Bertarelli Foundation British Indian Ocean Territory
Marine Science Expedition Report
4th – 20th April 2016

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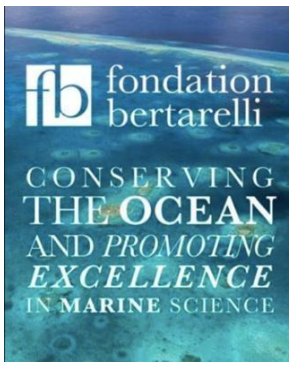

Acknowledgements
Many thanks to the Bertarelli Foundation for supporting the expedition by providing funding, equipment, complex logistical arrangements and their enthusiasm for the ambitious research and conservation objectives of the Consortium represented by the expedition team. The science team were incredibly privileged to conduct the expedition on the Vava II, for which we are enormously grateful to the Bertarelli family. The expedition would not have been possible without the expertise and support of the Captain, officers and crew who became an integral part of the science team and maximised the scientific outputs. The professionalism, enthusiasm and dynamism of the Vava II team made the expedition work in spite of the challenging mix of activities and the team were also an absolute pleasure to work with. We are also extremely grateful to the British Indian Ocean Territory Administration for the considerable administrative logistical support provided from London, Diego Garcia and from the Pacific Marlin that facilitated the delivery of a safe and successful expedition that achieved some very valuable scientific outputs.

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I. Expedition Highlights

- Deployed a total of **153 animals with 248 tags**: 49 grey reef sharks (*Carcharhinus amblyrhynchos),* 69 silvertip reef sharks (*Carcharhinus albimarginatus*), 1 blacktip reef shark (*Carcharhinus melanopterus*), 1 whitetip reef shark (*Triaenodon obesus*), 26 manta rays (*Manta alfredi*) and six species of teleosts including yellowfin tuna (*Thunnus albacares*), kawakawa (*Euthynnus affinis*), black saddled grouper (*Plectropomus laevis*), dogtooth tuna (*Gymnosarda unicolor*), sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*).
- **137** animals where equipped with **acoustic** tags, **85** with **conventional identification** tags for mark recapture analyses, **19** with **satellite** enabled tags and **7** with **camera** tags. Eight grey reef sharks were double tagged.
- **Four mantas** that were tagged in 2015 were **sighted again** with the acoustic tags still attached.
- Tagging data showed one **manta** completed a **round-trip journey of at least 400 km**, with the manta moving from Victory Bank to Egmont, a distance of ~160 km in ~ 4 days.
- **Two sharks were recaptured for the first time** during the expedition. A male grey reef shark and a female silvertip shark tagged in 2015. The animals were in excellent condition and these recapture data help statistical estimates of population abundance.
- Initial camera tagging data corroborated evidence from previous expeditions that suggest a **deep-water refuge for sharks** offshore to the **south of Peros Banhos**.
- For the two most frequently tagged sharks, **silvertip and grey reef sharks**, the measured total **lengths were significantly (albeit slightly) bigger** in this expedition than in previous years. It remains to be determined whether this is an effect of fishing in different locations with respect to previous expeditions or if there is a shift in the size structure of the shark assemblage (i.e. a sign of recovery).
- The surprising abundance of pelagic fish observed at **Speakers Bank** led Dr Barbara Block to refer to it as a **"tuna hotel".**
- **63 acoustic receivers** (VR2, VR4-UWM, VR4G) in the existing array were serviced and downloaded. Downloads of the receivers resulted in **99,814 detections from 92 animals**. We deployed **30 new receivers** to expand the array to cover additional habitats and regions of the archipelago. At the conclusion of the expedition, the array included a total of 93 acoustic receivers deployed across the archipelago.
- To study **reef processes and seawater chemistry**, the first data of this kind in the BIOT region were collected - **117 water samples** across the archipelago.
- To apply a **new molecular technique** in BIOT, water samples (n=81) for **environmental DNA** studies were collected from 40 sites throughout the archipelago to determine recent genetic presence of a) pelagic animals and b) microbes, invertebrates (including corals) and fish from the reefs.
- The **first SeaFet long-term pH logger** was installed in BIOT to track changes in ocean chemistry and help detect and quantify ocean acidification.
- Instruments are designed to record data on environmental physics and chemistry for a full year were installed, including **13 temperature sensors, 2 pressure sensors, 1 salinity/temperature sensor, a pH logger, and an Acoustic Doppler Current Profiler (ADCP).**
- As a pilot effort for BIOT we used an **aerial camera system to collect several hours of shallow reef habitat imagery**. We will use these images to explore automated information extraction.
- The reef team surveyed **17 key reef sites**, amounting to **~1010 mins of dive time**, **~875 mins of video footage** recorded and **~1000 photographs recorded** along transects.
- Over **2000 m^2 of reef was surveyed** using a mixture of photography and videography to allow analysis of the 3D structural complexity, composition and function of the shallow reefs of the Chagos Archipelago.

- Detailed data were collected from 14 coral reef sites across the archipelago to **record the incidence of bleaching and disease** on reef-forming coral communities.
- Initial observations are that **while much of the structure of the reefs is still intact, much of the reef has suffered heavily from last year's bleaching and is still being affected by the current El Niño warming event.**
- **Coral recruitment levels are high** at a majority of sites, and if recruit survival is high, reef functioning will likely recover, as occurred following the 1998 bleaching event.
- A **new 'structure from motion' technique** was applied and initial analysis shows it effectively capture the reef organisms surveyed in sufficient detail to record colonies to genus level, and enable measurement of a number of metrics known to be important to sustaining healthy reefs.
- We conducted the **first twilight reef surveys** (reefs slopes deeper than 30m) conducted in BIOT since the 1980s. Reefs in the 30-60m depth range were surveyed at 12 sites around the Chagos Archipelago. Surveys indicate high coral cover (>80%) in some locations, implying deeper reefs may act as a refuge for threatened shallow reef species and have a crucial role in overall reef resilience.
- We identified several shallow reef species **not previously reported** from twilight reefs, including the **endemic Chagos clownfish (*Amphiprion chagosensis*) at 37 metres**. In addition, we regularly observed sharks on twilight reefs, suggesting these **deep reefs have an important role for larger pelagic species**.
- An unusual sighting of a **pod of false killer whales** (*Pseudorca crassidens*) was documented at Egmont atoll.

II. Communications

- Daily Powerpoint presentations were submitted to the Captain and crew, Bertarelli Foundation and BIOTA that documented science activities and observations of note.
- Blogs were submitted by several expedition team members and published online and further communications are planning following the expedition.
- Updates were also shared via Twitter, using #BIOTExped16.
- Video footage was captured and a short film compiled by Prof Dunbar, Stanford University.

III. Summary of Achievements against Objectives

	Expedition objective	Status
1	Maintain existing Vemco acoustic receiver array	**Complete** – 63 receivers serviced and downloaded.
2	expand the array by adding additional moorings and receivers	**Complete** – 30 new receivers installed plus one new VR4 Global Unit.
3	Conduct electronic tagging of teleosts and elasmobranchs that utilize the habitat in and around the receiver array.	**Complete** - 153 animals deployed with 248 tags.
4	sample tissues for isotopic analyses to provide information on the trophic ecology and habitat use of species within BIOT	**Complete** – samples and data collected from tagged animals.
5	Collect water samples to analyse for environmental DNA (eDNA) signatures	**Complete** - Water samples (n=81) for eDNA were collected from 40 sites throughout the archipelago to determine recent genetic presence of a) pelagic animals and b) microbes, invertebrates (including corals) and fish.
6	Collect deployed sensor instruments (temperature and oxygen), and redeploy additional units for collecting environmental data	**Partially complete** – due to logistical constraints, existing sensor instruments were not collected. New instruments were deployed designed to record data on environmental physics and chemistry for a full year and include 13 temperature sensors, 2 pressure sensors, 1 salinity/temperature sensor, a pH logger, and an Acoustic Doppler Current Profiler (ADCP).
7	To obtain a detailed baseline of key reef variables during the expected 2016 bleaching event.	**Complete** - 117 seawater samples were collected from 15 reef sites throughout the archipelago for carbon system measurement. The reef team surveyed 17 key reef sites, amounting to ~1010 mins of dive time, ~875 mins of video footage recorded and ~1000 photographs recorded along transects. An aerial camera was used to collect imagery of larger portions of the reef system than can be captured by divers in the water. This allows work in specific locations to be placed into a whole reef system context.
8	To quantity three-dimensional reef complexity to examine the impacts of beaching on reef functioning and habitat provision.	**Complete** - Over 2000 m^2 of reef from 14 sites across the archipelago was surveyed using a mixture of photography and videography.
9	To provide the first surveys of the lower mesophotic zone (60 – 150m) using a mini-ROV unit.	**Complete** - Surveyed reefs in the 30-60m depth range at 12 sites.
10	To document changes in key reef fish species related to the expected 2016 bleaching event.	**Complete** – 17 key reef sites were surveyed for reef fish populations using videography that can be compared to previous years of reef fish population assessment.
11	To inform the BIOT Administration on the findings to inform management of the marine reserve, as well as to contribute high calibre science from a globally important coral reef reference sites	**In progress** – Report and follow up meetings planned with the BIOT Administration. Science outputs being developed as data are analysed and publications prepared. Results will also be shared at a series of international conferences this year.

IV. Background

It is widely acknowledged that the global ocean is in decline with marine ecosystems expected to approach terrestrial rates of extinction in response to increasing industrialisation. The effects of climate change, global fisheries exploitation and the expansion of activities such as deep-sea mining mean that there is an urgency to move to more sustainable systems of ocean management. Spatial conservation measures such as marine protected areas (MPAs) are seen as essential to shift the oceans from decline to recovery and to ensure that the critical services provided by marine ecosystems remain in perpetuity. Several States have declared large-scale MPAs, most notably the UK, the USA and small islands dependent States such as Palau. Despite this apparent progress there is strong opposition to MPAs particularly from the fishing industry and States with distant-water fishing fleets. We need to increase our capacity to observe the seas today to enable predictions of changes to the biogeographic range and ecology and function of these productive ecosystems over the next century.

The Chagos Archipelago, also known as the British Indian Ocean Territory (BIOT), is the world's largest no-take marine reserve, declared by the UK Government in 2010. It was primarily established to protect the shallow-water coral reef ecosystems of the archipelago, which remain in excellent condition compared to most reefs in the world. The marine reserve also includes a huge area of pelagic and deep-seabed habitat (>200m depth) including ~10% of all Indian Ocean seamounts. Fishing interests have generally remained opposed to the reserve as they claim it provides no protection to target and bycatch species and limits their opportunities to fish. However, only 3% of the marine ecosystems of the BIOT MPA, by area, have been studied which means that justifying its wider conservation benefits has been difficult. This also reflects a general lack of study and understanding of the effects of establishing large-scale marine reserves on ocean biota. In addition, it has become apparent through recent work funded by the Bertarelli Foundation that there are serious issues related to enforcement within the reserve which may be increasing and which are compromising its conservation value. These issues have thrust the BIOT MPA into the frontline of both scientific understanding and management of large-scale marine reserves now subject to intense scrutiny by the international community involved in ocean governance.

From 2013 to 2015, the Bertarelli Foundation supported leading multi-disciplinary marine science organisations from the UK, USA and Australia to establish a consortium that developed a 5 year science plan to substantially advance knowledge of all aspects of the physical and biological oceanography of the BIOT MPA as well as to establish a new global standard in its monitoring and enforcement. The consortium has set out to test and establish the efficacy of such large-scale marine reserves globally in order to improve management and conservation, but also provide fundamental scientific advances in marine biodiversity, ecosystem function and human impacts on the ocean. Such scientific endeavour sets the BIOT MPA on a trajectory to become a global exemplar for large MPAs, underpins the future conservation management of the reserve and will act as a baseline against which to evaluate and predict environmental changes.

Our approach is to integrate international partners into our collaborative, cross disciplinary programme of activities, each of which addresses knowledge and information gaps outlined in the BIOT Interim Conservation Management Plan (BICMP 2014). The research closely maps onto the BICMP priorities. We will help a) monitor sentinel species population, habitats, and migration corridors, in order to promote their conservation and protection (BICMP Priority areas 1 and 2); b) reduce threats to sentinel species and their habitats, for example through assessment of threat of mortality of species during migration to distant areas (BICMP Priority area 3); c) drive informed decisions to minimise adverse human impacts on the environment by rigorously assessing habitat and increasing effectiveness of the reserve (BICMP Priority area 4); and d) communicate the science and associated conservation benefits of our work widely (BICMP Priority area 5).

In this expedition, our key objectives were to:

a) Monitor and track sentinel species, with a focus on those vulnerable to illegal fishing, particularly sharks and manta rays.
b) Assess the full extent and severity of bleaching around the Chagos Archipelago during maximum due to the current El Nino which is causing a global coral bleaching event.
c) Establish biogeochemical monitoring to document changes over short and long time periods to detect and quantify ocean acidification as well as the magnitude of natural metabolic cycles on selected reef sites.

V. Project Summary Reports

a) Sentinel Species Tracking

Team: Barb Block, Taylor Chapple, Aaron Carlisle, Jon Dale, Francesco Ferretti, Luke Gardner, Robbie Schallert, Dave Tickler.
Stanford University, University of Western Australia

Introduction

The British Indian Ocean Territory (BIOT) is the world's largest no-take marine protected area (MPA) and effective long-term biodiversity conservation and regional fisheries management strategies are only possible with extensive knowledge of how and when species use the MPA. The Bertarelli Foundation has funded the Chagos Archipelago Science Consortium to assess how mobile species such as elasmobranchs and teleost fishes use this area. To achieve these goals, researchers from Stanford University, the University of Western Australia (UWA) and the Zoological Society of London (ZSL) have used electronic tag technology to study the residency, habitat use and connectivity of fish, sharks and manta rays within and around BIOT. This research is vital for understanding the importance of the archipelago as a refuge for pelagic fish and elasmobranchs, as well as the more residential reef associated animals. Telemetry data obtained from acoustic and satellite tags of marked animals can be used to estimate home range and habitat use of the focal species. Such geospatial data are vital for understanding habitat use, identifying aggregation hot spots and estimating shark density throughout BIOT. Electronic tag data are also a critical component of mark recapture models that are being used to assess the population size of different species as well as aiding in the development of new technology, such as the FAST tag. In addition, the tagging effort at this location provides information on large-scale, long-term movement patterns and connectivity of populations of reef and pelagic species across with wider Indian Ocean basin. The Consortium team is also developing the site as an ocean observatory for monitoring important abiotic variables pertinent to climate change by placing instruments with a long-term environmental data acquisition potential.

