The REV Ocean Mesophotic and Deep-Sea Research Cruise

Cruise Report

Executive Summary

The REV Ocean mesophotic and deep-sea research cruise mapped and surveyed deep-sea and shallow-water ecosystems of the western seaward reefs and slopes of Peros Banhos and Eagle Island. In addition, surveys were undertaken by snorkellers of Middle Brother Atoll. Two submersibles were deployed from the *RV Odyssey* and undertook video transects and sampling from depths of 500m, 250m, 120m and 60m depths. Contrasting biological communities were observed across depth zones.

At 500m slopes, terraces and walls of carbonate rock hosted rich communities of corals, sponges, anemones and mobile fauna such as sea cucumbers and sea stars. A submarine spur extending from the seaward slope of Peros Banhos was found to host cold-water coral framework sufficiently large to be identified as deep-water reefs. This is a habitat rarely observed in the Indian Ocean. Other areas were covered in highly bioturbated fine carbonate sediments inhabited by soft-shelled urchins and large marine snails. Predatory fish, usually associated with seamounts were also observed, likely feeding on small swimming animals that migrate into deep water during the day and become trapped against the seafloor. The existence of this trapping zone is likely of significance for large predators in the Chagos Marine Reserve.

At 250m depth sediments were interspersed with large boulders and debris fallen from reef slopes above. Biota was relatively scarce at these depths and mainly confined to rocky habitat. At slightly shallower depths fields of carbonate chimneys were discovered that may be evidence of methane seepage and carbonate accretion through the action of chemosynthetic bacteria forming stromatolites. This is to be confirmed through analysis of rock samples and its significance is unknown.

Complex rocky reef at 120m depth is inhabited by a rich seafloor fauna and fish communities. Where these reefs continue to 60m depth they also support rich communities which include zooxanthellate corals and seaweeds. However, the exposure of the western boundary of these atolls to extreme weather mean that low diversity coarse sands cover much of the 60m depth zone.

Shallow-water observations revealed a mixture of exposed low-diversity reef (seaward reefs of Peros Banhos and Eagle Island) versus rich coral reefs and high

associated biodiversity of fish and other animals in Middle Brother Lagoon. Healthy colonies of the endangered coral *Ctenella chagius* were found to live in this location.

A collection of common seafloor organisms and seaweeds were collected for taxonomic work to elucidate the biodiversity of the investigated localities in the Chagos Archipelago. Many of the seaweeds were small and fleshy algae, usually found elsewhere in the Indian Ocean, were missing probably because of the isolation of the archipelago and extremely high grazing pressure on the reefs of the marine reserve. Samples were also collected for genome sequencing of *Ctenella chagius*, genetic connectivity studies to examine shallow-to-deep connectivity of coral populations and environmental DNA to genetically fingerprint biodiversity.

Résumé

L’expédition scientifique sur les ecosystèmes mésophotiques et les environnements profonds menée par REV Ocean a permis de cartographier et d'étudier les écosystèmes profonds et peu profonds des récifs côté vers la mer et des pentes occidentales de Peros Banhos et de l'île de l'Aigle. De plus, des relevés ont été effectués par des plongeurs en apnée sur l'atoll Middle Brother. Deux submersibles ont été déployés à partir du *RV Odyssey* et ont entrepris des transects vidéo et des échantillonnages à des profondeurs de 500m, 250m, 120m et 60m. Des communautés biologiques contrastées ont été observées à travers les différentes zones de profondeur.

À 500 m, des pentes, des terrasses et des parois de roches carbonées abritaient des communautés diverses de coraux, d'éponges, d'anémones et de faune mobile comme par exemple des holothuries et étoiles de mer. Un éperon ou une crête sous-marine abritant une structure de coraux profonds suffisamment grande pour être identifiée comme un récif d'eau profonde a été découverte. Il s'agit d'un habitat rarement observé dans l'océan Indien. D'autres zones étaient couvertes de sédiments carbonés fins fortement bioturbés, habités par des oursins à coquille molle ainsi que de grands gastéropodes. Des poissons prédateurs, généralement associés aux monts sous-marins, ont également été observés, se nourrissant probablement de petits animaux nageurs qui migrent vers les eaux profondes durant la journée et se retrouvent piégés contre le plancher océanique. L'existence de cette zone de piégeage est probablement importante pour les grands prédateurs de la Réserve Marine des Chagos

À 250 m de profondeur, les sédiments étaient parsemés de gros blocs et de débris tombés des pentes récifales situées au-dessus. Les espèces étaient relativement rare à ces profondeurs et principalement confinées aux habitats rocheux. A des profondeurs légèrement inférieures, champs de cheminées carbonées on été découvertes, pouvant être la preuve d'une infiltration de méthane et d'une accrétion de carbonate par l'action de bactéries chimiosynthétiques formant des stromatolites. Ceci doit être confirmé par l'analyse d'échantillons de roche et son importance reste inconnue.

Un récif rocheux complexe à 120 m de profondeur habité par une riche faune des fonds marins et des communautés de poisons a été observé. Ces récifs se poursuivent jusqu'à 60 m de profondeur, abritant également de riches communautés comprenant des coraux photosynthétiques et des algues. Cependant, l'exposition de la limite occidentale de ces atolls à des conditions climatiques extrêmes signifie que des sables grossiers peu diversifiés couvrent une grande partie de la zone de 60 mètres de profondeur.

Les observations en eaux peu profondes ont révélé un mélange de récifs exposés aux courants comprenant une faible diversité (récifs du côté de la mer de Peros Banhos et de l'île de l'Aigle) et de récifs coralliens riches avec une biodiversité associée élevée de poissons et d'autres animaux dans le lagon de Middle Brother. Enfin, des colonies saines du corail en voie de disparition *Ctenella chagius* ont été observes.

Une collection d'organismes communs du plancher océanique et d'algues a été collectée pour un travail taxonomique visant à élucider la biodiversité des zones étudiées dans l'Archipel des Chagos. Cependant, la majorité des algues étaient petites, et les algues charnues que l'on trouve habituellement ailleurs dans l'océan Indien étaient absentes, probablement en raison de l'isolement de l'archipel et de la pression de pâturage extrêmement élevée sur les récifs de la réserve marine. Enfin, des échantillons ont été collectés pour le séquençage du génome de *Ctenella chagius*, pour des études de connectivité génétique afin examiner la connectivité des populations coralliennes entre les eaux peu profondes et les eaux profondes ainsi que de l'ADN environnemental pour établir des empreintes génétiques de biodiversité.

Contents

Authors

Alex David Rogers[1*], Olivier de Clerk[2], Clara Diaz[3], Kimly Do[4], Nicola Foster[3], Lawrence Hislop[1], Richard Jeong[5], Jyodee Pilly[6], Leighton Rolley[1], Denise Swanborn[7], Laura Warmuth[7], Bryan Wilson[7], Eva Ramirez-Llodra[1]

REV Ocean and Science Team

From left to right: Olivier de Clerke, Nils Baadnes, Laura Warmuth, Leighton Rolley, Alex Rogers, Nicola Foster, Clara Diaz (Back Row); Eva Ramirez-Llodra, Lawrence Hislop, Denise Swanborn, Jyodee Pilly (Middle Row); Bryan Wilson (Front).

Captain and Crew of the Ship

Captain Nicholas Inglis, Hermie Alelis, Kiara Bridge, Rachel Coleman, Vladimir Druro, La Mae Jandoc, Richard Jeong, Marvin Legrimas, Thomas Leighton, Argie Maloloy-On, Arlie Penera, Domineka Plecas, Jan Van Rijkevorsel, Scott Slagel, Shaun Smith, Jack Taylor, Severino Tubon, Rafael Vazquez.

Submarine Team

Kimly Do, Troy Engen, Javier Gomez Guindos, Kelvin Magee, Barry McGowan.

Affiliation of Authors:

1. REV Ocean, Oksenøyveien 10, NO-1366 Lysaker, Norway

2. University of Gent, Krijgslaan 281, S8 9000 Gent, Belgium

3. University of Plymouth, School of Biological and Marine Sciences, Davy Building, Drake Circus, Plymouth, PL4 8AA, United Kingdom

4. Deep R&D Ltd., Belmont House Station Way, Crawley, RH10 1JA, United Kingdom

5. Ocean Conservation, Exploration and Education Foundation, 511 Avenue Of The Americas Unit 268, New York NY 10011-8436, USA

6. School of Ocean Sciences, Bangor University, Bangor, Gwynedd, LL57 2DG, United Kingdom

7. University of Oxford, Department of Biology, 11a Mansfield Road, Oxford OX1 3SZ, United Kingdom

* Corresponding Author

Email: alex.rogers@revocean.org

Phone: +44 (0)7927 645 546

Introduction

The Chagos Archipelago includes the world's largest submerged atoll structure, the Great Chagos Bank, as well as about 58 islands, associated banks and an estimated 86 seamounts and 243 deep-sea knolls (Yesson et al., 2011; Sheppard et al., 2012; Hays et al., 2020). In 2010 a marine reserve was established around the archipelago and is one of the largest strict no-take marine protected areas in the world (550,000 km^2; Sheppard et al., 2012). Because of the absence of humans on all but one of the islands (Diego Garcia, where a large military base is present) the archipelago is thought to have low levels of local anthropogenic disturbance not only making it a refuge for marine life but also an important location for marine scientific research (Hays et al., 2020). This research has focused over the last four decades on monitoring of change in coral reef ecosystems but recently has also focused on fundamental aspects of reef ecology as well as the beneficial effects of very large marine protected areas on aquatic/marine megafauna (e.g. seabirds, turtles and sharks; Hays et al., 2020).

