36	13.76	33.2	33.4
Plot 2	8.58	10.74	33.4	33.8
Plot 3	9.20	16.87	41.8	37.4
Water pool east end	9.09	13.73	38.2	33.2

Table 3. Water quality parameters recorded at each location

6.3 Terrestrial invertebrates

The expedition gave an opportunity to easily visit a wide range of islands and to move between them quickly. Having extensively reviewed all records made of any invertebrates observed on any island in BIOT through history, aggregated in this database, it is clear that many islands have either very few or no invertebrate records at all.

Collections of terrestrial invertebrates were made on: Ile verte, Ile Parasol, Ile Longue, Mapou, Vache Marine, Ile Grande Coquillage and Moresby. The first four of the islands have no previous records of terrestrial invertebrates on file.

A total of more than 300 specimens have been preserved for further analysis and identification. Notable records are a nematode collected on Vache Marine and a tick collected on Parasol that are both new records for BIOT.

Fig 28. Aranae sp. Collected on Isle Verte, Peros Banhos

Fig 29. *Amblyomma cajennense* collected on Parasol a new record for BIOT

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