To support these goals, the Consortium team undertook two research expeditions in March and April 2016 aboard the M/Y Vava II. During these expeditions, the scientific team aimed to maintain and expand the current acoustic monitoring array and deploy additional acoustic, satellite and biologging tags on pelagic and reef associated teleost and elasmobranch fishes. We also collected water samples to detect the genetic signatures (environmental DNA; eDNA) of animals associated with the archipelago and serviced and deployed physical oceanographic instruments to monitor the physical and biogeochemical processes shaping the ecosystems of BIOT.

Methods

After capture, sharks <200 cm TL were taken onboard, sexed, and measured for total length (TL), fork length (FL) and pre-caudal length (PCL). If the individual was male, internal and external clasper lengths were also taken. For larger sharks, these procedures were done while the animal was in the

water alongside the boat. On average, sharks were handled onboard for the sampling, tagging and measurement operations within 6.3 minutes. Handling time was longer in the water and when sharks had to be equipped with SPOT or camera tags (9.2 minutes).

Figure 1: Researchers tagging mantas (left) and sharks (right) with the support of the crew from Vava II.

Tagging operations (Figure 2) consisted of making a small incision in the abdominal region of the animal to create an opening for insertion of an acoustic tag into the peritoneal cavity. An identification "conventional" tag was then inserted at the base of the first dorsal fin, a small piece of muscle tissue was biopsied for isotopic analyses, and a small fragment of pectoral (or dorsal) fin was removed for DNA analysis.

Figure 2: Tagging operations. a) Morphometric measurements on a recaptured grey reef shark. b) Spaghetti tags were used as "conventional tags" for mark-recapture analyses. c) A grey reef shark double tagged with a SPOT and acoustic tag. Note the small abdominal incision for acoustic tag insertion.

Results and Discussion

Our Consortium's tagging team deployed a total of 153 animals with 248 tags: 49 Grey Reef Sharks (*Carcharhinus amblyrhynchos*), 69 Silvertip Reef Sharks (*Carcharhinus albimarginatus*), 1 Blacktip Reef Shark (*Carcharhinus melanopterus*), 1 Whitetip Reef Shark (*Triaenodon obesus*), 26 Manta Rays *(Manta alfredi*) and six species of teleosts including Yellowfin Tuna (*Thunnus albacares*), Kawakawa (*Euthynnus affinis*), Black saddled Grouper (*Plectropomus laevis*), Dogtooth Tuna (*Gymnosarda*

unicolor), Sailfish (*Istiophorus platypterus*) and Blue Marlin (*Makaira nigricans*). 137 animals where equipped with acoustic tags, 85 with conventional identification tags for mark recapture analyses, 19 with satellite enabled tags and 7 with camera tags. Eight grey reef sharks were double tagged (i.e. equipped with acoustic and satellite tags) (Table 1, Figure 3) with locations shown in Figure 4.

Species	Total Individuals	Acoustic	Conventional	Satellite	Camera
Silvertip Shark	69	67	49	0	3
Grey Reef Shark	49	48	34	8	2
Manta	26	16	0	8	2
Yellowfin Tuna	2	1	0	1	0
Whitetip Reef Shark	1	1	1	0	0
Kawakawa	1	1	0	0	0
Black Saddled Grouper	1	1	0	0	0
Dogtooth	1	1	0	0	0
Blacktip Reef Shark	1	1	1	0	0
Sailfish	1	0	0	1	0
Marlin	1	0	0	1	0
Total	**153**	**137**	**85**	**19**	**7**

Table 1. Summary of species tagged

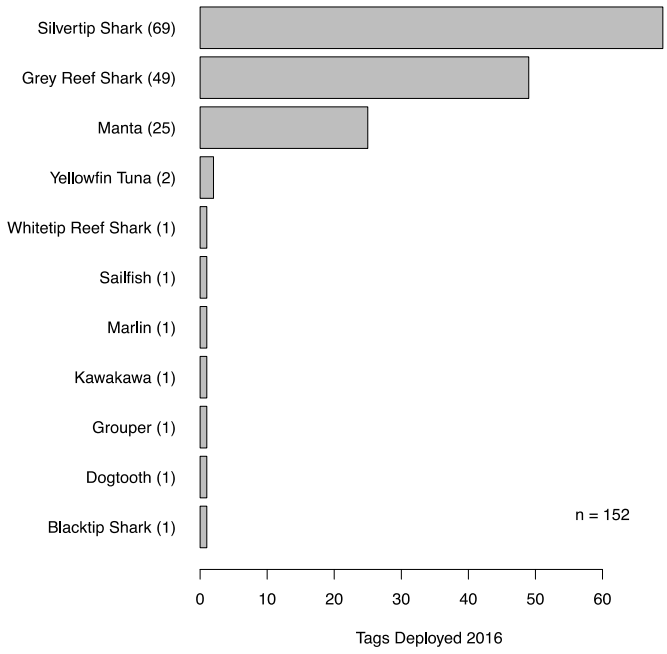


Figure 3. Bar-plot of the number of each species tagged in the 2016 expeditions.

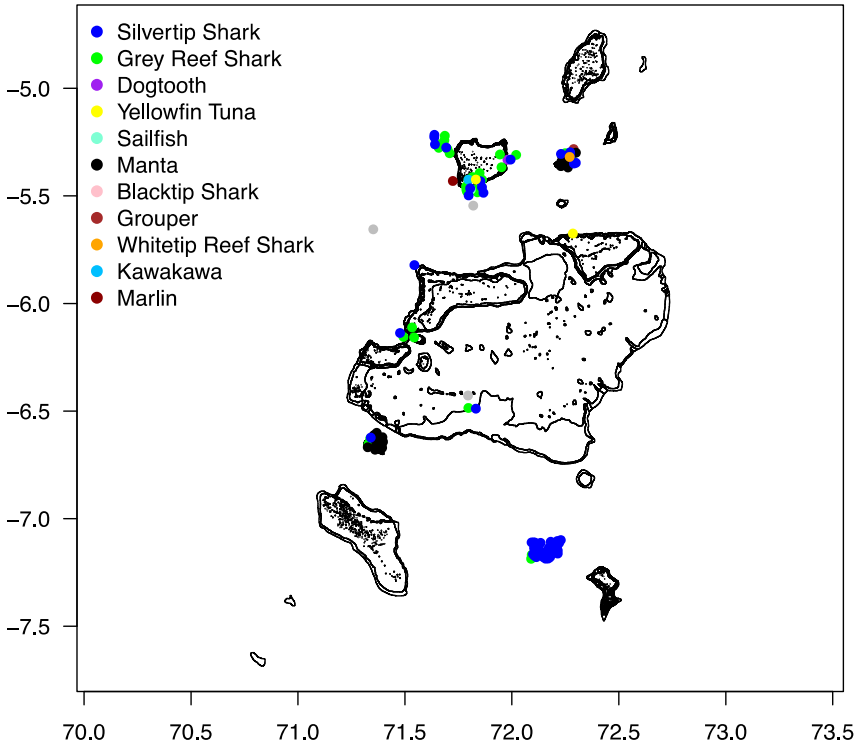


Figure 4. A map of capture locations for sharks and bony fish tagged in the 2016 expeditions. Capture locations have been slightly altered to avoid total overlap between catches occurring in the same locations.

On the 2016 expedition, four of the mantas that were tagged in 2015 were sighted again with the acoustic tags still attached (Figure 5). A quick analysis of one of the manta's movements shows a round-trip journey of at least 400 km, with the manta moving from Victory Bank to Egmont (in the last part of the track), a distance of ~160 km, in ~ 4 days (Figure 6).

Figure 5. A manta acoustically tagged in 2015 re-sighted in 2016 with the tag still attached.

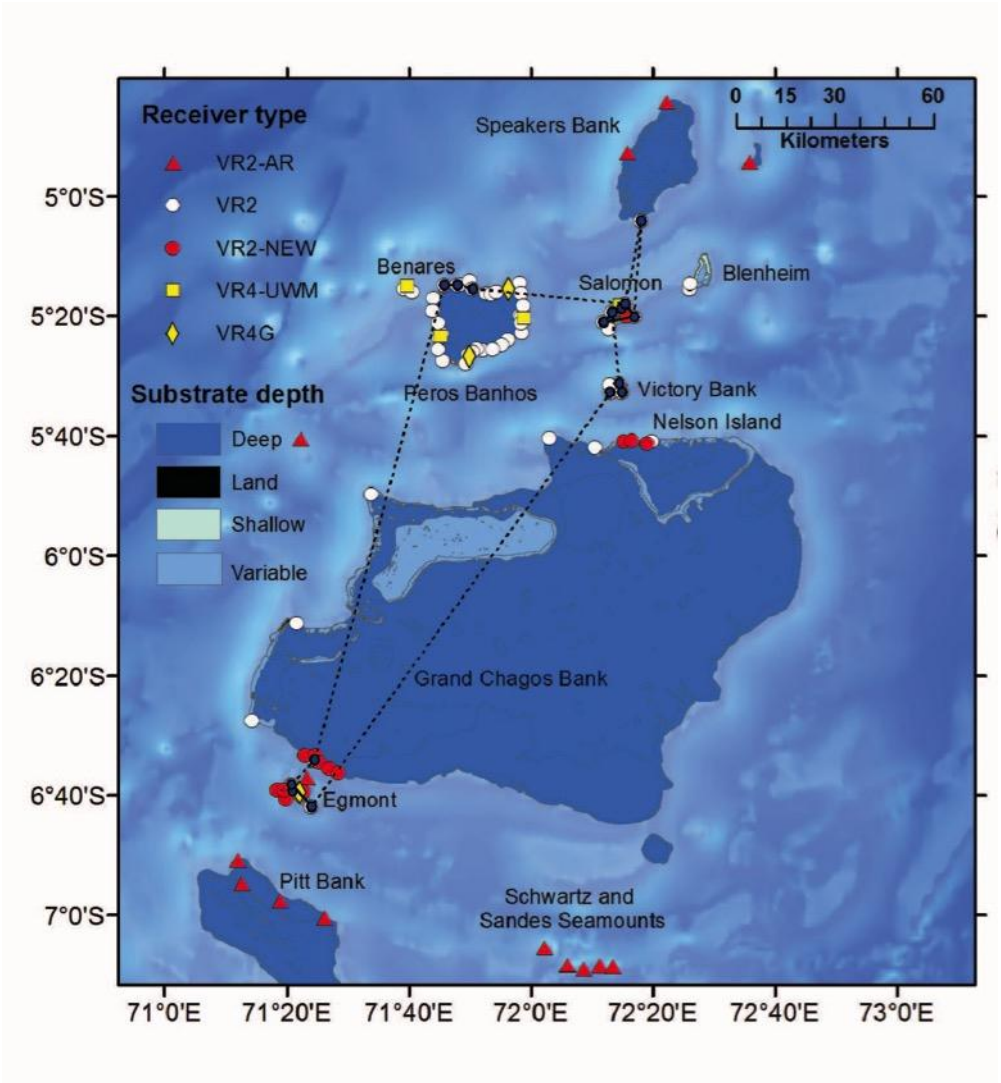


Figure 6. An overlay plot of a manta detected by acoustic tag at the array system throughout the archipelago. The manta was tagged during the 2015 Consortium expedition at Egmont.

Two sharks were recaptured during the expedition. A male grey reef shark tagged in 2015 with a CATS CAM and acoustic tag at Egmont was recaptured in the same location. The animals were in excellent condition and these recapture data are vital for estimating statistical estimates of population abundance. A female silvertip shark tagged the previous day at Solomon was recaptured at the same location. These represent our first recaptured animals.

Camera Tag Deployments

CATS CAM tags (Customized Animal Tracking Solutions, Australia) were deployed on 5 sharks (2 grey reef and 3 silvertip; Figure 7 & Table 2) for up to 20 hrs. Initial data corroborated evidence from previous trips that suggest a deep-water refuge for sharks offshore to the south of Peros Banhos. Use of this previously unknown refuge has been a consistent behaviour observed in grey reef sharks tagged at Peros Banhos. This refuge may be a source of recovery for exploited areas of the archipelago. Analyses regarding use of this refuge, energetics, inter- and intraspecific interactions and behaviour are ongoing.

Figure 7. A silvertip shark with a biologging CATS CAM tag.

Species	Size (TL cm)	Sex
Grey Reef	145	Female
Grey Reef	152	Female
Silvertip	203	Male
Silvertip	148	Female
Silvertip	144	Female
Manta	Unknown	Unknown
Manta	Unknown	Female

Table 2. Animals with biologging CATS CAM tags.

During this expedition, we had our first successful deployments of CATS CAM tags on mantas enabled by the development of a non-invasive attachment method using suction cup mounts (Figure 8). CATS CAMS were deployed on two mantas for short periods (Table 2). These deployments represent major steps forward in our ability to collect non-invasive biologging data from these large elasmobranchs.

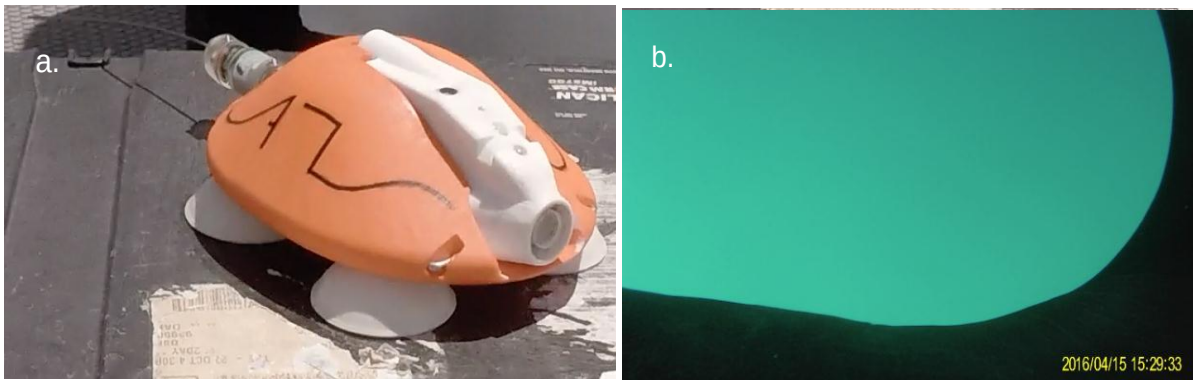


Figure 8. a) A suction cup CATS CAM tag developed by Stanford scientists to non-invasively attach to mantas and b) the first ever manta-view of Chagos: the view from a successful CATS CAM tag deployment showing the head (left side of photo) and upturned wing (right side of photo) of the swimming manta.