In general terms, the Indian Ocean has been neglected in terms of deep-sea research, acquiring the reputation of being "the forlorn ocean" among marine scientists (Rogers et al., 2017). The Chagos Archipelago is no exception and despite investigations in the early 20th Century by the Percy Sladen Trust Expedition to the Indian Ocean in 1905 (Andradi-Brown et al., 2019), there is little understanding of the biological communities of the Chagos Archipelago below SCUBA-diving depths (Hays et al., 2020). The Percy-Sladen Expedition undertook dredging operations to 150m depth and results suggested that zooxanthellate (photosynthesizing corals) were not found below 90m depth (Gardiner, 1905). Ten dredges below 90m depth on the seaward slopes of Salomon Atoll recovered Antipatharia, Octocorallia and mobile invertebrates (Gardiner & Cooper, 1907; Cooper, 1909). In the 1970s the three Joint Service Expeditions of 1972, 1974-1975, and 1978-1979 surveyed reefs in Chagos down to 60m depth (Baldwin, 1975; Griffiths, 1981; Sheppard, 1981). Since then, some diver surveys have been conducted to 50m depth, but increasingly restrictive health and safety regulations have subsequently curtailed such exploration. Instead, scientists have resorted to remote methods of study of mesophotic reef ecosystems including the use of Baited Remote Underwater Video (BRUVs) for study of fish communities, Remotely Operated Vehicles (ROVs) for benthic surveys to 150m

depth and the use of biological acoustics to study pelagic ecology (Letessier et al., 2016; Tickler et al., 2017; Andradi-Brown et al., 2019; Hosegood et al., 2019; Hays et al., 2020).

Diver observations of seaward reefs in Chagos have indicated a slope of varying gradient depending on location giving away to sandy slopes or shelves descending into deeper water (Andradi-Brown et al., 2019). On the southern side of Peros Banhos, at depths of 40-50m an 8-meter vertical rocky cliff has been reported to also include caves reaching back at least 10m into the wall (Winterbottom et al., 1989). More recent unpublished work using ROVs by the University of Plymouth has included observations of depths down to 160m depth at Egmont Atoll. At depths from 80-90m rocky walls were present with plate corals, Octocorallia and Antipatharia as well as a considerable diversity of fish. At depths of 110 – 120 m depth the seafloor was either flat and sandy or presented rocky walls colonized by Octocorallia, Antipatharia, Stylasterida, sponges and again diverse fish communities. At 150-160m gentle slopes with large boulders were observed with burrows in the sand and a lower-diversity rock community including *Tubastrea*, brisingid seastars and other organisms. Pelagic studies have indicated that seamount ecosystems within the Chagos Archipelago are hotspots for pelagic predators such as sharks. Acoustic surveys have suggested that the biomass of back scatterers is up to 100 times the background in the surrounding ocean (Letessier et al., 2016). Physical oceanographic studies suggest that internal waves associated with these seamounts may flush the summits with cool nutrient rich deep water (Hosegood et al., 2019). For this to explain the concentrations of deep scatterers and predators this would either have to lead to enhanced food supplies on the seamounts or act otherwise to concentrate the biomass of micronekton and other zooplankton (Hosegood et al., 2019). The fact that an enhancement of deep scatterers has now also been detected on the slopes of the islands suggests that a similar mechanism is operating both on seaward atoll slopes in addition to seamounts.

The presence of the *RV Odyssey* belonging to the Ocean Conservation, Exploration and Education Foundation (OCEEF) in the central Indian Ocean in Autumn of 2022 to undertake the Nekton Maldives Expedition presented an opportunity to deploy this vessel to the Chagos Archipelago. For the Nekton expedition, this vessel was

equipped with two Triton submersibles, a Triton 3K3 and a Triton 7K3, capable of diving to 1,000m and 2,300m respectively (although only currently certified to 1,000m). These submersibles are excellent platforms for submarine survey of seafloor ecosystems. The Triton 3K3 was equipped for seafloor survey with paired foreward and downward orientated high-definition Teledyne cameras. The 7K3 was equipped with a science skid containing a comprehensive suite of instrumentation for sampling biota, taking sediment cores and for undertaking video transects. In addition, *RV Odyssey* has a multibeam mapping system for mapping topography necessary to operate the submersibles safely. Given this opportunity an application for Permits for a deep-sea expedition to the Chagos Archipelago was submitted as soon as funding was made available for the expedition by REV Ocean.

Three locations in the Chagos Archipelago, western Peros Banhos Atoll, Eagle Island (Great Chagos Bank) and Pitt Bank, were selected for seafloor survey (see Figure 1). These locations were selected because the western side of the archipelago offered the greatest opportunity for sheltering in the lee of islands and atolls at this time of year when the prevailing winds tend to be from the southeast. They also offered contrasting localities (i.e. off islands on the seaward side of atolls or off a submerged atoll). The sites were also relatively near each other to minimize loss of time to transits within Chagos. The intention was to deploy the submersibles to undertake video survey and sampling of benthic communities include sessile and mobile benthic fauna and seaweeds as well as near seafloor fish communities. These surveys were directed to match depths with the Nekton Maldives Expedition so a direct comparison could be made between the surveys in the two locations. These depths included mesophotic communities (upper mesophotic at 60m and lower mesophotic at 120m), rariphotic communities (250m depth) and upper bathyal communities (500m depth). The surveys were intended to characterize habitat at these depths to test specific hypotheses related to the Chagos Archipelago and in relation to the wider western Indian Ocean region (see below). Overall, the expedition was aimed at transforming our current knowledge of the deep-water benthic ecosystems of the Chagos Archipelago and allowing an initial assessment of the importance of these in relation to the overall functioning of marine ecosystems within the marine protected area.

Figure 1. Sampling locations for the REV Ocean Mesophotic and Deep-Sea Research Cruise.

The hypotheses to be tested in the proposed expedition were developed from the review of current understanding of mesophotic reef communities in the Chagos Archipelago by Andradi-Brown et al. (2019) and also on studies of mesophotic and rariphotic communities from a broader geographic perspective (e.g. Laverick et al., 2018; Stefanoudis et al., 2019a,b).

(i) Coral communities show significant change with depth so that shallow (40 – 60m) and deep (60-90m+) mesophotic reefs are dominated by different species (e.g. *Pachyseris* and *Leptoseris* spp.) to those in shallow-water. This means that deep-water coral communities are limited as refugia for shallow coral reef species but are important in terms of the distinct biodiversity they harbour adding to overall reef biodiversity. Mesophotic coral communities are similar to those observed elsewhere in the Indian Ocean.

(ii) Fish communities of the Chagos reefs decline in biomass and abundance with depth but show less of a change of species diversity with depth indicating that mesophotic reefs may act as a refugium for shallow-water fish species (e.g. the endemic Chagos clownfish) from the impacts of climate disruption. Mesophotic fish communities are similar to those observed elsewhere in the Indian Ocean. The Coelacanth, *Latimeria chalumnae*, is present in deep mesophotic or rariphotic depths in the Chagos Archipelago.

(iii) There will be a switch in feeding strategy of fish with a decline in herbivory and a relative increase in the proportion of zooplanktivores moving from shallow to deep water.

(iv) Seaweed diversity of the Chagos reefs declines in biomass and abundance with depth. At the lower boundary of the photic zone diversity will be dominated by a combination of slow-growing persistent crustose species (coralline red algae, peyssonnelioid crusts, green gelatinous crusts, and the brown alga *Lobophora*) and ephemeral fast-growing species. From a taxonomic point of view mesophotic seaweeds are well-differentiated from those of shallow-water habitats. A link with temperate seaweed flora's of South Africa and the Arabian upwelling region is expected.

(v) Rariphotic reef communities (150-300m) are distinct from upper and lower mesophotic reef communities and are dominated by calcareous algae, octocorals, black corals, sponges and also fish of shallow water families but endemic to deep water.

(vi) The bathyal zone is distinct from the rariphotic zone and comprises families of organisms typical of the deep sea.

In addition to the mesophotic and deep-sea work planned for the expedition the availability of a launch on the *RV Odyssey* (the *Northwind*) offered the potential to facilitate shallow water work at Peros Banhos and Eagle Island. Here the focus was to assess reef health and to gather additional coral material for genetic connectivity and genomic studies. In particular, the species *Ctenella chagius* is amongst the most threatened reef-forming coral species in the world[1]. The expedition therefore offered the opportunity to collect genetic material from this coral species to support and ongoing full genome sequencing analysis funded by the Bertarelli Foundation's Marine Science programme and a QIAGEN Global NGS Profile Award. Given that we are likely entering the sixth global extinction event, largely driven by human activities, preservation of the genetic heritage represented by threatened species is increasingly a priority for scientists (Cowie et al 2022). The expedition was also an opportunity to carry out the first comprehensive assessment of seaweed biodiversity in the Chagos Archipeago.

Finally, the REV Ocean *Odyssey* Mesophotic and Deep-Sea Research Cruise formed an important contribution to the UN Decade of Ocean Science for Sustainable Development (https://www.oceandecade.org/). In particular, it was aimed to contribute to the Ocean Decade programme Challenger 150 (Howell et al., 2020a,b), an international effort to increase knowledge on the deep sea. This programme has a specific Indian Ocean working group. The Ocean Decade also has an emphasis on the career development and training of Early Career Researchers (ECRs). To this end we opened the expedition to Ph.D. students working on the Chagos Archipelago.