Size distributions

For the two most frequently tagged sharks, silvertip and grey reef sharks, the measured total lengths were slightly bigger in this expedition than in the previous years (Figures 9 & 10; both statistically

significant). For silvertip sharks average TL was 144 cm (SD: 27) an increase from 128 cm (SD: 23, t = 3.84, df = 129.3, p-value < 0.001); for grey reef sharks average TL was 128 cm (SD: 18), an increase from 121 cm (SD: 20, t = 2.3307, df = 91.223, p-value = 0.02). It remains to be determined whether this is an effect of fishing in different locations with respect to previous expeditions or if there is a shift in the size structure of the shark assemblage (i.e. a sign of recovery).

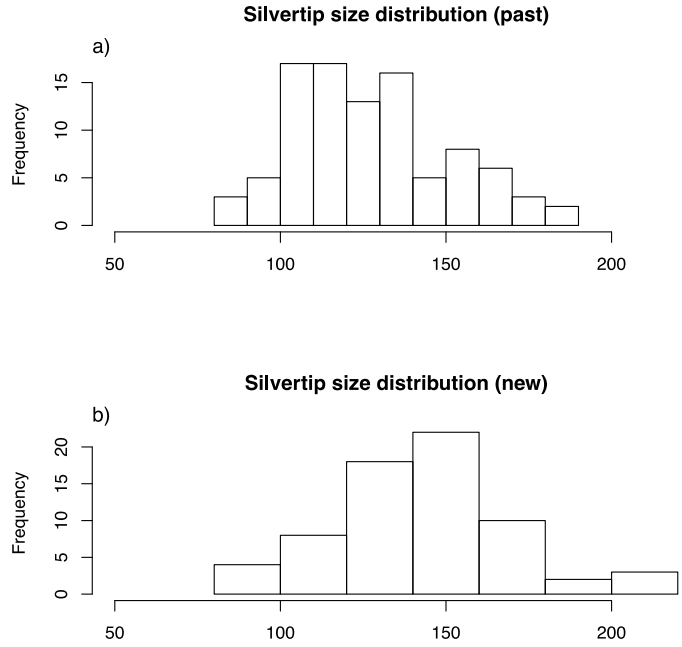


Figure 9. a) Size distribution of Silvertip Sharks tagged during the 2016 expeditions compared with b) the 2015 expedition.

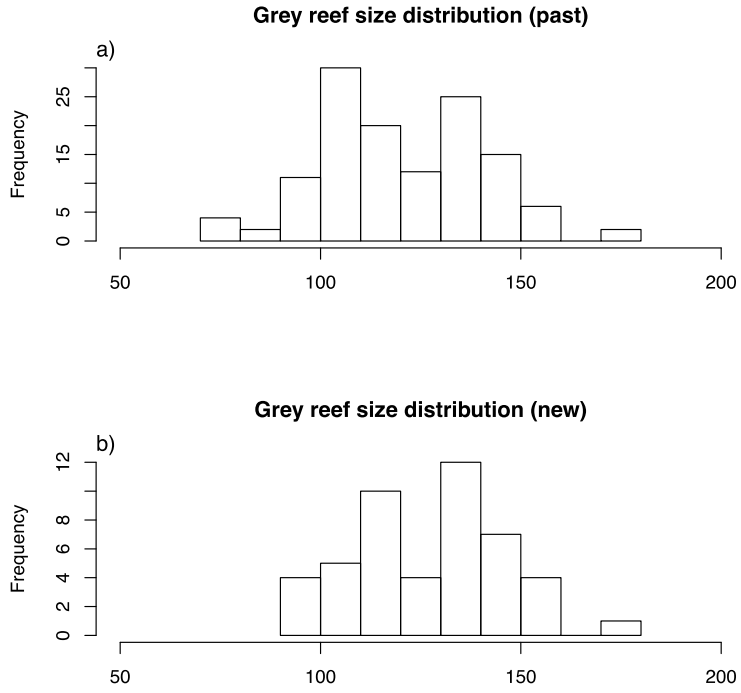


Figure 10. Size distribution of Grey reef sharks tagged in the 2016 expedition (a) compared with the one from previous years (b).

Servicing of Acoustic array

In total, 63 acoustic receivers (VR2, VR4-UWM, VR4G) in the existing array were serviced and downloaded. Downloads of the receivers resulted in 99,814 detections from 92 animals (Appendix 1). We deployed 30 new receivers to expand the array to cover additional habitats and regions of the archipelago (Figure 11; Appendix Table A1). The new receivers added to the array include 16 VR2-Acoustic release receivers that were deployed in deep water habitats and 13 VR2 receivers in shallower depths within diving range (<= 25 m). Additionally one new VR4 Global unit was deployed inside the lagoon at Egmont to provide real-time data on manta movements. At the conclusion of the expedition, the array included a total of 93 acoustic receivers deployed across the archipelago.

The maintenance and expansion of the array and tagging of additional animals are critical steps for understanding how species use these important habitats, how much protection the MPA provides to pelagic and reef fishes, as well as information on patterns of connectivity between species within the Archipelago and other parts of the Indian Ocean. Further analyses of the samples and data collected during this expedition are currently being processed at Stanford University.

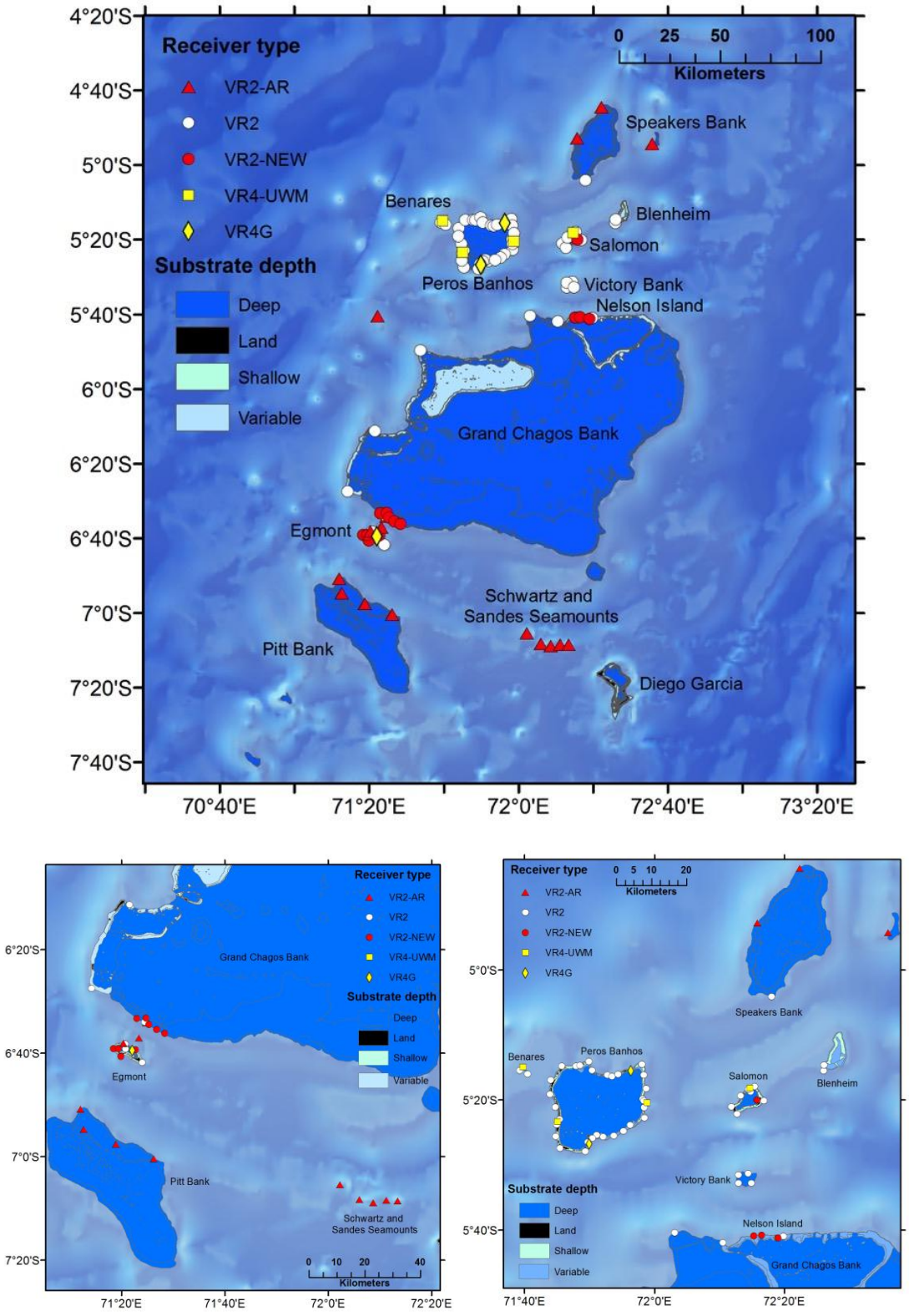


Figure 11: Location of receivers serviced and deployed during the 2016 expedition. VR2-AR are acoustic release receivers that were deployed in deeper habitats beyond diving depths. Red symbols indicate new deployments. The additional receivers greatly expanded the range of habitats and area of the archipelago monitored by the array.

Appendix 1: Acoustic detections from receiver downloads

Station	Region	Receiver type	Latitude	Longitude	Depth (m)	Substrate
AR01	Peros Banhos-GCB Channel	VR2-AR	-5° 40.449	71° 22.359	300	UNKNOWN
BE01	Benares	VR2	-5° 15.403	71° 39.281	23	RUBBLE
BE02	Benares	VR2	-5° 15.972	71° 40.490	18.8	RUBBLE
BEUWM01	Benares	VR4-UWM	-5° 14.949	71° 39.776	16	RUBBLE
BL01	Blenheim	VR2	-5° 15.470	72° 26.032	20	REEF
BL02	Blenheim	VR2	-5° 14.590	72° 26.084	18	REEF
EG01	Egmont	VR2	-6° 39.145	71° 20.851	20	SAND
EG02	Egmont	VR2	-6° 38.238	71° 20.754	15	REEF
EG03	Egmont	VR2	-6° 41.795	71° 24.062	15	REEF
EG04	Egmont	VR2-NEW	-6° 39.291	71° 22.742	25	REEF
EG05	Egmont	VR2-NEW	-6° 39.109	71° 18.499	25	REEF
EG06	Egmont	VR2-NEW	-6° 40.595	71° 19.924	25	REEF
EG07	Egmont	VR2-NEW	-6° 39.104	71° 19.476	25	SAND
EG08	Egmont	VR2-AR	-6° 38.072	71° 20.411	25	UNKNOWN
EG09	Egmont	VR2-AR	-6° 37.07	71° 23.40	300	UNKNOWN
EG10	Egmont-GCB Channel	VR2-AR	-6° 34.109	71° 24.796	75	UNKNOWN
EG4G01	Egmont	VR4G	-6° 39.460	71° 22.144	20	SAND
GCB01	GCB-Nelson Island	VR2	-5° 40.936	72° 19.851	20	REEF
GCB02	GCB North	VR2	-5° 41.900	72° 10.529	20	REEF
GCB03	GCB Nelson	VR2	-5° 40.366	72° 03.115	20	REEF
GCB04	GCB Northeast	VR2	-5° 49.710	71° 33.778	20	REEF
GCB05	GCB Eagle Bank	VR2	-6° 11.274	71° 21.563	20	REEF
GCB06	GCB West	VR2	-6° 27.494	71° 14.260	20	REEF
GCB07	GCB North of Egmont	VR2	-6° 34.003	71° 24.526	20	REEF
GCB08	GCB North of Egmont	VR2-NEW	-6° 33.268	71° 23.023	25	REEF
GCB09	GCB North of Egmont	VR2-NEW	-6° 33.230	71° 24.783	25	REEF
GCB10	GCB North of Egmont	VR2-NEW	-6° 34.429	71° 25.318	25	REEF
GCB11	GCB North of Egmont	VR2-NEW	-6° 35.442	71° 26.860	25	REEF
GCB12	GCB North of Egmont	VR2-NEW	-6° 36.197	71° 28.447	25	REEF
NI01	GCB Nelson Island	VR2-NEW	-5° 40.833	72° 15.282	20	REEF
NI02	GCB Nelson Island	VR2-NEW	-5° 40.768	72° 16.494	22	REEF
NI03	GCB Nelson Island	VR2-NEW	-5° 41.211	72° 19.006	27	REEF
PB01	Peros Banhos	VR2	-5° 16.901	71° 44.055	24.5	REEF
PB02	Peros Banhos	VR2	-5° 14.756	71° 45.790	19.5	REEF
PB03	Peros Banhos	VR2	-5° 14.765	71° 47.953	18.5	REEF
PB04	Peros Banhos	VR2	-5° 14.688	71° 48.549	20.1	SAND
PB05	Peros Banhos	VR2	-5° 14.063	71° 49.960	18.5	REEF
PB06	Peros Banhos	VR2	-5° 15.403	71° 50.430	19.8	SAND
PB07	Peros Banhos	VR2	-5° 16.161	71° 52.774	18.3	SAND
PB08	Peros Banhos	VR2	-5° 16.360	71° 53.501	20.4	SAND
PB09	Peros Banhos	VR2	-5° 14.499	71° 58.101	21.7	REEF
PB10	Peros Banhos	VR2	-5° 19.809	71° 58.186	23	SAND
PB11	Peros Banhos	VR2	-5° 20.063	71° 58.362	20.8	SAND
PB12	Peros Banhos	VR2	-5° 22.734	71° 58.473	19.5	REEF
PB13	Peros Banhos	VR2	-5° 23.825	71° 56.312	19.7	SAND/REEF
PB14	Peros Banhos	VR2	-5° 25.623	71° 52.111	22.1	SAND
PB15	Peros Banhos	VR2	-5° 25.372	71° 51.145	24	SAND
PB16	Peros Banhos	VR2	-5° 25.847	71° 50.481	22.7	RUBBLE
PB17	Peros Banhos	VR2	-5° 26.437	71° 49.763	20.7	RUBBLE
PB18	Peros Banhos	VR2	-5° 27.388	71° 45.492	22.5	REEF
PB19	Peros Banhos	VR2	-5° 22.937	71° 45.465	29.5	SAND
PB20	Peros Banhos	VR2	-5° 21.071	71° 44.806	20	REEF
PB21	Peros Banhos	VR2	-5° 16.169	71° 58.399	20	REEF
PB22	Peros Banhos	VR2	-5° 18.233	71° 58.773	20	REEF
PB23	Peros Banhos	VR2	-5° 21.127	71° 58.448	20	REEF
PB24	Peros Banhos	VR2	-5° 24.753	71° 55.254	20	REEF
PB25	Peros Banhos	VR2	-5° 25.458	71° 53.755	20	REEF
PB26	Peros Banhos	VR2	-5° 27.861	71° 49.367	20	REEF
PB27	Peros Banhos	VR2	-5° 23.737	71° 44.985	18.9	REEF
PB28	Peros Banhos	VR2	-5° 19.088	71° 43.940	20	REEF
PB29	Peros Banhos	VR2	-5° 16.007	71° 54.405	23.1	REEF
PB30	Peros Banhos	VR2	-5° 25.566	71° 44.841	20	REEF
PB4G01	Peros Banhos	VR4G	-5° 15.491	71° 56.385	19.6	RUBBLE
PB4G02	Peros Banhos	VR4G	-5° 26.699	71° 49.974	20	RUBBLE
PBUWM01	Peros Banhos	VR4-UWM	-5° 20.397	71° 58.869	20	REEF
PBUWM02	Peros Banhos	VR4-UWM	-5° 23.339	71° 45.118	20	SAND
PITT01	Pitt Bank	VR2-AR	-6° 50.826	71° 12.117	20	GRAVEL
PITT02	Pitt Bank	VR2-AR	-6° 57.572	71° 18.956	64	GRAVEL
PITT03	Pitt Bank	VR2-AR	-6° 54.698	71° 12.741	74	GRAVEL
PITT04	Pitt Bank	VR2-AR	-7° 00.429	71° 26.280	31	GRAVEL
SA01	Salomon	VR2	-5° 18.710	72° 14.818	19.2	RUBBLE
SA02	Salomon	VR2	-5° 18.338	72° 14.457	19.2	REEF
SA03	Salomon	VR2	-5° 17.910	72° 15.413	20.8	REEF
SA04	Salomon	VR2	-5° 20.089	72° 16.861	21.5	REEF
SA05	Salomon	VR2	-5° 21.022	72° 11.879	13.7	REEF
SA06	Salomon	VR2	-5° 22.131	72° 12.720	18.0	REEF
SA07	Salomon	VR2	-5° 19.347	72° 13.259	18	REEF
SA11	Salomon	VR2-NEW	-5° 19.959	72° 15.814	25	SAND
SAUWM01	Salomon	VR4-UWM	-5° 18.180	72° 14.705	13.6	REEF
SB01	Speakers Bank	VR2	-5° 04.073	72° 18.053	20	UNKNOWN
SB02/AR02	Speakers Bank	VR2-AR	-4° 52.800	72° 15.880	43	UNKNOWN
SB03/AR05	Speakers Bank	VR2-AR	-4° 54.260	72° 35.970	140	UNKNOWN
SB04/AR06	Speakers Bank	VR2-AR	-4° 44.390	72° 22.380	39.3	UNKNOWN
SS01	Schwartz/Sandes Seamounts	VR2-AR	-7° 05.429	72° 02.311	300	UNKNOWN
SS02	Schwartz/Sandes Seamounts	VR2-AR	-7° 08.294	72° 06.115	250	UNKNOWN
SS03	Schwartz/Sandes Seamounts	VR2-AR	-7° 08.876	72° 08.756	95	UNKNOWN
SS04	Schwartz/Sandes Seamounts	VR2-AR	-7° 08.444	72° 11.313	350	UNKNOWN
SS05	Schwartz/Sandes Seamounts	VR2-AR	-7° 08.542	72° 13.516	370	UNKNOWN
VB01	Victory Bank	VR2	-5° 32.727	72° 12.954	25	REEF
VB02	Victory Bank	VR2	-5° 31.244	72° 14.432	25	REEF
VB03	Victory Bank	VR2	-5° 31.483	72° 12.935	25	SAND/REEF
VB04	Victory Bank	VR2	-5° 32.760	72° 14.930	25	SAND/REEF

Table A1: Metadata for acoustic receivers deployed during 2016 expedition. GCB is Grand Chagos Bank, VR2s are existing VR2 receivers that were serviced during the expedition, VR2-NEW are new VR2 receivers deployed during the expedition, and VR2-AR are new VR2 acoustic release receivers. Substrate indicates the general bottom type at the receiver location.

Figure A1a. Acoustic detections of all animals from receiver downloads

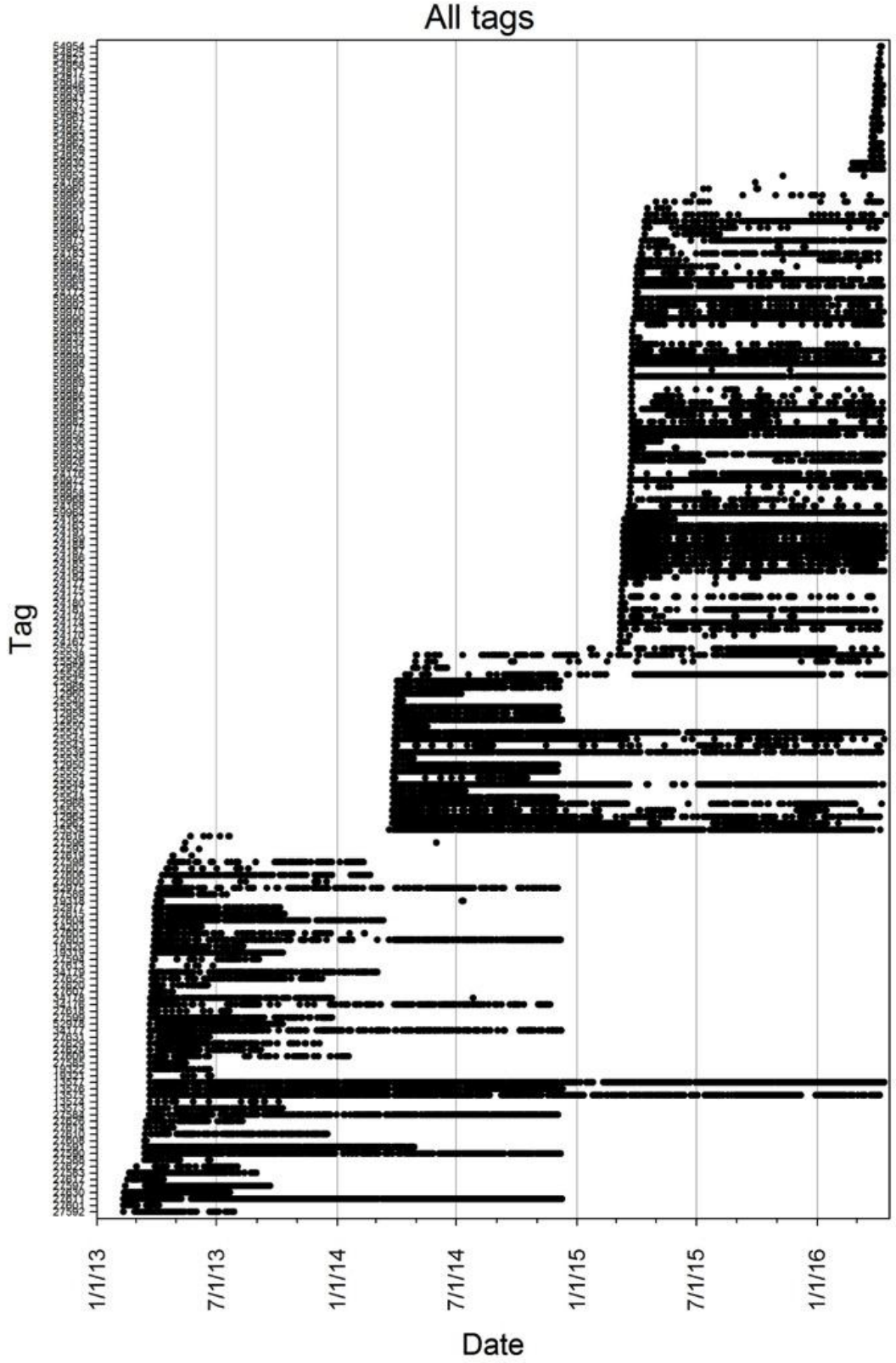


Figure A1b. Acoustic detections of Grey Reef Sharks from receiver downloads.

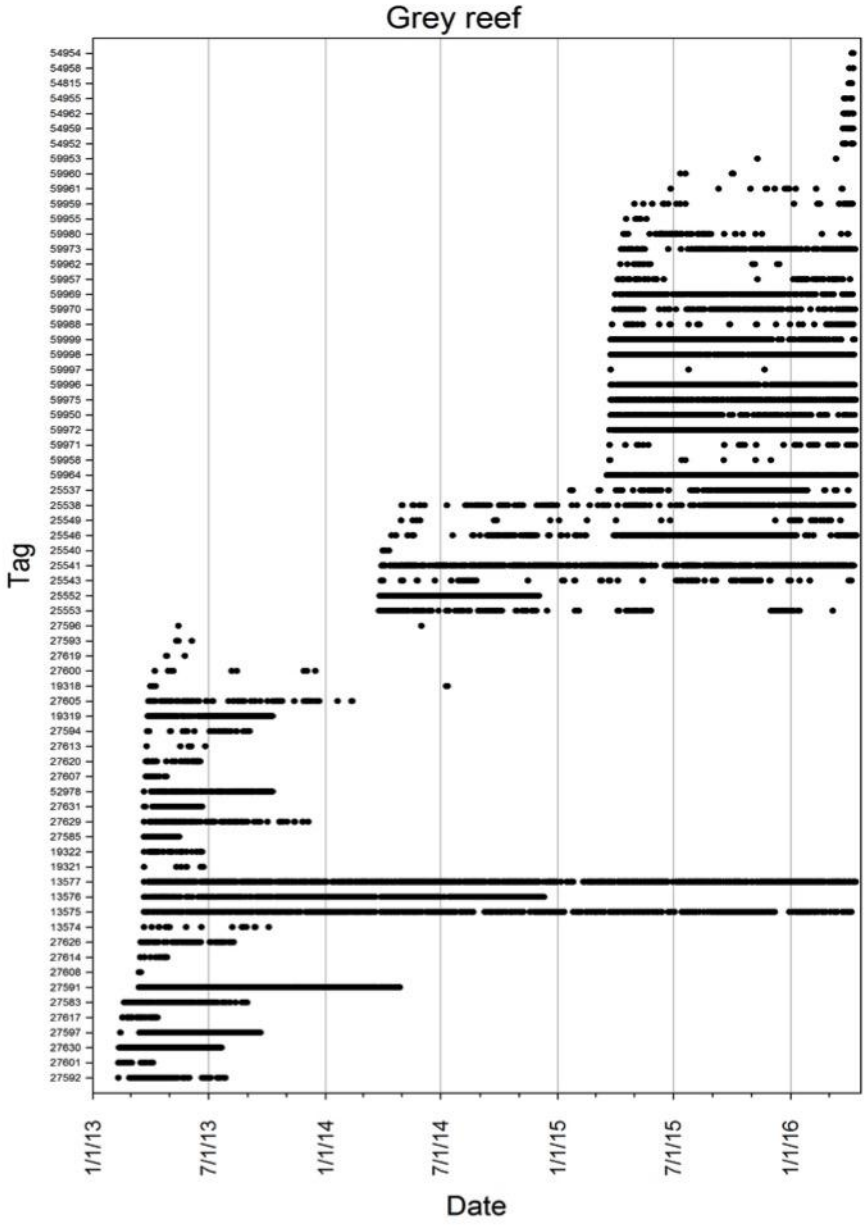


Figure A1c. Acoustic detections of mantas from receiver downloads.