[1] See: http://www.edgeofexistence.org/species/ctenella-chagius/#overview

Methodology

Deep Sea Ecology

Submersibles

The prime tools for deep-sea ecology during this expedition were two Triton human Deep Submergence Vehicles (DSVs) or submersibles. These submersibles are characterised by having acrylic spheres which give excellent near all-round vision for the occupants of the vehicles. The Triton 3K3 can carry a pilot and two passengers and dive to 1,000m depth (3,000 feet; Fig. 2). This is the most numerous type of Triton submersible manufactured to date and was originally constructed for the superyacht / leisure market. The Triton 7K3 submersible, *Aurelia* is also built for a pilot and two passengers and is capable of diving to 2,300m depth (7,000 feet; Fig. 3). However, this is a purpose-built submersible for science and the deepest diving DSV with a sphere constructed entirely of acrylic (36cm thick). It is the first of its type and has been built specifically for deployment from the Superyacht / Research vessel *REV Ocean* currently in construction in Norway.

Figure 2. Triton 3K3 submersible undergoing preparations for diving during the expedition. The hydrolek arm is in an upright position (yellow cloth for visibility) and both survey and pan and tilt cameras visible.

Figure 3. Triton 7K3 submersible, *Aurelia* showing the Titan 4 hydraulic arm, the prime tool used for sampling deep-sea organisms during the expedition.

During the expedition *Aurelia* had only been approved for diving to 1,000m depth by DNV (Det Norske Veritas), the world's leading classification society for the maritime industry as the submersible was recently manufactured in the Triton facilities near Barcelona. Because the submersibles are designed to operate at different depths, they use different buoyancy systems the 3K3 using compressed air and the 7K3 a combination of compressed air and mineral oil-based systems. *Aurelia* is also equipped with a Titan T4 hydraulic arm compared to the Hydrolek electronic arm on the 3K3 submersible (Figs. 2, 3). It is also equipped with a science skid which includes a CTD system (Conductivity, Temperature, Depth), a hydraulically-operated science drawer, corers, and a suction sampler. Cameras include paired 4K SubC Rayfin 4K cameras and a pan and tilt Deep-Sea Power and Light 4K camera located on the port Variable Ballast Tank (VBT). There is also a downward-pointed Imenco HD camera. Both the stereo and downward cameras are equipped with laser dots for

scaling. For further details of these systems and their performance see technical report.

Submersibles were deployed over the stern of the *RV Odyssey* by being lifted into the water under the A-frame of the vessel (Fig. 4). Although the submersibles are steadied using tugger-lines on either side of the aft working deck calm conditions are required for safe deployment and recovery of the vehicles (Fig. 5). This is one of the disadvantages of DSVs compared to ROVs. Once underwater the submersibles are tracked in real time using acoustic transponders. This not only allows safe operation of the vehicles but also enables mapping of survey tracks and the precise identification of sampling and other points of interest during scientific deployments. Dive launch and recovery is always under control of the Surface Officer (SO) who is on the deck during launch and recovery operations and who communicates directly with the officer in charge on the Bridge of the vessel (usually the Captain during submersible operations; Fig. 5). The SO also communicates with the submersible pilots via underwater telephone. Verbal communications checks (Comms Checks) are undertaken every 15 minutes between SO and submersible pilots. Submersible pilots also communicate to the SO sampling events, the start and end of each transect and any unusual observations.

Figure 4. Photograph of the Ocean Conservation Exploration and Education Foundation (OCEEF) vessel *RV Odyssey* showing stern A-frame.

Figure 5. Recovery of Triton 7K3 *Aurelia* from the stern of the *RV Odyssey*. Note the tugger lines on either side and the tow line attached to the back of the submersible.

Deep-sea survey

Video survey data were collected using paired stereo cameras mounted on the front the Triton 3K3 submersible, one pair forward facing and one pair downward facing. The four cameras were L3C-HD Teledyne Bowtech fixed-focus 1080p resolution recording at 60 frames per second (Fig. 6). The cameras recorded into a power and recording unit tailor made for this purpose which was contained within a 500m depth-rated SVS Power and Record Pressure vessel (Fig. 6). The stereo video cameras were set originally at 80º angle for stereo camera recording although the cameras were knocked out of alignment during the previous expedition and had to be recalibrated using a SeaGIS calibration cube (https://www.seagis.com.au/index.html). Also mounted on the front of this submersible were a pair of forward-facing lasers (Teledyne Bowtech OceanLaser Dot 10-30Vdc) in 6000m depth-rated titanium housings. The Triton 3K3 was also equipped with a Teledyne Bowtech Surveyor HD-Pro pan and tilt camera placed within a 4000m depth rated titanium housing although this was not used. Lighting on the submersible included four Teledyne Bowtech V-Light, high-powered deep-water LED panel floodlights with a beam angle of 80º and

a strength of 20,000 lumens each. These were housed in anodised aluminium housings with a 280Vdc, ON/OFF control, MCBH-3M-SS Seacon connector rated to 6,000m depth.

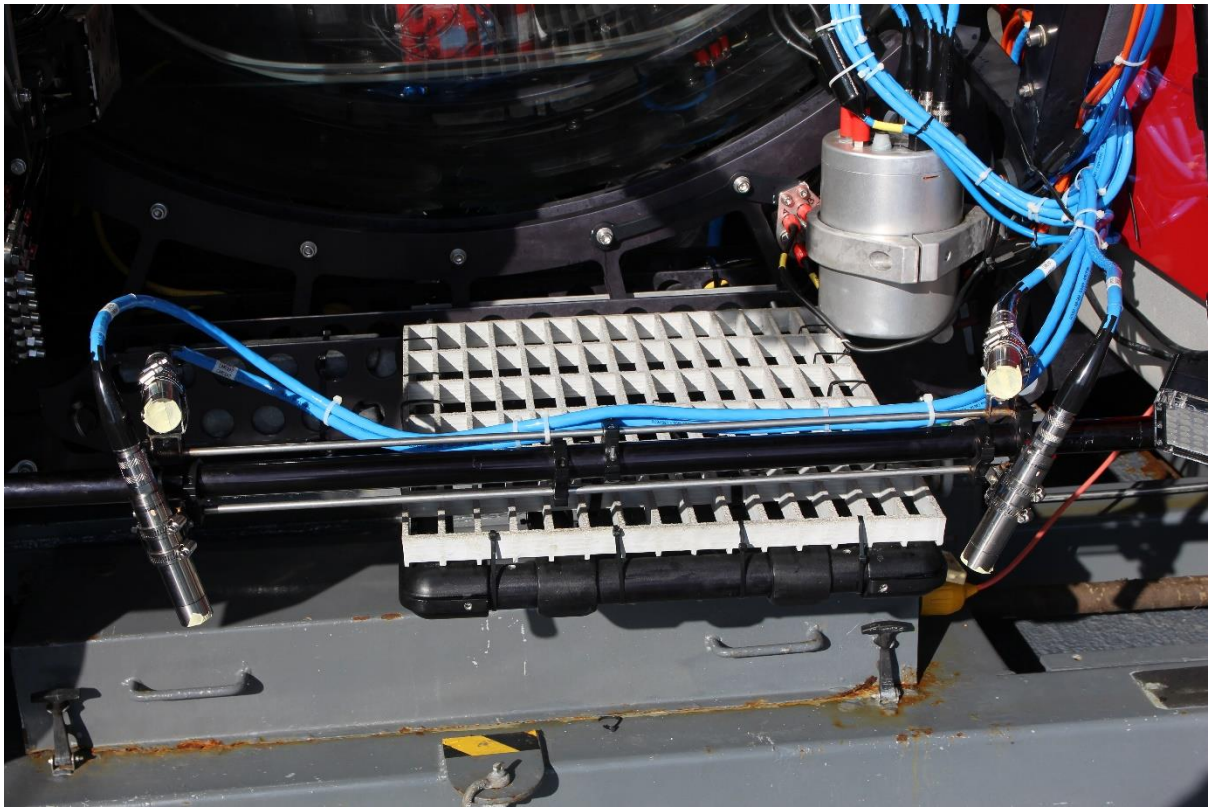

Figure 6. Front of Triton 3K3 submersible with paired L3C-HD Teledyne Bowtech fixed-focus cameras, one pair forward facing and the other downward facing. The Power and Recording Unit is located behind the cameras on the righthand side of the photo.

A minimum of three replicate transects were undertaken at each depth of 60m, 120m, 250m and 500m at each site. The distance of transects were measured by the SO using the submersible tracking system to 250m linear distance. Submersibles moved across the transect slowly (1-1.5 Kts) close to the seafloor (1 – 1.5m height) so that images could resolve the seafloor, seabed biota, fish and other swimming organisms. Often transect lines followed seafloor topography to maintain a close distance to the bottom and so distance travelled may exceed the linear distance where the isobath is convoluted (e.g. a submarine cliff following a winding course). As stated above, transect start and end was called up to the SO and recorded in Mission Control on a Dive Record sheet as well as noted down on a Submersible Dive Record sheet by a passenger, usually a scientist in the submersible (see Appendix I).