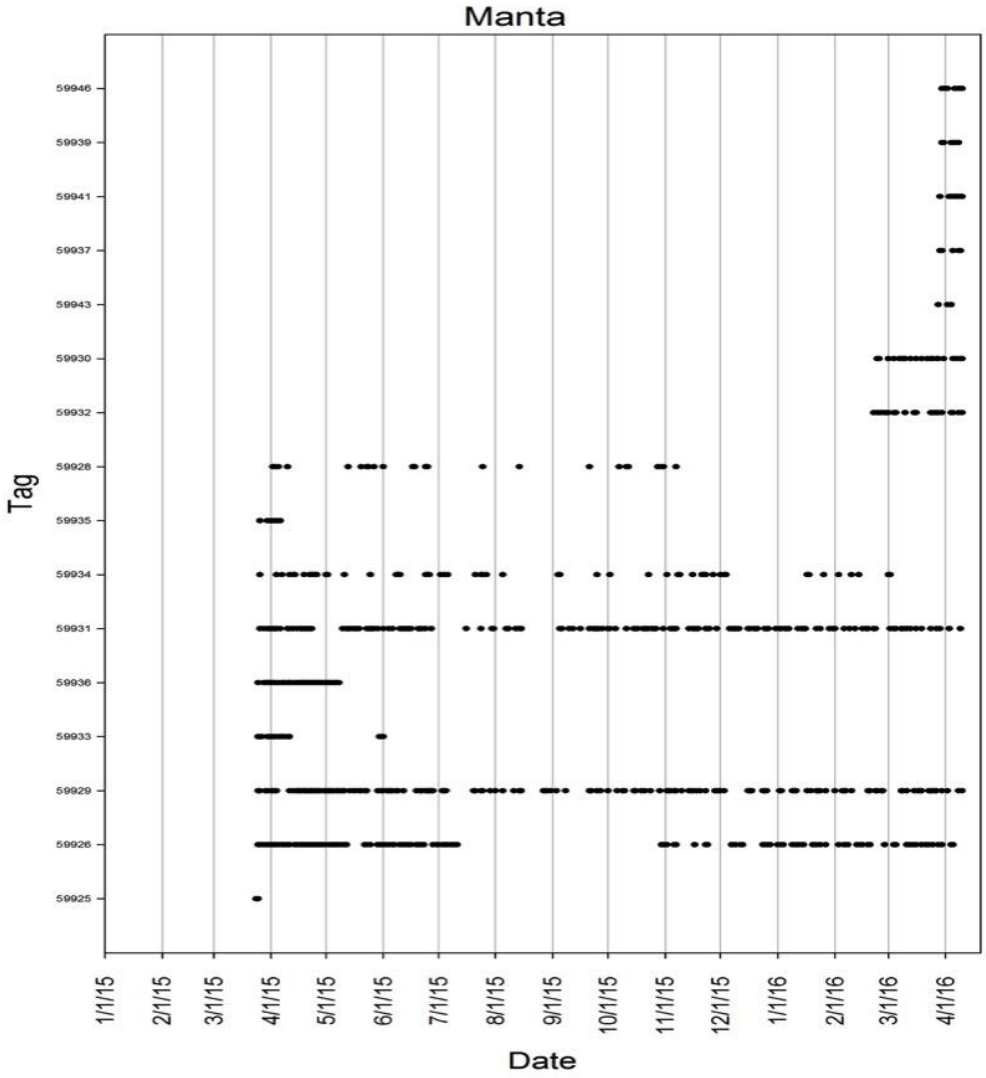
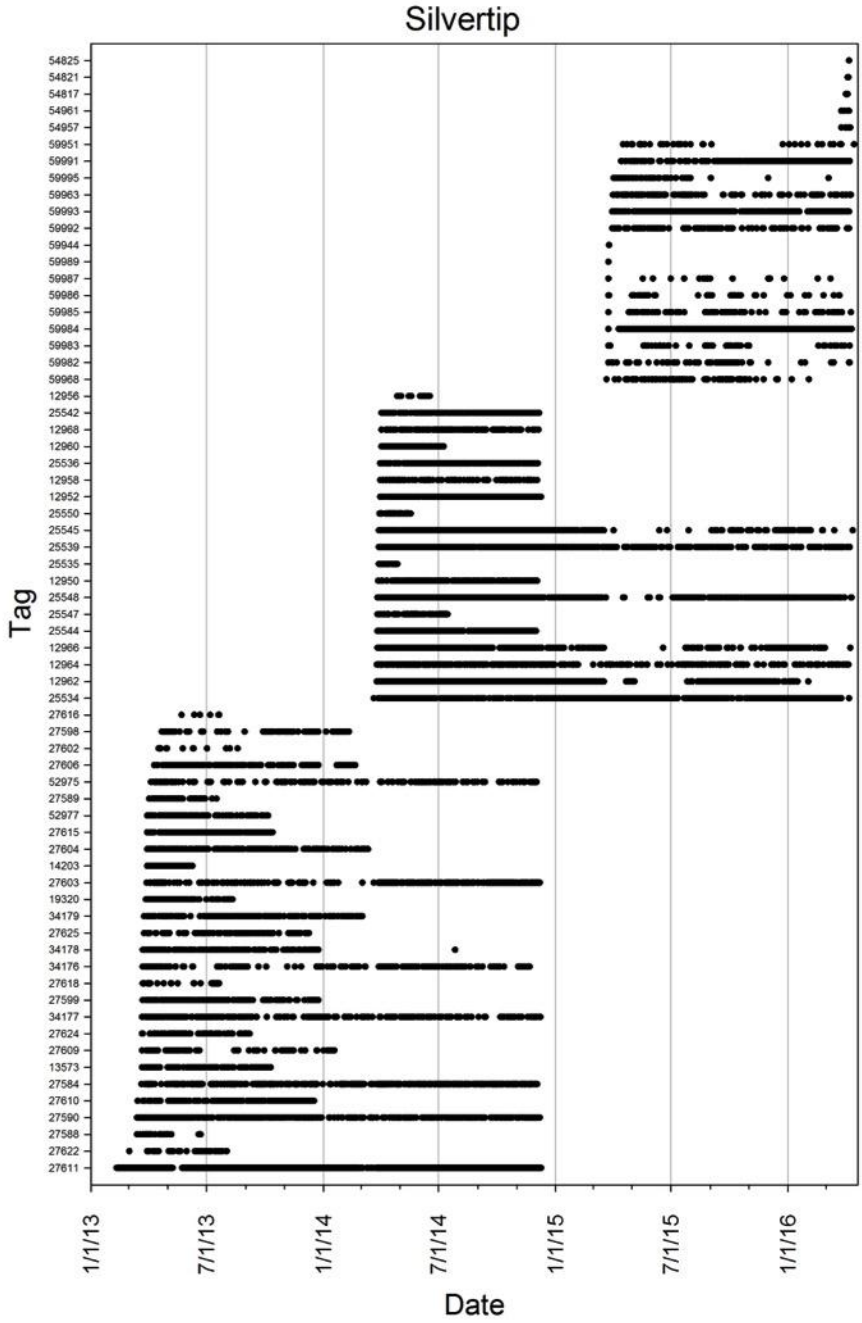


Figure A1d. Acoustic detections from Silver Tip Sharks.



b) Long-term monitoring of the ecological trajectories and resilience of reefs in the Chagos Archipelago

Ronan Roche and Heather Koldewey, Bangor University, Zoological Society of London

Summary

The Chagos Archipelago has particular value as baseline for reef condition and functioning within the Indian Ocean and globally. During April 2016 data were collected from key long-term monitoring sites identified to enable a rapid assessment of the condition of reefs of the Chagos Archipelago. Coral bleaching is currently underway on all reefs surveyed within the Archipelago, although the overall level is moderate, and there is high inter-site variability in the proportion of coral affected. Coral bleaching events due to high sea-surface temperatures are a global phenomenon, and impacted the Chagos Archipelago during the 1998 El Niño event. Following that event, most of Chagos Archipelago reef recovered which was attributed to the lack of other stressors on the reef. The impact of two El Niño events resulting in bleaching occurring in successive years is unknown and a current focus of global coral reef research.

Introduction

The absence of many localised anthropogenic stressors within the Chagos Archipelago allows a better understanding of the impacts of globally distributed stressors on these reefs, which, for the Indian Ocean region are likely to approach maximum resilience and recovery potential. The data collected during the April 2016 expedition will allow analysis and comparison with existing datasets of reef condition—data on Chagos Archipelago reef condition extend back to the 1970s, and regular video transect monitoring has taken place since 2006.

Coral bleaching was documented at sites in Perhos Banhos and Salomon Atolls during expeditions (Darwin expedition on the Pacific Marlin, Living Oceans Foundation, Pangaea and BIOTA assessments) in 2015. Up to the point of the Bertarelli Foundation 2016 expedition it has not been possible to determine how this event affected the coral reefs of the archipelago, and how severe the event impacted reef benthic communities. Whilst satellite temperature data recording degrees above the threshold bleaching temperature are available, many additional factors interact with temperature to influence if and where bleaching actually occurs.

We aimed to explore the potential interaction between bleaching severity and depth, therefore data were collected from 5 m to 25 m, to enable comparison between depth ranges. The project was also designed to integrate with existing reef datasets, and to determine where bleaching impacts from 2015 are most apparent, as this is likely to further influence the severity of bleaching during 2016. The dive team focused on repeating videography surveys of the reef habitat that have been conducted at the same sites since 2006 (Figure 12, Table 3). In addition, diver operated stereo videography was conducted to collect fish biomass data that can be compared to the long term dataset of Nick Graham, Newcastle University. These collaborative and comparative approaches and optimal site sampling were established prior to the expedition.

Figure 12. Coral surveying method at 25m during April 2016

Dive Number	Date	Site	Method	25m	10m
1	06-Apr	Ile Diamant Lagoon	Test Monit		
2	06-Apr	Ile Diamant Seaward	Test 3D		
3	07-Apr	Petite Coq	Monitor	1	
4	07-Apr	Petite Coq	10m		1
5	08-Apr	Bernard Knoll	Monitor	2	
6	08-Apr	Ile Poule	10m		2
7	09-Apr	South Brother	Monitor	3	
8	10-Apr	Egmont South	Monitor	4	
9	10-Apr	Egmont South	10m		3
10	11-Apr	Egmont North	Monitor	5	
11	11-Apr	Egmont Mid	10m		4
12	13-Apr	Salomon Ile Anglaise (Mid)	Monitor	6	
13	13-Apr	Salomon Ile Anglaise (Mid)	10m		5
14	14-Apr	Salomon Courts Knoll	Monitor	7	
15	14-Apr	Salomon Ile Fouquet	10m		6
16	15-Apr	SAMS Knoll	Monitor	8	
17	15-Apr	SA Ile Passe	Monitor	9	
18	16-Apr	PB Fouquet	10m		7
19	16-Apr	PB Moresby	Monitor	10	

Table 3. Reef monitoring surveys during April 2016. The Monitor sites were 25m to 5m video surveys while the 10m method focused on documenting fish at key sites (corals were also surveyed).

Results and Discussion

Current status of reefs of the Chagos Archipelago

From the video transect surveys carried out during April 2016, it is apparent that the current El Niño event is resulting in moderate levels of bleaching on reefs in the Chagos Archipelago. Many reefs have high levels of standing dead coral cover, particularly dead table *Acropora* and *Pocillopora.* These are the same genera which are currently undergoing bleaching (Figure 13) which suggests that impacts from the 2015 bleaching event are driving this pattern on the reefs. More detailed analysis will allow quantification of the changes in dead standing coral cover and morphological and genus characteristics between 2015 and 2016.

The sites surveyed during April 2016 which exhibited least bleaching were those which were at lagoon mouths and dominated by coral communities adapted to higher turbidity levels, where branching corals were largely absent (Figure 14). Additionally there may be a relationship with current flow/degree of flushing and the severity of bleaching on seaward sites around atolls. Further analysis of these patterns across the Archipelago will allow this hypothesis to be fully tested.

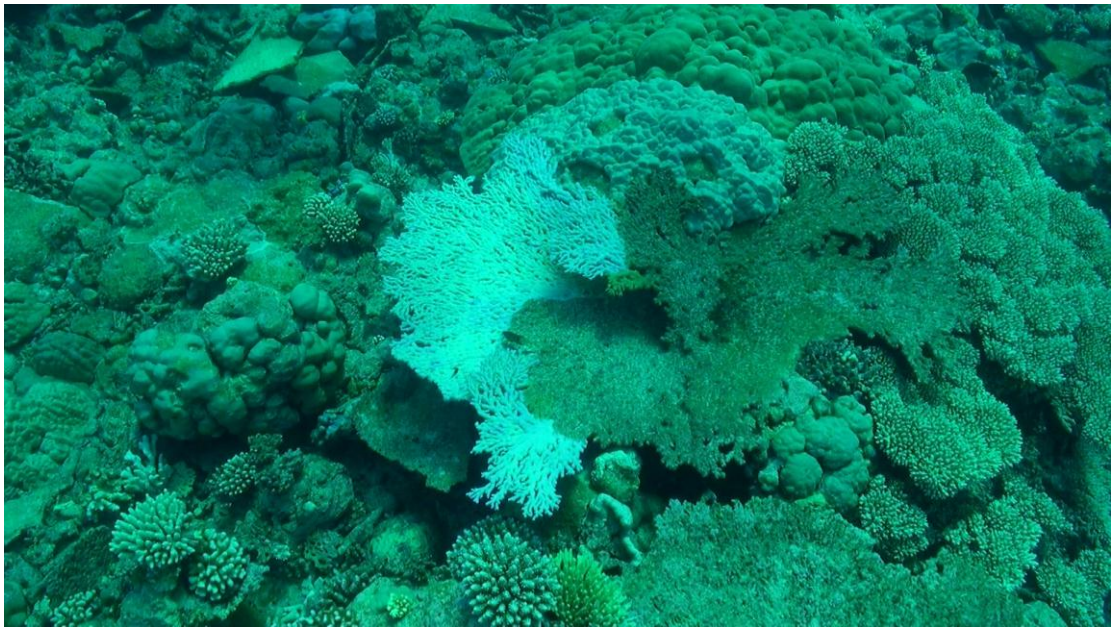


Figure 13. Partially bleached Table Acropora, Ile Poule

Future trajectories

There is emerging evidence which suggests that where moderate bleaching which does not result in widespread mortality occurs prior to a subsequent bleaching event, survivorship is increased relative to reefs which have not previously experienced bleaching. This may confer a degree of resistance to corals which experienced, but survived bleaching during the 2015 event. A key unknown is the ability of the reefs of BIOT to rebound from bleaching events occurring at this frequency. Coral recruitment levels are high at a majority of sites, and if recruit survival is high, reef functioning will likely recover, as occurred following the 1998 bleaching event. A rapid publication of the coral bleaching data is planned, as well as more in depth studies which will require further analysis (Appendix 2).

Figure 14. Unusual community dominated by heterotrophic feeding corals at Court's Knoll, Salomon Atoll

Appendix 2

Examples of planned analysis:

1. Integration with existing datasets e.g:

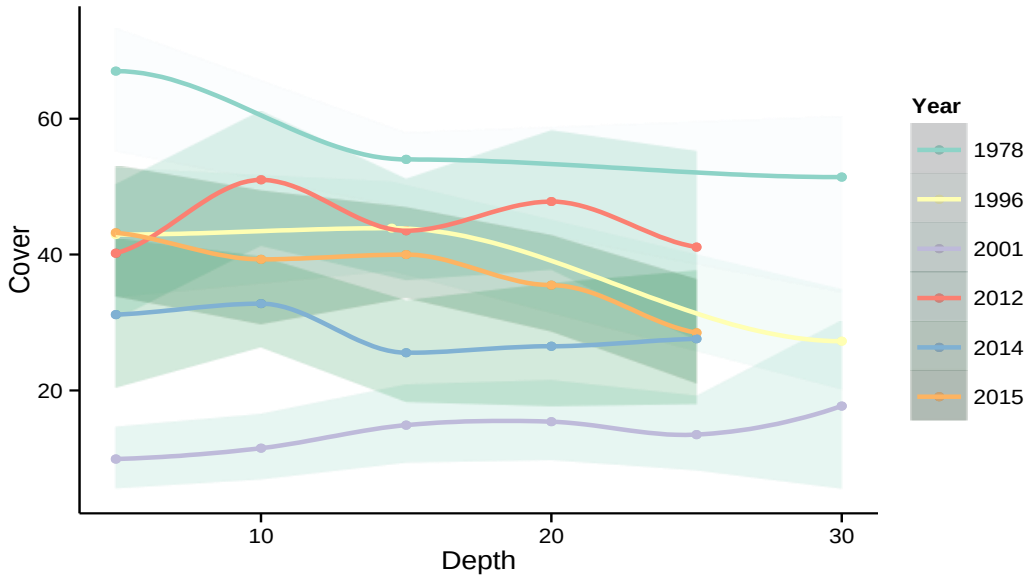


Figure A2a: Long-term trends in coral cover within the Chagos Archipelago

2. Multivariate analysis of benthic community variability e.g:

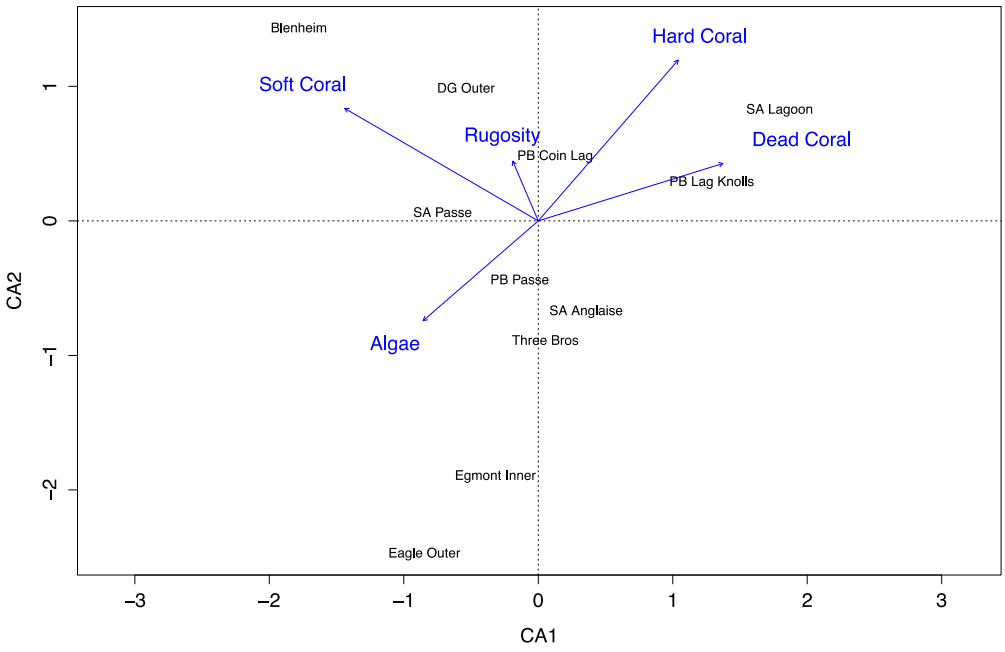


Figure A2b. Relationships in the variability of benthic communities within the Chagos Archipelago

c) Assessing the impact of the 2015 / 2016 El Niño heating event on the structural complexity and associated functional diversity of the Chagos archipelago

Catherine Head & Dominic Andradi-Brown, University of Oxford, Dan Bayley, University College London, Natural History Museum, ZSL.

Executive Summary

Data were collected from 14 sites across the archipelago to enable us to produce 3D models of reef structure and compare this detailed view of the reef structure to fish species richness and abundance. This will increase our understanding of how reef fish diversity is connected and dependent on the reef structure itself. In addition, to track the health of the reef, specifically the recovery from last year's bleaching event and the extent of bleaching this year, surveys of these sites will now be analysed. Signs of coral bleaching were witnessed across the archipelago on this expedition, as well as high mortality of coral, presumably as a result of last year's bleaching. However a high density of young corals was also seen, which was also the case after the 1998 bleaching event, which resulted in strong recovery of the reefs. Surveys carried out on the March (also see March expedition report) and April expeditions will give a picture of the health of the reef prior to this year's bleaching (March) and during the bleaching (April), providing a much needed time-series. The data will now be analysed and will be disseminated in at least two peer-reviewed publications, and the 3D models created can be used in outreach activities.

Over 2000 m^2 of reef was surveyed using a mixture of photography and videography to allow analysis of the 3D structural complexity, composition and function of the shallow reefs of the Chagos archipelago. Initial analysis shows the new 'structure from motion' technique to effectively capture the reef organisms in the area surveyed in sufficient detail to record colonies to genus level, and enable measurement of a number of metrics known to be important to sustaining healthy reefs. The remaining collected data will now be processed to allow analysis of the reefs' current status. This analysis will further allow detailed comparisons with future and potentially previous years' data to assess the trajectory of the reef condition in the long term following heat-induced bleaching.

Introduction

The resilience of reefs to potential shifts in state from coral to algal dominance following disturbance have recently been shown to be attributed to a number of key attributes, including: structural complexity, depth, high density of juvenile corals and herbivorous fishes, and low nutrient loads. Of these factors, high structural complexity is shown to be of primary importance, and has been shown historically to be integral to a number of ecological processes on the reef, and the services it provides. The strongest of these ecological relationships to 3D structure is a positive correlation to fish biomass and density (significantly within the Pomacanthidae, Pomacentridae and Scaridae families), with weaker negative correlations to algal cover and urchin density.

The aim of this work is to: 1) record the incidence of bleaching and disease on reef-forming coral communities with a focus on *Acropora cytherea* and *Porites lutea*, 2) quantify three-dimensional reef complexity to examine the relationship between benthic fish diversity and habitat provision of the reef and how that changes over time and 3) test Structure from Motion' (SfM) photogrammetry techniques to capture a permanent and detailed quantitative digital record of the 3D structure of the reef.

Bleaching Assessments

Coral bleaching, evident by the loss of the coral's colour, is a stress-response to changes in environmental conditions, most notably increases in sea surface temperatures (SST). The coral expels its zooxanthellae (single-celled algae cells), which provide the coral with energy, and are therefore vital for the coral's survival in the long-term. The coral cells can recover the zooxanthellae

from the water column, but if they don't coral bleaching results in mortality of the coral. Bleaching occurred in the archipelago last year but we do not know if this resulted in mortality or not, and another bleaching event was predicted to start at the beginning of April this year due to the current El Nino climate event causing increases in SST. This bleaching was witnessed as the expedition progressed. Our transects will quantity mortality from last year's bleaching event and record the extent of current bleaching. Surveys carried out in March (by Catherine Head) will also allow a comparison of coral health pre- this month's 2016 bleaching (March 2016) and during 2016's bleaching occurring on this expedition. The University of Western Australia team working on the Pangaea expedition have kindly agreed to collect bleaching data using comparable methods at key sites during the May expedition as part of the Consortium collaboration.

Reef structural complexity and reef fish diversity

Coral reef structural complexity, the three-dimensional structure making up the reef architecture, is a crucial habitat for many reef fauna. Losses of structural complexity are often associated with changes in reef fish communities. More broadly reef complexity is associated with key fish groups that provide ecosystem functions, such as herbivores, with grazing 'halos' reported around areas of structural complexity. In addition, complex reef structures have been identified as important for diversity in understudied reef cryptofauna in BIOT. Many of these small cryptic crustaceans are preyed upon by invertebrate feeding fish species that are also reliant on the reef structure. Reef architectural complexity is made up of many components, across many spatial scales including rugosity, reef height, diversity and size of holes in the reef. Traditional methods to measure rugosity normally involve laying a chain over the surface of the reef. This however limits the scale that complexity can be measured. In response, there has been an increase in the use of 3D modelling to reconstruct reefs, and providing an opportunity to assess complexity with multiple morphometrics.

We further record the associated biodiversity (richnesss and composition) of reef and demersal organisms. Repeat surveys were conducted at shallow depths across reef types (Flat, crest, slope, lagoon) immediately preceding the current El Niño event and we plan to repeat the method following the heating event to quantify the resulting effects on the reef physical structure and composition, as well as on individual species' characteristics in these conditions.

We have successfully captured a total of approximately 2300 m^2 of coral reef structural data from across 3 separate atolls (in both lagoon and seaward environments) across the Chagos archipelago. Current and future data will be processed and subsequently analysed for a number of structural and composition metrics. We also plan to test if historic footage can be used to compare the current structure to previous years.

To quantify the 3D reef complexity six quadrats were located at 5m intervals along each transect. GoPro cameras were placed filming the quadrat for 10 minutes to record the fish community associated with the reef. The reef contained within the quadrat was then filmed using a DSLR camera to provide high quality imagery for 3D model reconstruction (Figure 15).

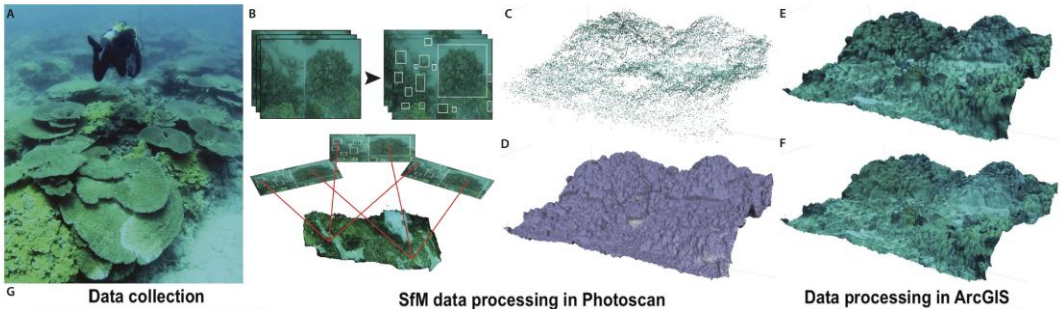


Figure 25. Primary steps of the SfM workflow for three-dimensional reconstruction of the benthic substrate. (A) Image collection; (B) keypoints matched and aligned to develop a photo mosaic of the substrate; (C) ortho-rectified point cloud created using SfM;(D) A triangulated irregular network (TIN) mesh is created; (E) shaded or; (F) textured using images. (Adapted from Burns et al. 2015)

Results & Discussion

Data were gathered from 14 sites across the archipelago (Table 5).

Atoll	Site	No. of bleaching transects	No. of 3D complexity quadrats
Peros Banhos	Ile Diamont lagoon	3	12
Peros Banhos	Petite Coquillage	3	12
Peros Banhos	Bernard's Knoll lagoon	3	12
Peros Banhos	Ile Poule South	3	12
Great Chagos Bank	South Brothers	3	12
Egmont	Egmont South	3	12
Egmont	Egmont Mid	3	12
Salomon	Ile Anglaise Mid	3	12
Salomon	Court's Knoll lagoon	3	12
Salomon	Ile Fouquet	3	12
Salomon	Ile Jacabin	3	12
Salomon	Sam's Knoll lagoon	3	12
Peros Banhos	Ile Fouquet	3	12
Peros Banhos	Moresby	3	12

Table 5. List of sites from which data were gathered.

3D models will now be generated from the quadrat surveys (Figure 16) using a custom made Python pipeline built by our research group and extensively tested on previous reef footage from our work in the Honduras and Indonesia. GoPro fish videos will be analysed using EventMeasure video annotation software, allowing the maximum number of individuals of each fish species at each quadrat to be recorded. Permutational analysis of variance will be used to test for differences in reef structural complexity with depth, and the associations of different fish species with metrics of complexity.

Video footage of bleaching and disease transects will now be analysed using Coral Point Count software to assign 10 random points to each still image from the footage. The benthic cover, e.g. coral, rock, under each randomly assigned point will be recorded and if a coral is identified it's health, e.g. bleached/diseased/dead/alive, will also be recorded. From this information we can quantitatively establish the health of the reef and compare this to data collected using the same methodology in 2012 and 2013 to provide us with information on the extent of damage caused by last year's bleaching. Surveys undertaken in March this year can also be compared to the surveys collected on the expedition (April) to quantify the extent of bleaching so far this year.

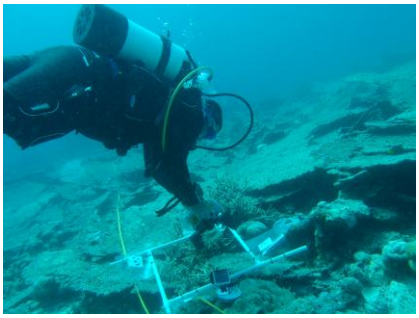


Figure 16. Videoing a quadrat to create 3D models, and a GoPro set up to record fish diversity associated with that section of the reef.

Initial observations are that while much of the structure of the reefs is still intact, much of the reef has suffered heavily from last year's bleaching and is still being affected by the current heating event. It is likely that there will be significant physical and bio-erosion over the course of this year which will reduce the 3D complexity and overall volume of the reefs. Erosion will likely be higher within the seaward sites as these were observed to be suffering higher stress and mortality than those of the lagoons. Mortality is also likely to be higher in the rapidly growing 'weedy' coral species such as the *Acropora* and *Pocillopora* which were observed to be affected most from the heating stress. Reef-associated fish populations appear to still be present in good numbers, however analysis of how their populations will be affected by the predicted loss of coral habitat is still uncertain.

d) Exploring the Twilight Reefs of the Chagos Archipelago as a potential source of resilience
Dominic Andradi-Brown, University of Oxford

Achievements and outputs
We conducted the first twilight reef surveys (reefs slopes deeper than 30m) conducted in the Chagos Archipelago since the 1980s. Using the Deep Trekker remote underwater vehicle (ROV), kindly provided by the Bertarelli Foundation and the Vava II, we surveyed reefs in the 30-60m depth range at 12 sites around the Chagos Archipelago. Surveys indicate high coral cover (>80%) in some locations, implying deeper reefs may act as a refuge for threatened shallow reef species and have a crucial role in overall reef resilience. We identified several shallow reef species not previously reported from twilight reefs, including the endemic Chagos clownfish. In addition, we also regularly observed sharks on twilight reefs, suggesting these deep reefs have an important role for larger pelagic species. Full video analysis is underway with the aim of producing a scientific paper shortly.

Introduction
Twilight reefs, known formally as mesophotic coral ecosystems, are reefs found from 30-150m depth. They are characterised as light dependent reef communities, however are highly adapted to the low light levels received at these depths. Most coral reef research is focused on shallow reefs due to the difficulties in accessing deeper reefs. Many reef threats disproportionately affect shallow depths, for example, coral bleaching is caused by a combination of high temperature and high light intensity. Therefore, twilight reefs, by virtue of being deeper appear to be more protected from the impacts of bleaching than shallow coral reefs. Other direct stressors to reefs including storm impacts and marine debris pollution also decline with depth. Twilight reefs may therefore have an important role in overall reef resilience.