Sampling

Sampling was achieved in deep water using the Titan T4 manipulator (Fig. 7). For the duration of the cruise the suction sampler of *Aurelia* was not functioning (see Technical Report below). Also, limited presence of sediments suitable for sampling with the corers led to the decision to remove these from the science skid and replace them with extra lighting for the cameras. However, sediments were encountered in later dives. Both sessile and mobile megafauna were collected by the co-pilot using the Titan T4 manipulator under direction of what to sample by the scientist on board (Fig. 7). Some organisms were too delicate to be sampled using the manipulator (e.g. benthic Ctenophora) or to fast to be caught (most crustaceans). However, the hydraulic drawer of the science skid was found to function excellently providing a good seal. This prevented the loss of organisms once sampled, a common issue with both ROVs and DSVs especially during surface recovery and also prevented the escape of more mobile animals such as urchins. Each sampling event was recorded on the Dive Record Sheet including time in UTC, depth of collection and a description of the organism (i.e. octocoral, sea urchin etc.). Sampling events were called up to Mission Control by the pilot and recorded by a scientist present there who recorded the events with time and position on another Dive Record Sheet (these were slightly different for the Triton 7K3 and 3K3; See Appendix I). Sampling events were also filmed by the dive scientist using a mobile phone to assist with assigning specimens to sampling events post dive and to provide information on the habitat in which the organisms lived.

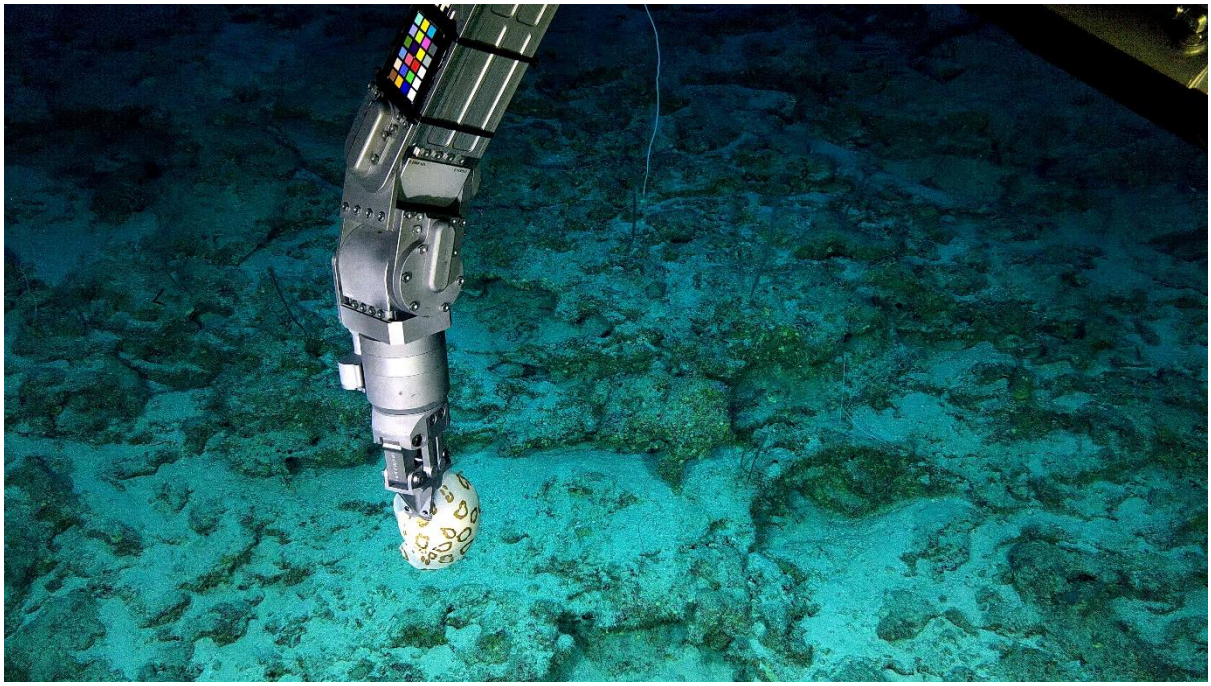

Figure 7. Titan T4 manipulator taking Sample ID 351, a large nudibranch from the genus *Pleurobranchaea* from 248m depth off Eagle Island.

Post sampling organisms were retrieved from the sampling drawer into buckets of seawater cooled prior to the dives in a cold room. Different buckets were used for the different compartments of the drawer to help with the assignment of specimens to sampling events recorded during the dive. Samples were moved to the cold room and processed immediately. This involved photographing each organism with a label upon which was written a unique sample number, the date, the deployment number and what the sample was (e.g. black coral). Labels were preprinted with ethanol-resistant ink to ensure that numbers were unique and consecutive. Samples were sub-sampled for genetics in 100% ethanol and/or RNA later and then fixed either in 10% formalin and transferred 48 hours to 70% ethanol or fixed in 100% ethanol. Treatment depended on taxon (see Appendix II). For sponges a subsample was taken for drying (up to 3cm by 3cm). Maintaining the link between sampling event and the unique identity of samples was critical to ensure that the sampling location of each specimen was known.

Multibeam Mapping

General Methodology

The *RV Odyssey* is fitted with a Teledyne Reson Multibeam Echosounder which was used to conduct seabed surveys. The maximum depth mapped at both sites was

around 2,000m. Data acquisition was done through QPS Qinsy and initial data processing was conducted in QPS Qimera. Although backscatter data were acquired, because of lack of software availability no backscatter processing was conducted during the cruise. Sound velocity profiles were obtained through XBT (1,000m) and CTD (500m) deployments

Seabed surveys were conducted to identify operationally safe and ecologically interesting targets for the submersible dives. Therefore, mapping was conducted directly upon arrival at the intended dive sites. As limited information is available on the bathymetry of the operating areas, surveys generally started with an initial line perpendicular to the coast to find the minimum operating depth. This informed subsequent survey lines.

The goal of the seabed surveys was to maximise the amount of information obtained at each site. Therefore, the MBES was also run opportunistically in between deployments or in evenings. For these survey activities survey lines were not always set out.

Snorkel Surveys

On seaward (Peros Banhos and Eagle Island) and lagoonal (Middle Brother) reefs, snorkelling was carried out in at least buddy pairs, within line-of-sight of the surface support craft *Northwind*. Snorkels were routinely 45 min in duration with minimum surface intervals of 30 min. Snorkelers were correctly weighted for subsurface diving. The lead snorkeler carried a mesh bag containing a diving slate, pre-labelled ziplock bags, gavel, scale bar (dive weight) and underwater camera. Visual surveys of reefs for *C. chagius* (Fig. 8). were performed from the surface, covering as much area as was possible within the snorkel period.

Figure 8. Hemispherical *Ctenella chagius* colony (above centre) observed at 7m depth from surface snorkel surveys

Colonies of *C. chagius* were to be photographed in wide-angle from surface, from 1m above (with scale bar), close-up macro and finally, with pre-labelled ziplock bag for sampling. The depth limitations of snorkeling, however, meant that these photographs were not always possible; where they were, samples (approximately 1-5 cm^3) were collected using the gavel and placed into pre-labelled ziplock bags (Fig. 9). Immediately following tissue sampling, snorkelers returned to the surface support craft and transferred samples into pre-labelled 50ml polypropylene tubes (corresponding to labels on pre-labelled ziplock bags) and tissue samples fully immersed into RNALater proprietary storage reagent and stored in a shaded coolbox. Upon return to the *RV Odyssey*, samples were stored in the cold room at 4-8°C for 24h (to allow for complete penetration of RNALater into tissues).

Figure 9. Sampling tissues from *Ctenella chagius* by snorkel

For surface surveys for general reef assessment, snorkellers with GPS watches filmed triplicate 50m transects of reef substrate from the surface in depths >1m (and more usually 5m), with cameras pointing directly downwards. For all snorkel surveys, relevant environmental data recorded as per REV Ocean Snorkel Record, with "Comments on the Snorkel" section to include water temperature, visibility and any currents observed.

Samples of *C. chagius* collected during this expedition were stored in RNALater for extraction of total RNA and DNA upon return to Europe. These extracted nucleic acids will then be sequenced by RNA-Seq and whole genome sequencing (using long-read technologies), both of which will support ongoing genome assemblies of *C. chagius* samples collected from other parts of the Chagos Archipelago (including Moresby Island and Diego Garcia).

Seaweed sampling

Sampling was achieved at Peros Banhos (Ile Pierre, Ile Manon, Ile Soeur), Eagle Island and Three Bother Islands. The seaward slopes of the reefs from 0m to 6m depth were surveyed by snorkelling teams containing 3 to 4 people. Surveys consisted of 50m transects, recorded using underwater GoPro video technology in

combination with random searches covering a larger area of the reef. Sampling (predominantly seaweed) was done by hand. For each sample the depth and potential association with other biota was recorded. Seaweed samples were preserved as herbarium vouchers, silica-dried samples (for DNA extraction), formalin (morphological analyses) and RNALater (genomics and microbiome characterisation). Specimens were photographed using either an Olympus Tough 6 camera or a Leica S9i dissecting microscope with light base.

eDNA

Environmental DNA (eDNA) refers to traces of DNA left in the water column by organisms, along with whole organisms (microscopic eukaryotes and prokaryotes), including larvae (Taberlet et al., 2012). eDNA sourced from seawater can be used as a method to investigate and monitor the breadth of biodiversity present within a habitat (Stat et al., 2017), particularly less accessible habitats, such as the mesophotic and rariphotic zones and the deep sea. Recent advances in next generation sequencing combined with metabarcoding mean that eDNA offers a fast an efficient insight into biodiversity at multiple depths, and these data can be used to enhance visual survey efforts that focus on conspicuous organisms (Nichols et al., 2022). In addition, eDNA can detect more mobile species, such as cetaceans, that may pass through an area and be missed by visual surveys. Here, eDNA will be used to enhance our understanding of biodiversity over the depth gradient in the Chagos Archipelago.