Studies in the Caribbean have indicated many upper-twilight reefs (30-60m) contain threatened shallow reef species. If this is the case in BIOT, it suggests twilight reefs may provide local sources of fish and coral recruits/individuals to aid recovery following shallow reef damage. This idea, known as the deep reef refugia hypothesis was first proposed in the mid-1990s, but was not formalised and widely considered until 2010. There are two crucial components for this to be the case: (i) threatened shallow reef species must also be found on twilight reefs, and (ii) twilight reef and shallow reef populations must be connected.

During the Bertarelli Foundation April 2016 Science expedition we used an ROV unit to conduct point surveys at twelve locations around the Chagos Archipelago. The ROV was lowered into water at 60m depth and 5-10 minutes surveys were conducted in the 45-60m depth band. The ROV was then piloted up the reef slope to survey in the 30-45m depth band for the same time period. Where possible the ROV then slowly navigated up the reef slope further into the shallow reef community, and was recovered once it reached 15m depth. By using this quick point survey method it was possible for multiple drops to be conducted in same general area and many sites to be quickly surveyed.

Results and discussion
Preliminary observations of the ROV footage revealed a species rich twilight reef community. Table 6 shows the full list of sites surveyed and the number of ROV drops at each site.

Atoll	Site	Number of drops
Peros Banhos	Ile Fouquet Seaward	3
	Ile Diamant Anchorage	2
	Ile Diamant Seaward	2
	Ile Petite Coquillage	6

	Ile Fouquet Anchorage	2
Egmont	North Seaward	1
	Mid Seaward	1
Sandes seamount	Mid-water shark survey	2
Salomon	Ile de Passe	4
	Ile Anglais Mid	3
	Ile Fouquet	2

Table 6. ROV survey locations and number of replicate drops completed

During ROV surveys we identified particularly high coral coverage at Ile Anglais Mid, which had close to 100% live coral cover at 35-45m depth (Figure 17). This contrasted with the shallow reefs at that site which contained low levels of live coral cover.

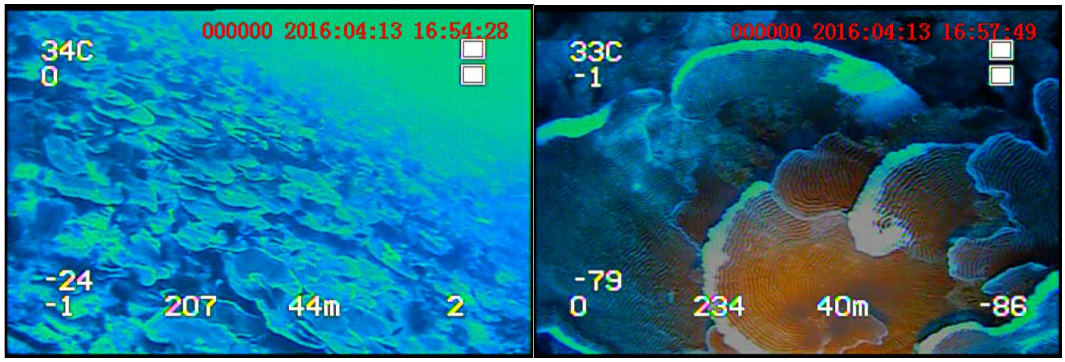


Figure 17. High coral cover at Ile Anglais Mid, Salomon Atoll in the 35-45m depth range.

Large numbers of fish were also identified, including Bluefin Trevally, at twilight depths (Figure 18). Fish families such as trevally are known to rove large distances over the reef and are common on shallow reefs as well. This suggests these fish populations are may be connected to the shallow reefs, with individuals moving across the depth gradient, though this requires more research. The know depth limits of several fish were expanded, including the Chagos clownfish (*Amphiprion chagosensis*), which had previously only been recorded to 25m depth. We identified individuals living at 37m depth.

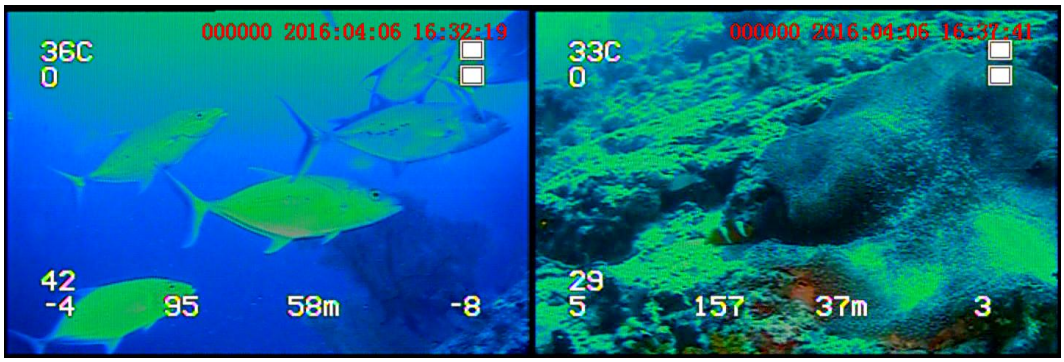


Figure 18. Bluefin trevally (left) were regularly observed on twilight reefs. Many small damselfish were found associated with twilight corals and anemones, including the Chagos clownfish (right).

Three species of sharks were also observed regularly on twilight reefs: silver tips, grey reef and black tip reef sharks (Figure 19). This suggests that sharks are using the full depth range of reefs in BIOT, highlighting the importance of deep reef to pelagic mobile predators. In addition, while surveying Sandes seamount we identified large numbers of silver tip and grey reef sharks that swam up to the ROV unit and circled it. These sharks followed the ROV down onto the top of the seamount, and also

as it returned to the surface, allowing many close up images to be captured by the twilight reef survey team.

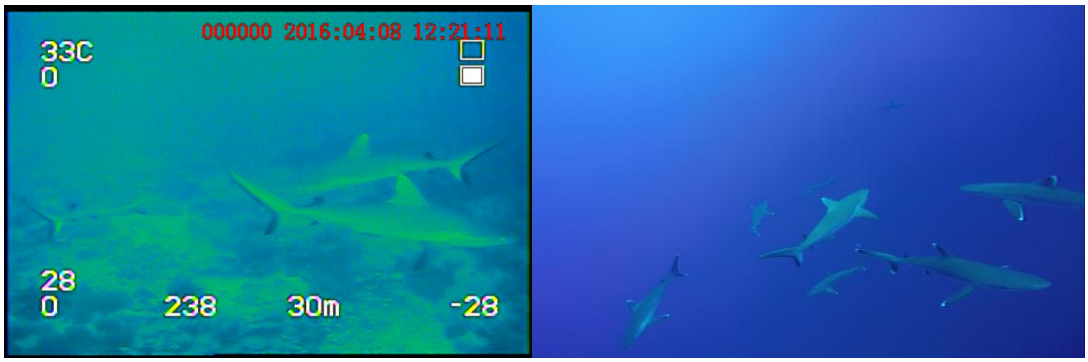


Figure 19. Grey reef sharks (left) were regularly observed on twilight reefs. Many silver tip sharks were observed by the ROV over Sandes seamount (right).

Video analysis of the ROV footage is underway. We aim to produce a scientific paper that identifies the coral cover at various depths on twilight reefs around the Chagos Archipelago, and compares this to the coral cover in the shallows. The only previous twilight reef surveys conducted in BIOT were in the 1980s, and we plan to compare how the twilight reef coral cover has changed between the 1980s and 2016.

e) Environmental DNA (eDNA) as a new approach to monitoring biodiversity
Team: Catherine Head and Luke Gardner, Oxford University, Stanford University

Introduction
Environmental DNA (eDNA) is the DNA from all organisms released into the water column in a host of ways including in their faeces, sperm, eggs and skin. These traces of DNA have the potential to be used to monitor the diversity of organisms that inhabit an area. In the marine environment initial studies, show promise for future non-invasive assessments and monitoring of marine biodiversity and resources.

a) Reef eDNA sampling
In BIOT we applied eDNA sampling on the reef to quantity the diversity of the microbiome, invertebrates (including corals) and vertebrates, and identify these species. The invertebrate species results can be validated using invertebrate species richness lists for BIOT, created as a result of Catherine Head's PhD over the last 4 years and using known coral and reef fish species lists for BIOT. Use of water sampling for the microbiome is well established and does not require validation. Taxonomically very few scientists would be able to identify the invertebrate component of biodiversity, particularly the sessile fraction, creating a bottleneck to establishing the true biodiversity of BIOT, but this can be overcome using eDNA sampling and next generation sequencing.

Water samples were collected from 13 sites across the archipelago and the Environmental DNA (eDNA) of microbes, invertebrates (including corals) and fish, from these samples will now be analysed back in the lab. The potential of this new technique for answering important questions about the inter-connectivity of trophic groups, from microbes to coral species to fish, is high and this study will act as a pilot study for the archipelago. We will be collaborating with Prof Rob Dunbar to compare environmental data with the eDNA results, to understand if the diversity of organisms is correlated with certain environmental parameters e.g. carbon and nitrogen in the water column. We will also be collaborating with Dr Luke Gardner (Stanford Uni) to combine their samples from the pelagic (open ocean) realm with our samples from the reef, to look at differences and connections between the communities of these two ecosystems. This will result in multiple publications.

We collected three 1 litre samples of water and 50 ml sediment cores from approximately 10m depth, and deeper depths were possible, at selected reef site. Stervix filters were used to isolate the eDNA from water samples and all samples were preserved in RNALater and stored in the freezer for the duration of the expedition.

Atoll	Site	Depth	Exposure
Peros Banhos	Ile Diamont Lagoon	10m	Lagoon
Peros Banhos	Petite Coquillage	10m	Seaward
Peros Banhos	Il Poule	10m	Seaward
Peros Banhos	Bernard's Knoll	10m	Lagoon
Great Chagos Bank	Brothers South	10m, 50m, 75m	Seaward
Egmont	Egmont South	10m, 25m, 50m, 75m	Seaward
Egmont	Egmont Mid	10m	Seaward
Salomon	Ile Anglaise Mid	10m, 25m, 50m	Seaward
Salomon	Ile Fouquet	10m	Seaward
Salomon	Court's Knoll	10m	Lagoon
Salomon	Sam's Knoll	10m	Lagoon
Peros Banhos	Ile Fouquet	10m	Seaward
Peros Banhos	Moresby	10m	Seaward

Table 7. List of sites and depths from which water and sediment samples were collected at reef sites.

Samples were collected from 13 sites around the archipelago, processed and taken back to Oxford for lab analysis. The next step is to extract the DNA from the preserved filters and use next generation sequencing to 'read' the DNA. The DNA reads can then be compared to global databases of DNA sequences to establish the number and identity of organisms in the samples from their DNA. The results will then be crosschecked with visual survey records from the archipelago.

Environmental data on the carbonate cycle (collected by Prof Rob Dunbar and Hans Dejong), such as pH, carbon levels and sea temperature, will be used to investigate if there is any correlation between species richness, particularly in the microbial realm, and environmental conditions. For instance, the presence of certain microbes is thought to be connected with the level of the carbon and nitrogen in the water column. This will help us unravel differences in community structure across sites and reefs across trophic groups. We will also be focusing on the differences in community structure (from the microbes to the fish) between lagoons and seaward reef sites, because lagoon reef communities are often very different to seaward reefs, for instance they are often more resilient to increases in sea surface temperature. In addition, we also have water samples collected in 2015 from BIOT, and have samples available to us form locations in the Atlantic and Pacific that will allow for rare spatial and temporal comparisons.

b) *Pelagic eDNA sampling*

Seawater samples were collected from a number of sites throughout the archipelago to determine recent genetic presence of pelagic animals. Water samples were collected either at the ocean surface or a depth of 10 m using a Niskin bottle apparatus (Figure 20). A total of 81 water samples were collected representing 27 geographical sites collected in triplicate (Figure 21). Of those 27 sites - 3 were collected in duplicate for comparative analysis of differing environmental DNA (eDNA) methods employed between Stanford and Oxford Universities including processing and bioinformatics. Sampling sites included locations were presence of pelagic animals is expected to be relatively high including Egmont channel for Manta rays and Swartz seamount for sharks. Likewise some of the locations sampled had no bathymetric structure or observational evidence for the presence of pelagic species including sampling sites between Peros Banhos and Speakers bank. We hypothesize that eDNA signatures of these pelagic animals will be detectable in a spatially gradated manner, increasing with proximity to local bathymetry features. All the water samples taken during the expedition were filtered and prepared for transport on-board the ship. Extraction, library preparation, sequencing and bioinformatic analysis will be conducted in laboratory conditions at Stanford University. These samples will be very valuable as a tool to compliment the sentinel monitoring component of the project, detecting the presence or absence of some of the more scarce pelagic animals without the need for a chance visual encounter.

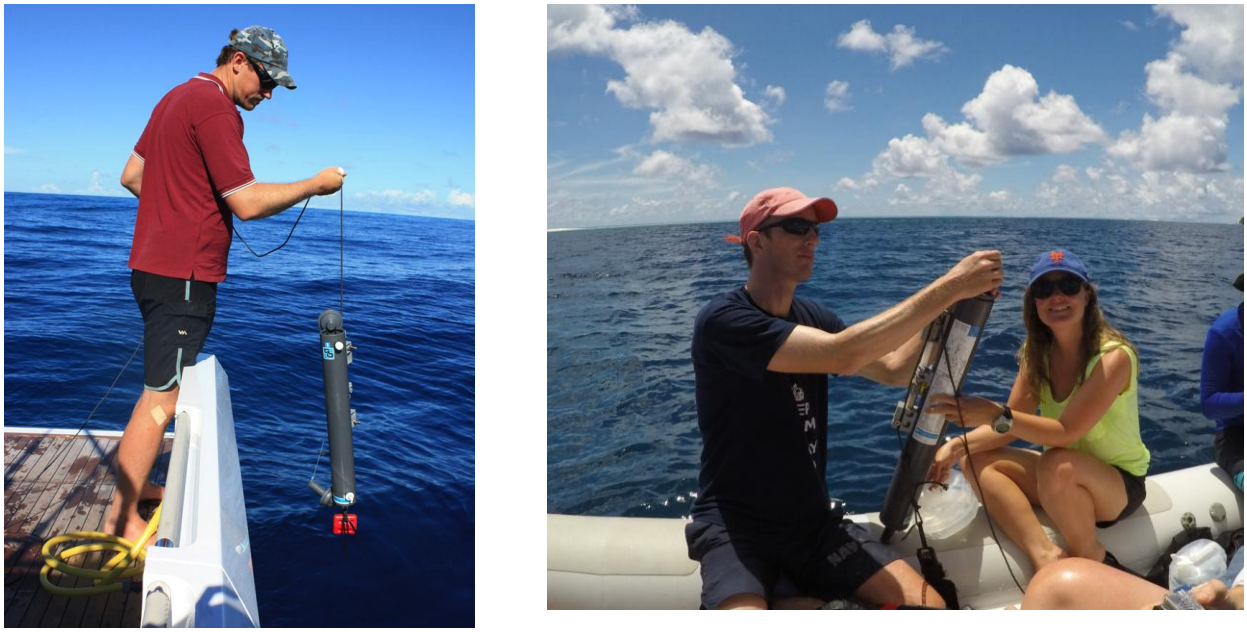


Figure 20. Niskin bottle deployment for collection of water from deeper depths (25m, 50m and 75m).

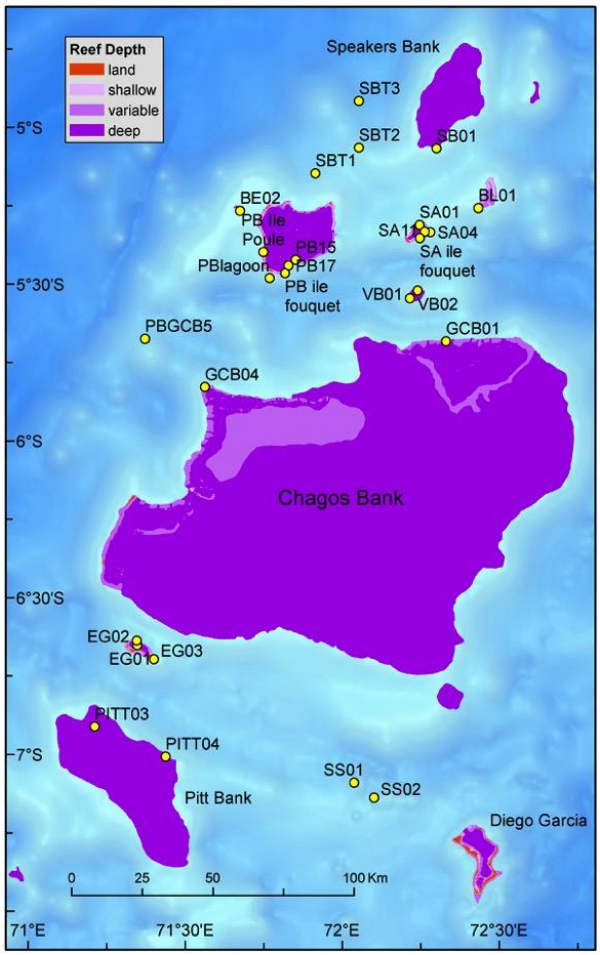


Figure 21. Location of pelagic eDNA sampling sites within BIOT.

f. Establishing approaches to biogeochemical monitoring
Rob Dunbar and Hans Dejong, Stanford University

Coral reefs consist of benthic substrates with very large specific surface areas because of the complexity of their architecture and the many different lifestyles maintained by their constituent organisms. They are also communities of strongly amplified productivity and skeletal growth rates relative to the surrounding ocean. This means that functionally, most coral reefs are heterogeneous chemical reactors wherein the metabolism of the community has a large impact on seawater chemistry. What is less well appreciated are the synergistic processes whereby the chemistry of the water column feeds back onto functionality and health of the reef.

This two-way coupling between reef processes and seawater chemistry is an important attribute of the reef ecosystem and one with built-in feedbacks that may confer elements of resiliency to reefs exposed to stress. To study these interactions and to collect the first data of this kind in the BIOT region, we collected 117 water samples across the archipelago. The water samples were sent back to Stanford University for total alkalinity and total CO_2 analysis.

Seawater samples were collected at 12 reef sites along depth transects (at 5, 10, 15, 20, and 25 m; Figure 22). In addition, we collected surface and bottom water along a transect across Salomon lagoon wherein we also collected vertical profiles of seawater temperature and salinity using a portable CTD unit. Seawater samples were also collected offshore (at 25, 50, and 75 m depth) using Niskin bottles. By comparing carbon system data from the reef and Salomon lagoon samples with information from the offshore source waters, we will assess to what extent these reefs are net calcifying. In addition, we will expect to gain insight into the spatial variability of carbon system variability among the different atolls around BIOT. Based on anecdotal observations in the Pacific, we expect that carbon chemistry (pH and aragonite saturation state) of the overlying seawater may influence the health and recovery of reefs after bleaching events.

We also designed and constructed an automatic water sampler for this expedition (Figure 23). Nearly all seawater samples collected from reef systems worldwide for chemical analysis are collected during daylight hours when the ecosystem is net autotrophic. An automated sampling system allows us to examine variability in the C system and oxygen levels at night when the entire system becomes heterotrophic. This new sampler pumps ambient seawater into collection bottles every 2 hours over a 24-hour period. We deployed the sampler at 3 reef sites and simultaneously collected data on oxygen concentration, temperature, salinity, light, and current flow. By studying day/night variability in the C system and oxygen levels, we can calculate the biogeochemical function of the reef, i.e. whether the reef is mainly calcifying or photosynthesizing.

To document variability in carbon chemistry over timescales of minutes to months, we deployed a SEAFeT DuraFeT-based pH logger along with a pressure sensor in a channel that enters the southwest side of Peros Banhos lagoon (on mooring PBUWM02; Figure 24). This is the first pH sensor to be deployed in BIOT and will allow us to include Peros Banhos in a larger set of study sites set up to detect and quantify ocean acidification as well as the magnitude of natural metabolic cycles on selected reef sites throughout the tropics. The pressure sensor records the tidal cycle, telling us when water enters or exits the lagoon. Therefore, we will have a long record of the pH of the source water that enters the lagoon as well as the lagoon water as it exits. Similarly, we deployed a salinity and pressure sensor in the main channel that enters Salomon lagoon (SAUWM01). We deployed 13 temperature sensors across the archipelago. These temperature sensors were deployed on existing moorings in locations that are adjacent to high priority coral reef

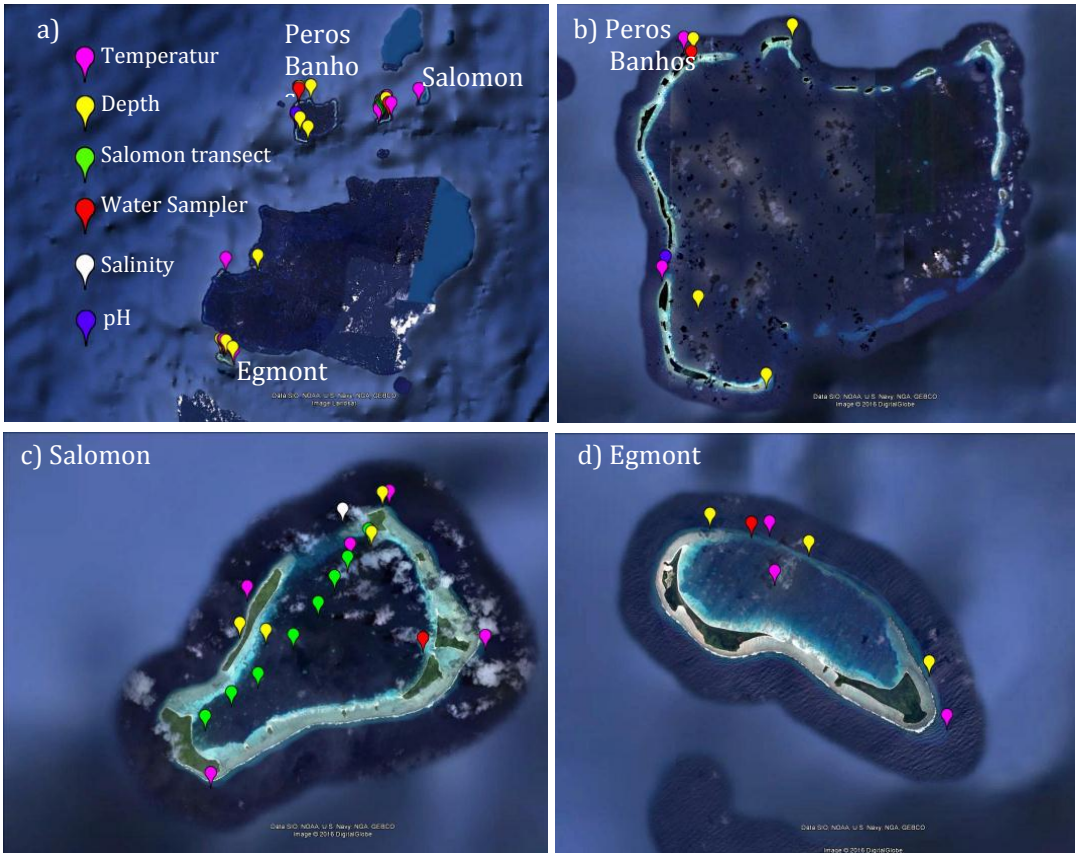


Figure 22. Locations of temperature loggers (pink), carbon system depth transects (yellow), automatic water sampler deployments (red), Salomon carbon system transect (green), pH logger with pressure logger (purple), and salinity logger with pressure logger (white). The maps represent (a) the entire Archipelago, (b) Peros Banhos, (c) Salomon atoll, and (d) Egmont atoll.

monitoring sites. In situ temperature sensors are crucial since there are often large discrepancies between satellite-derived temperature products and the actual temperature that the reef experiences. We deployed an RDI-Teledyne Acoustic Doppler Current Profiler to examine time-varying water column circulation as a function of depth in an area of known concentrations of mantas. Such a circulation data set allows us to include environmental energy in assessments of animal site preference and also provides data that can be used to test physical circulation models for the region if and when they are developed.

An additional 117 seawater samples were collected from 15 reef sites throughout the archipelago for carbon system measurements (Figure 12). The sampling program was done in coordination with the reef team transect program and nearly all water samples for carbon system chemistry were collected adjacent to long-term coral reef assessment sites. These data will provide insight into the two-way interactions between the reefs and the adjacent seawater. In particular, we will determine the degree to which the water column is saturated with respect to the carbonate minerals produced by the reef and by examining the ratio between the uptake of total dissolved CO_2 and carbonate alkalinity we will assess the degree to which the BIOT reefs are maintaining a healthy balance between primary production and the creation of reef carbonate. To support Consortium goals for the archipelago into the future we also deployed a variety of time series logging sensors on animal tracking moorings throughout the study area. These instruments are designed to record data on environmental physics and chemistry for a full year and include 13 temperature sensors, 2 pressure sensors, 1 salinity/temperature sensor, a pH logger, and an Acoustic Doppler Current Profiler (ADCP). The ADCP was deployed looking up in Manta Alley and is capable of resolving 3D water movement in bins of approximately 1 meter from the deployment depth to the sea surface. We also worked with consortium scientists to deploy and program the ADCP so that can track any large

animals that transit through the 4 ADCP sound cones. As a pilot effort for BIOT we used an aerial camera system to collect several hours of shallow reef habitat imagery. We will use these images to explore automated information extraction. Additional underwater imagery was collected wherever C system water samples were taken.

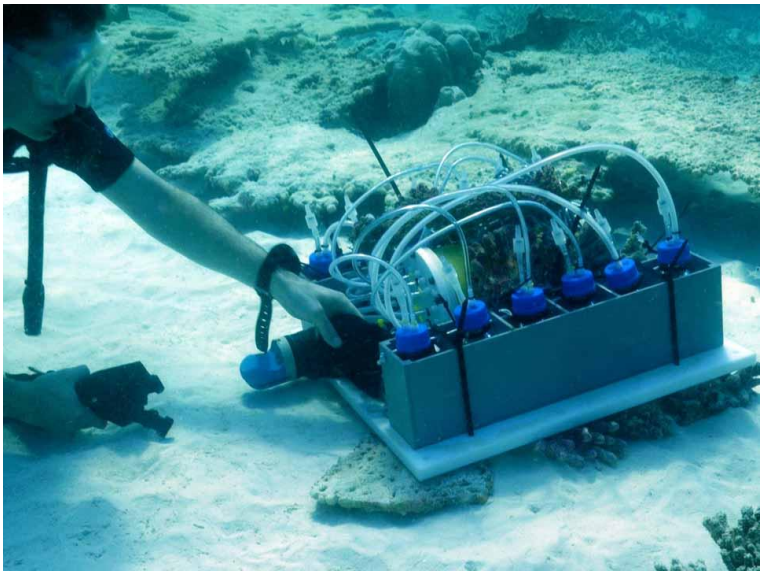


Figure 23. Innovative underwater time series water sampler developed and constructed for this project.

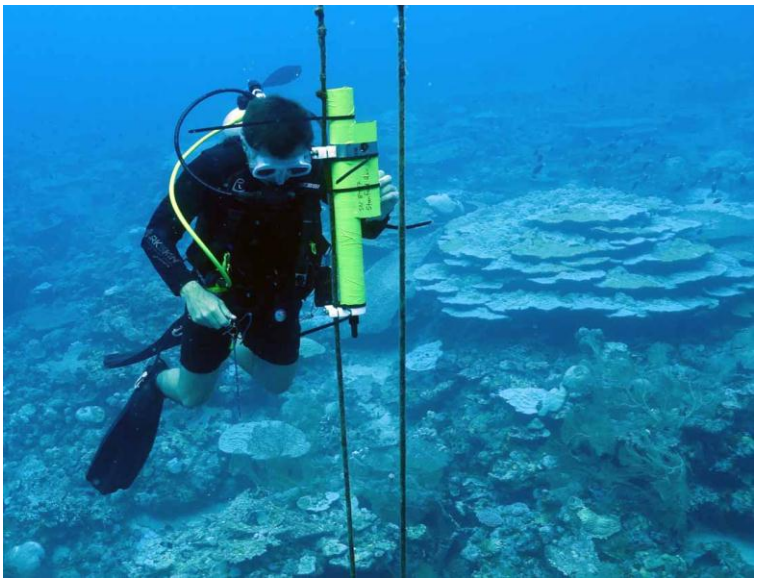


Figure 24. Installing an SBE-37 temperature, salinity, and depth logger on a mooring at Peros Banhos.