Water samples were collected from just below the surface using sterilised 10 L buckets. Once the samples were collected, a lid was placed on the bucket and it was transferred to the lab for immediate processing. Samples from 60 m, 120 m, 250 m and 500 m were collected using 1.8 L Niskin bottles attached to the port main ballast tank of the 7K3 submersible. At the appropriate sample depth, the Niskin bottles were triggered to close. Samples were processed as quickly as possible once the submersible was returned to deck.

Prior to processing the water samples, a vial (5 ml Eppendorf) was prepared for each sample (bucket or niskin bottle) and labelled appropriately with a unique sample ID and replicate number. All surfaces and equipment were sterilised using a 10% sodium hypochlorite (bleach) solution before filtration began and between samples. Following sterilisation of equipment and gloves, hot water was used to remove any

residue of bleach and cold water was used to cool the equipment. Gloves were worn and sterilised regularly during the filtration procedure. Each water sample was pumped through a microfibre filter (Fisher) using a millepore vacuum pump (Fig. 10). For surface samples, 6 L of water were filtered per replicate and for deep samples, between 1 and 1.5 L of water were filtered per sample. A control of 1 L of distilled water was pumped through a filter during each batch of sample processing. Each filter was removed using sterile forceps and placed in a 5 ml vial with 100% ethanol. Samples were stored in the cold laboratory.

Figure 10. Millepore pump setup for filtering seawater samples for eDNA analysis.

A total of 6 surface and 27 deeper water samples were collected during the expedition for eDNA analysis (Table 1). These samples will be returned to the UK for further analysis. DNA will be extracted from each filter using a DNeasy Blood and Tissue extraction kit (Qiagen). A minimum of 3 universal primers (including COI, 16S and ITS2) will be used to amplify DNA, and samples will be sequenced using an Illumina MiSeq platform (Nguyen et al., 2020; Alexander et al 2020). Sequences will be processed using appropriate pipelines in R. Results will be cross-referenced against relevant databases (e.g. GenBank and Barcode of Life Database) to enable sequences to be assigned to taxa, where possible. Biodiversity within water samples will be compared to that recorded using visual surveys of the seabed and fish communities at each depth.

Row Labels	Surface	60 m	120 m	250 m	500 m	Total
Great Chagos Bank - Eagle Island	3	4	4	3	6	20
Peros Banhos - Ile Pierre	3	2	3	2	3	13
Grand Total	**6**	**6**	**7**	**5**	**9**	**33**

Table 1. Number of water samples collected for eDNA analysis at each site and depth within the Chagos Archipelago.

Population Genetics

One possible refuge for coral reef species against the impacts of climate change and local threats is the presence of Mesophotic Coral Ecosystems (MCEs) (Glynn 1996; Bongaerts et al., 2010; Bridge et al., 2013). Some reef-forming species, and their associated biota, are found deeper than the brightly lit shallow waters normally associated with zooxanthellate corals, forming MCEs, which are defined as light-dependent corals and associated communities at depths of 30 m to >150 m in tropical and sub-tropical waters (Lesser et al., 2009). MCEs may occur over geographic distributions as extensive as shallow-water coral reefs (Puglise et al., 2009) and the potential importance of MCEs as refugia from the effects of climate change has only recently emerged (Bongaerts et al., 2010). MCEs have shown evidence of being in a relatively pristine condition and having largely escaped recent coral bleaching events and current anthropogenic threats (Puglise et al., 2009; Smith et al., 2010; Bridge and Guinotte 2013; Sinniger et al., 2013; Muir et al., 2017; Baird et al., 2018; Stefanoudis et al., 2019a,b), and thus, may play an important role in the recovery of impacted reef regions (Ridgway et al., 2002; Hoegh-Guldberg et al., 2008; Bongaerts et al., 2010; Bridge and Guinotte 2013).

The ‘Deep Reef Refugia Hypothesis’ was first postulated by Glynn (1996) and suggests that the perturbations (storms, bleaching) experienced by shallow reefs frequently diminish in strength with increasing depth, thus, deeper reefs could provide a spatial refuge for corals with a wide depth distribution and could serve as a source of larvae for the recovery of degraded shallow-water reefs. The potential connectivity between shallow reefs and their mesophotic counterparts depends on a number of factors, including the extent of species overlap between depths (Muir et al., 2015; Laverick et al., 2017), the oceanographic regime experienced by larvae at depth (Slattery et al., 2011) and possible changes in reproductive characteristics with depth (Baker et al., 2016).

Understanding the vertical and horizontal genetic connectivity among shallower and deeper reefs in the Chagos Archipelago will enable us to understand the potential that MCEs offer in terms of supporting the recovery of shallow-water reefs in the region. Recent research by the University of Plymouth on the mesophotic reefs around Egmont Atoll and Sandes Seamount has included sampling scleractinian coral colonies between 20 and 95 m. This expedition will expand this research to additional sites within the Chagos Archipelago, with coral sampling in Peros Banhos and Eagle Island.

Samples of Scleractinian corals were collected using the 7K3 submersible, *Aurelia* on dives between 60m and 120 m depth. The Titan T4 arm was used to break a small fragment of coral from the edge of a colony and this was placed within the sample box of the submersible (Fig. 11). The time, depth and location of the sampling was recorded on log sheets. On returning to the ship, samples were transferred to the cold laboratory, where they were labelled, photographed and preserved in 100% ethanol for genetics analysis and 70% formalin for morphological analysis.

A total of 10 plate corals were sampled from Peros Banhos and Eagle Island between 58 and 89 m depth. On return to the UK, DNA will be extracted from samples using a DNeasy Blood and Tissue kit (Qiagen) and samples will be identified using coral specific primers for the COI region of the genome, where possible. Population genetic analysis will be undertaken using Single Nucleotide Polymorphisms (SNPs) to assess vertical and horizontal connectivity of specific coral species among Peros Banhos, Eagle Island, Egmont Atoll and Sandes Seamount.

Figure 11. Titan T4 arm on the 7K3 submersible sampling a fragment of plate coral at 60m, Eagle Island, Chagos Archipelago.

CTDs

A Seabird SBE19 V2 Seacat Profiler mounted in a dedicated CTD cage was used to measure electrical conductivity, temperature and pressure/depth of seawater. It also had sensors attached for measuring dissolved oxygen, pH and chlorophyll fluorescence to obtain further detail on water chemistry. The CTD cage was fitted with an additional 25kg weight to prevent the CTD cage from floating and excessive drift, and a Sonardyne USBL beacon to monitor its position and depth

Using a hydrographic winch, the CTD was deployed off the stern of the vessel down to a depth of 500m. Before deployment, the CTD was turned on and caps were

removed from the relevant sensors. The CTD was submerged at 1m depth for 1 minute to allow the USBL signal to come in, before rolling out to 500m. Deployment depth was monitored both using the USBL beacon and the amount of wire rolled out from the winch. At 475m roll-out speed was slowed down allowing the CTD cage to stop at 500m. The CTD was kept at 500m for 30 seconds, before being rolled back in in one continuous motion.

Upon recovery caps were placed back on specific sensors and the whole instrument was washed with fresh water for future deployments. Data was transferred from the CTD to the computer using Seabird software (Seasoft V2) and outputted as a .hex file. These outputs were converted to visuals representing changes in the measured oceanographic parameters along the depth gradient. CTD outputs were also used to calculate Sound Velocity Profiles used for multibeam data processing.

A total of three CTDs were deployed, 1 at Peros Banhos and 2 at Eagle Island (Table 2).

Date	Location	Start Lat (DD)	Start Lon (DD)	Time of deployment (UTC)	Water depth below vessel	Distance drifted during deployment
18/10/2022	Peros Banhos	-5.3165	71.7173	09:18	725m	200m
20/10/2022	Eagle Island	-6.1843	71.2990	09:20	700m	355m
23/10/2022	Eagle Island	-6.1881	71.2944	09:22	800m	185m

Table 2. CTD casts using the surface-deployed CTD system during the REV Ocean Mesophotic and Deep-Sea Research Cruise.

Social Media

A communications and social media plan was arranged before the cruise so that daily postings highlighting the science, technology and teamwork onboard could be communicated to relevant partners and to people around the world interested in following the mission. The social media content was aligned with the Chagos mission calendar of activity, mission objectives and REV Ocean brand voice. Content was distributed on REV Oceans four main social media channels (Twitter, Facebook, Instagram and LinkedIn; see Table 3).

Lawrence Hislop, Communication Director of REV Ocean participated in the mission and produced photos, videos, and audio recordings daily and worked together with the team onboard to craft text highlighting the mission's core results. The Chagos

cruise directly followed the Nekton Mission in the Maldives and the same social media company (Communications Inc. in the UK) facilitated posting from on-shore. With limited internet capacity on the *RV Odyssey*, this provided the best opportunity for a regular stream of updates.

October	Activity
01	• First Press Release published about the Mission • Website goes live • Social media announcements • Mission Content across Digital Channels begins to be published (tagging all partners)
10th	• Chagos Mission starts, Male, Maldives
15 – 25th	• Mission – at sea – producing and publishing content on digital channels and to international news media. • Content created by REV Ocean and shared with Partners directly for promoting on their channels. • Content highlighting ECR's work, Partner's role on Expedition and amplified as part of the Digital Communications Campaign. • Content produced during mission for later publication.
26th	Mission ends
End-October	• REV Ocean shares photographic and video content produced from the mission to share with partners. • Tailored educational film produced for regional community. • REV Ocean participates in events (in person and /or virtual) promoting the key discoveries, messages and core accomplishments of the mission.
2022-2023	• REV Ocean and partners attend global ocean, science, environmental events highlighting the discoveries and impacts from the mission.
2023	• First scientific results from the mission announced.

Table 3. Social Media Calendar for the REV Ocean Mesophotic and Deep-Sea Research Cruise

Summary of Mission Deployments

DAY	Deployment	Activity	Location	Site	Depth (m)	LAT/LON
16 Oct 2022						
	001	XBT	Peros Banhos	Ile de Pièrre	1000	5°09.350S 71°46.510E
	002	Snorkel	Peros Banhos	Ile de Pièrre	0-10	5°18.1 S 71°43.9 E
	003	Omega162	Peros Banhos	Ile de Pièrre	500	5°18.633S 71°43.447 E
	004	Aurelia32	Peros Banhos	Ile de Pièrre	500	5°18.633 S 71°43.447 E
17 Oct 2022						
	029	XBT	Peros Banhos	Ile de Pièrre	1000	05°15.454S 71°38.467E
	005	Snorkel	Peros Banhos	Ile de Pièrre	0-10	5°18.9675 S 71°43.946 E
	006	Omega163	Peros Banhos	Ile de Pièrre	60, 120, 250	05°18.680 S 71°43.632 E
	007	Aurelia33	Peros Banhos	Ile de Pièrre	60, 120, 250	05°18.680 S 71°43.632 E
18 Oct 2022						
	008	Snorkel	Peros Banhos	Petite soeur	0-10	5°21.33 S 71°45.06 E
	009	Omega164	Peros Banhos	Ile de Pièrre	60, 120, 250	05°19.118 S 71°43.502 E
	010	Aurelia34	Peros Banhos	Ile de Pièrre	60, 120, 250	05°19.118 S 71°43.502 E
	011	CTD	Peros Banhos	Ile de Pièrre	500	05°18.9915 S 71°43.0355 E
19 Oct 2022						
	012	Snorkel	Great Chagos Bank	Eagle Island	0-10	6°10.35 S 71°20.06 E
	013	Omega165	Great Chagos Bank	Eagle Island	60, 120, 250	06°11.1577 S 71°19.1015 E
	014	Aurelia35	Great Chagos Bank	Eagle Island	60, 120, 250	06°11.273S 71°19.190 E
20 Oct 2022						
	015	XBT	Great Chagos Bank	Eagle Island	1000	06°08.460S 71°30.960E
	016	Snorkel	Great Chagos Bank	Middle Brother	0-10	6°09.164 S 71°31.053 E
	017	Omega166	Great Chagos Bank	Eagle Island	500	06°11.27326S 71°19.10177E
	018	Aurelia36	Great Chagos Bank	Eagle Island	500	06°11.27326S 71°19.10177E
	019	CTD	Great Chagos Bank	Eagle Island	500	06°11.0587 71°17.909E
21 Oct 2022						

	020	Snorkel	Great Chagos Bank	Middle Brother	0-10	6°09.164 S 71°31.053 E
	021	Omega167	Great Chagos Bank	Eagle Island	60, 120, 250	06°11.24631 S 71°19.12424E
	022	Aurelia37	Great Chagos Bank	Eagle Island	60, 120, 250	06°10.979S 71°19.339E
22 Oct 2022						
	023	XBT	Great Chagos Bank	Eagle Island	1000	6°35.880S 71°11.400E
	024	Omega168	Great Chagos Bank	Eagle Island	500	06°10.45369 S 71°18.91750E
	025	Aurelia38	Great Chagos Bank	Eagle Island	500	06°10.45369 S 71°18.91750E
23 Oct 2022						
	026	Omega169	Great Chagos Bank	Eagle Island	60, 120, 250	06°11.9195 S 71°18.6898 E
	027	Aurelia39	Great Chagos Bank	Eagle Island	60, 120, 250	06°11.9195 S 71°18.6898 E
	028	CTD	Great Chagos Bank	Eagle Island	500	06°11.29782S 71°17.67185E
	030	XBT	Great Chagos Bank	Eagle Island	1000	06°11.220S 71°17.598E

Table 4. Summary of mission deployments.

Preliminary results

Multibeam

Peros Banhos

An area of 245.606 km² was surveyed around the northwestern side of Peros Banhos, including Benares Bank. The focal area of the surveys was initially Ile Pierre as this was the intended dive site, however, the survey area was expanded when more interesting bathymetry was observed to the north and south of Ile Pierre.

The survey uncovered a narrow ridge extending from Ile Pierre to the Benares Bank, with steep drop offs on either side of the ridge (Fig. 12). The seabed survey showed a wide canyon system to the north of this ridge, which was mapped down to 2,000m depth, which large rocky structures in the basin (Fig. 12).

Bathymetry was comparable along the islands (Ile Diamant, Ile Pierre, Grande Soeur, Petite Soeur) with a narrow shallow ridge, with a vertical wall starting at 100m depth dropping down to about 200m where a more gradual slope began, interspersed with rocky features from 1,000m onwards. However, steep narrow channel/chute structures were observed off Grande Soeur and Petite Soeur between 400m-800m depth (Fig. 12).

Mass wasting events were observed to the south of Benares Bank, with pronounced gullies at similar depths as the channels at Grande Soeur and Petite Soeur (400m-800m; Fig.12)

Some evidence of a shallower flat (800m) was observed from one survey line further to the north, however, further surveys will be required to confirm.

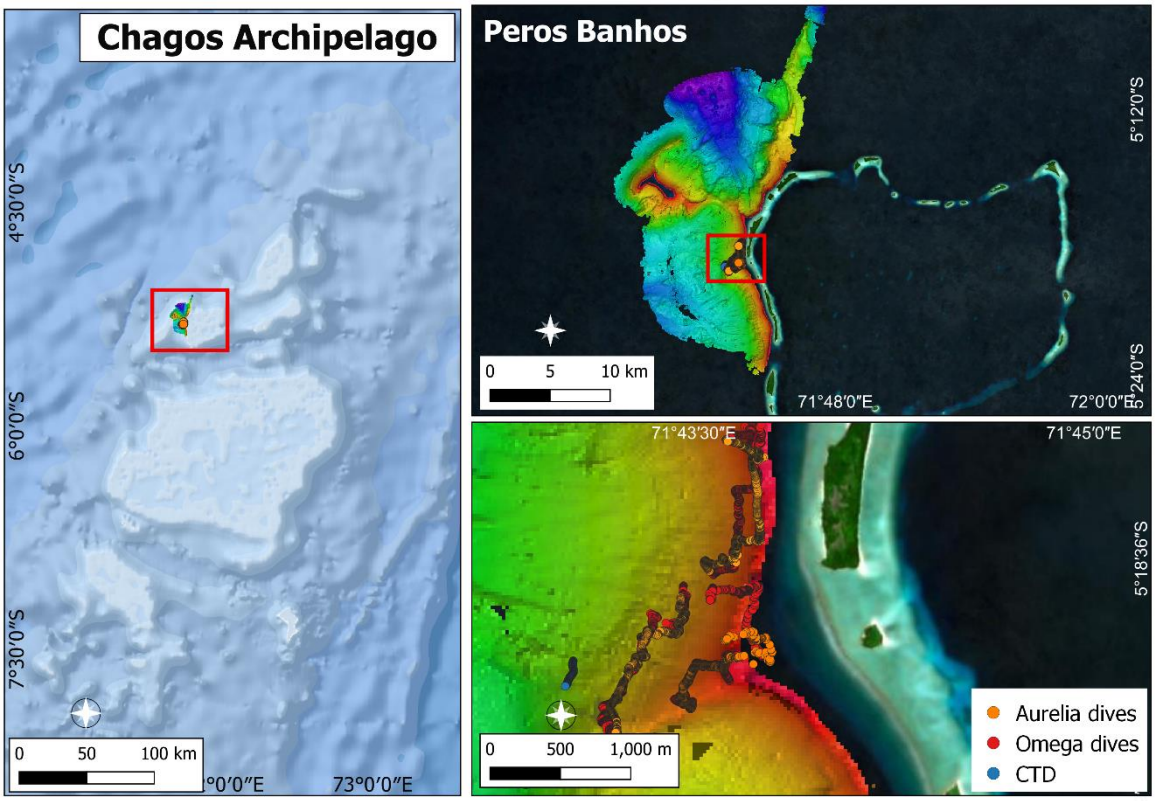

Figure 12. Multibeam bathymetry data for Peros Banhos showing general location (left panel), overall multibeam map (upper right) and detail showing tracks of submersible dives (lower right). Colours run from shallow (red) to deep (purple). Note that the submersible track for the Triton 3K3 500m (deepest) dive ends at the tip of the submarine spur from the atoll where coral frameworks were observed (see Preliminary Results).

Eagle Island

A total area of 256.233 km² was surveyed around Eagle Island. This included the north of the atoll extending to Middle Brother, and the western side of the atoll extending to Danger Island (Fig. 13).

The survey revealed an extensive canyon system in the northern side of the atoll, extending between the Brothers and Eagle Island. The canyon system has three main forked branches joining a main basin between 1,300-1,500m depth (Fig. 13).

Off Eagle Island the survey revealed a steep wall from about 100m to 250m which flattened out in a more-gentle slope riddled with smaller gullies until about 600m (Fig. 13). Evidence of mass wasting events and chute structures were observed at 600m resulting in a steeper drop off until 1200m-1400m (Fig. 13). This was also observed along the western side of the atoll past Cow Island down to Danger Island. Here, pronounced mass wasting and chute structures were observed (Fig. 13).

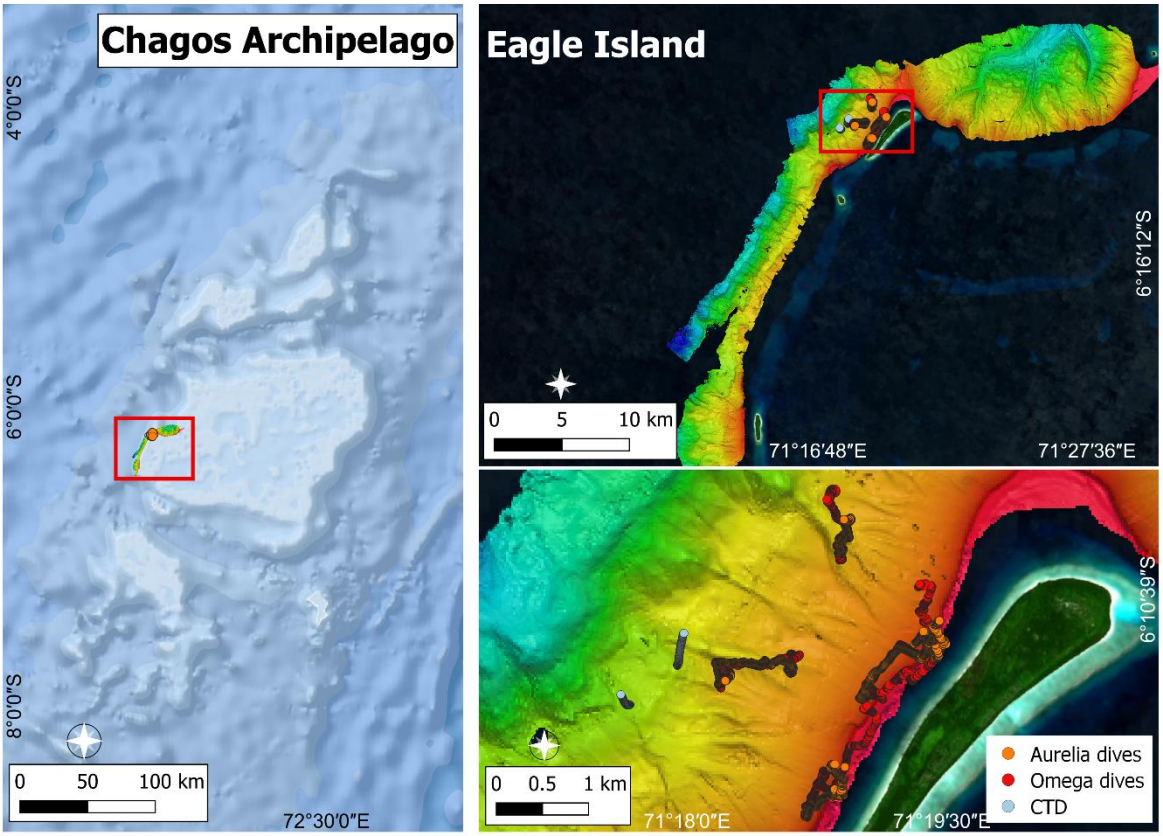

Figure 13. Multibeam bathymetry data for Eagle Island showing general location (left panel), overall multibeam map (upper right) and detail showing tracks of submersible dives and CTD positions (lower right). Colours run from shallow (red) to deep (purple).

Pitt Bank

68.69875 km² was mapped around the northwest corner of Pitt Bank. As weather conditions did not allow deployment of the submersible the area mapped here was smaller than areas around Peros Banhos and Eagle Island (Fig. 14).

The northwest side of the bank featured a short ridge at around 200m depth followed by a steep slope with chute structures and channels between 400m and 800m (Fig. 14). Limited evidence of a shallower bank at 400m and steep drop to 1600m was

observed to the north of Pitt Bank (Fig. 14).

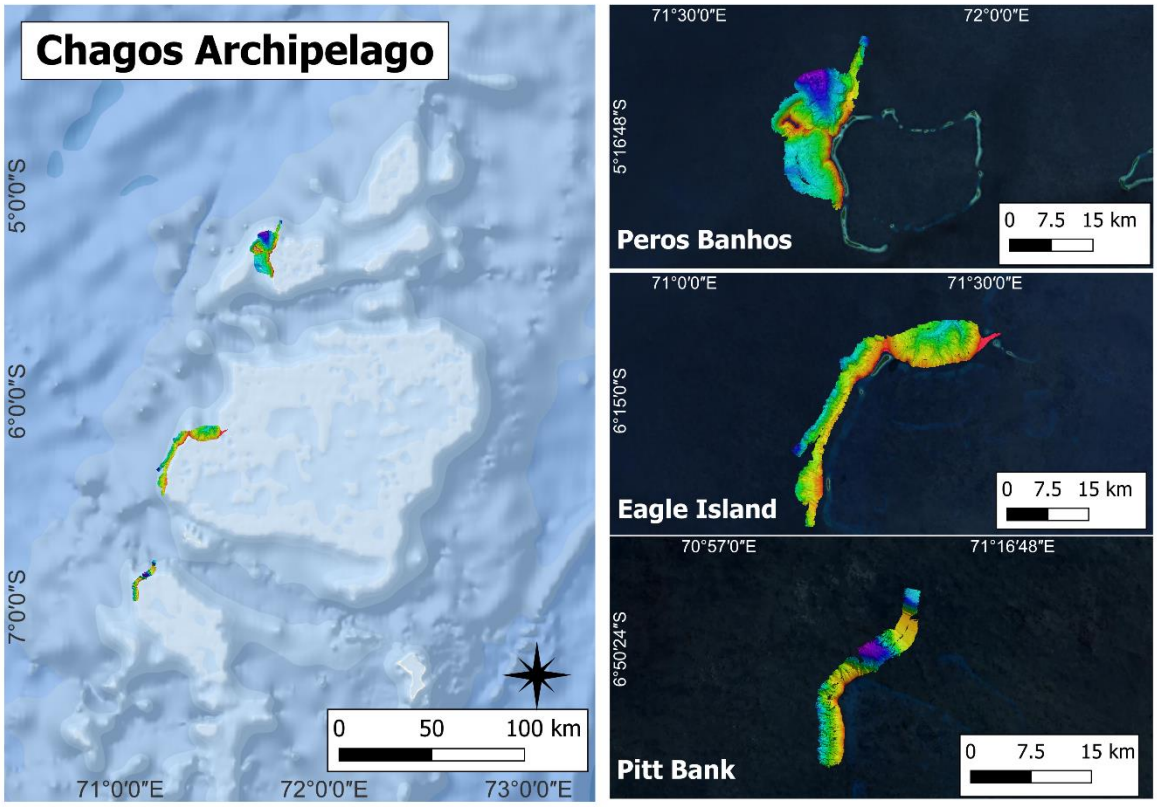

Figure 14. Map showing where all multibeam mapping was undertaken.

Issues

Depth readings were found to be inaccurate when compared with the USBL data from the submersible dives. The MBES depth was around 70m deeper than it should be. The depth values given in the above section are corrected depth values.

CTDs

Results for the salinity, temperature and fluorescence data are shown graphically on figures 15, 16 and 17 for Peros Banhos and Eagle Island. Whilst we do not undertake water mass analysis here a number of features are apparent from the data presented. Depth profiles for salinity, temperature and fluorescence vary markedly between Peros Banhos and Eagle Island particularly in the shape of the subsurface salinity maximum and the thermocline between 20-50m depth. At Peros Banhos there is a surface mixed layer down to 20-30m below which is a marked thermocline from ~30 to 50m where the temperature drops from ~28^{o}C to 21^{o}C. Temperature then declines to ~ 9^{o}C at 500m depth, the maximum depth of

submersible operations (Fig. 15). A marked salinity maximum between 20-150m is prominent at Peros Banhos but less so at Eagle Island (Fig. 15,16,17). This feature was not apparent from physical oceanographic studies at Sandes Seamount where salinity is relatively uniform to 60m depth and then markedly declines down to 80m forming a distinct pycnocline (Hosegood et al., 2019). Fluorescence is detectable to 100 – 150m depth, an unsurprising result given the very clear waters and deep light penetration around the Chagos Archipelago (Figs. 15, 16, 17). However, the marked chlorophyll maximum observed at Sandes Seamount (see Hosegood et al., 2019) is not apparent on any profiles taken during this expedition although there is a slight subsurface chlorophyll peak at around 20m depth in the first CTD cast at Eagle Island (Fig. 16). Very different results obtained during this expedition at different locations and also between this expedition and previous expeditions points to the very dynamic oceanography of the archipelago both temporally and spatially. The CTD also had pH and oxygen sensors and further CTD readings were taken on a CTD mounted on the DSV *Aurelia*. These will be further analysed by a physical oceanographer in the future to delineate water mass structure and other information.

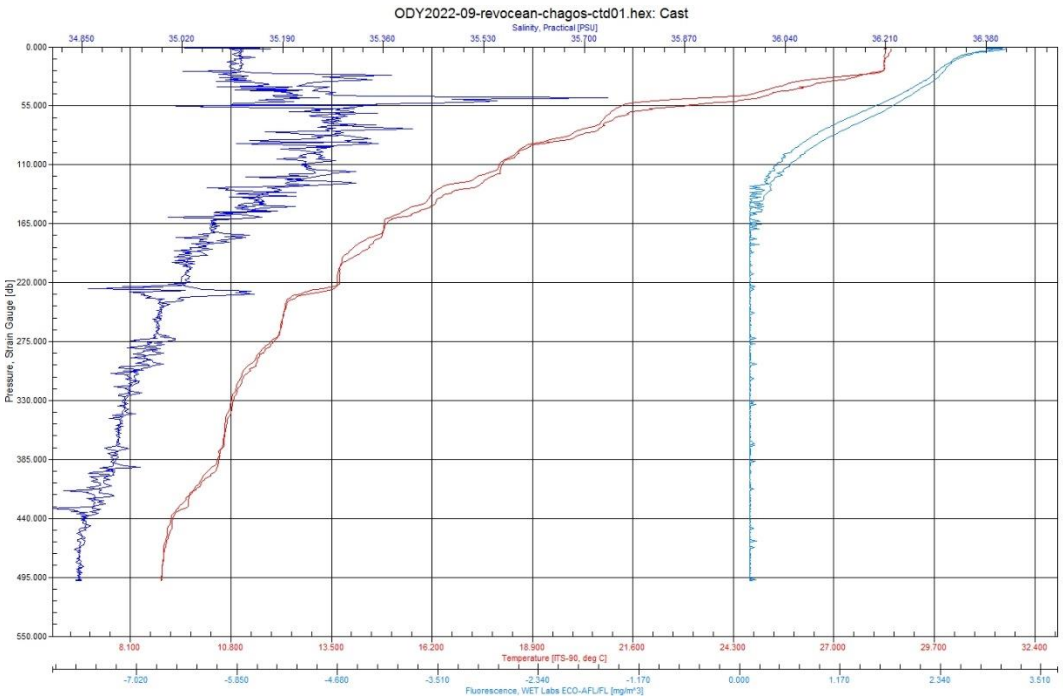

Figure 15. CTD cast to 500m depth at Peros Banhos showing salinity (dark blue), temperature (red) and fluorescence (turquoise).

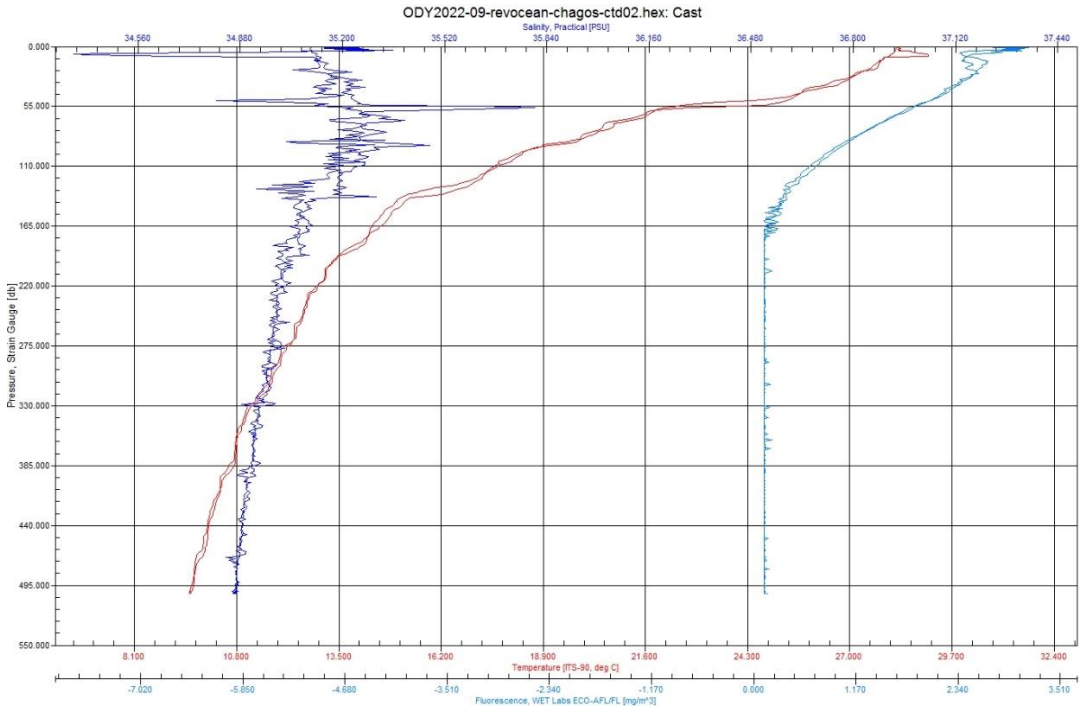

Figure 16. CTD cast to 500m depth at Eagle Island showing salinity (dark blue), temperature (red) and fluorescence (turquoise).

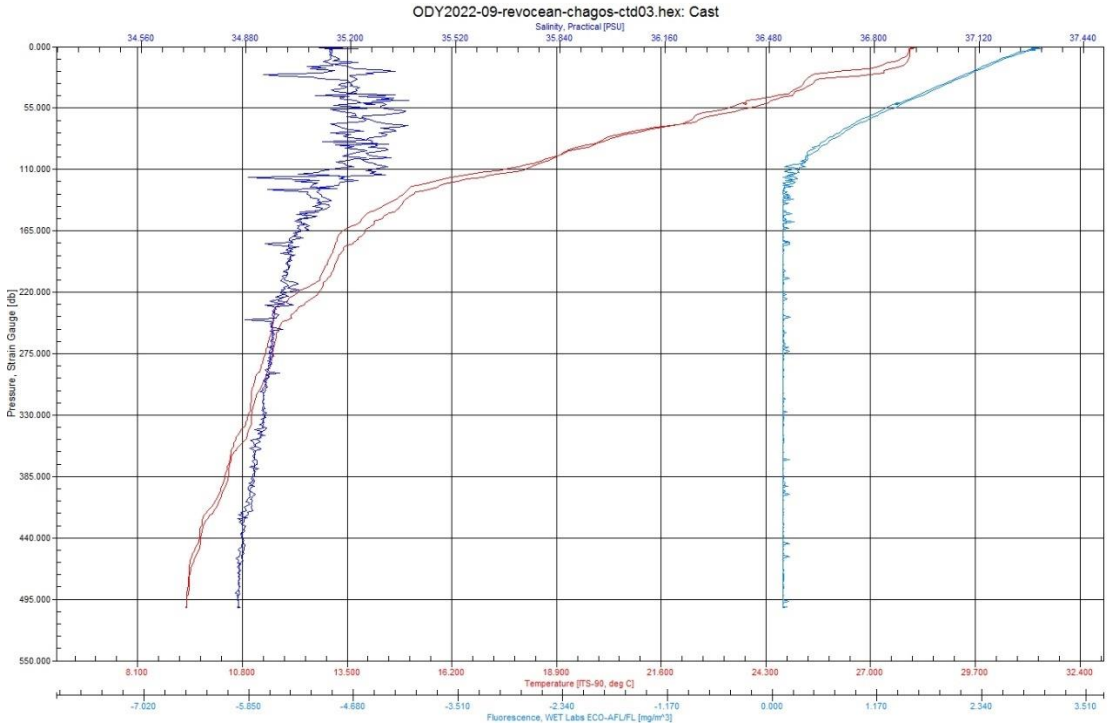

Figure 17. CTD cast to 500m depth at Eagle Island showing salinity (dark blue), temperature (red) and fluorescence (turquoise).

Deep-Sea Ecology

1. Peros Banhos

The submarine topography of Peros Banhos is extremely complex (Fig. 12) and the dive observations undertaken during this expedition can only be taken as preliminary. There are likely to be a larger range of habitats and biological communities than documented in just six dives over three days.

500m Depth

This dive took place on a steep rocky slope which also comprised terraces, short blind gulleys and vertical walls (Fig. 12). The substratum was mainly carbonate rock which in places was fissured with long cracks and small holes and caves. A thin draping of sediment was present in areas and towards the end of the dive with the Triton 3K3 an area of huge stone blocks, rock pinnacles and gulleys. Fish fauna included a variety of predators which have been seen at similar depths in the Maldives and also elsewhere at rariphotic depths (150-300m; e.g. Bermuda). These included tinsel fish (*Grammicolepis brachiusculus*; Fig. 18), Darwin's slimehead (*Gephyroberyx darwini*; Fig. 19), coffin fish (*Chaunax* cf *pencillatus*), cardinal fish (*Epigonus* sp.), spikey oreo (*Neocyttus rhomboidalis*), rattail (*Trachyrhychus* sp + others) and a common unidentified fish (possible Ophidiidae). Several of these fish species are known to feed on micronektonic fish, squid and crustaceans (see below).

Figure 18. Tinsel fish, *Grammicolepis brachiusculus,* filmed on a steep rocky slope at ~ 500m off Eagle Island.

The invertebrate fauna at 500m depth at Peros Banhos appeared sparsely distributed throughout much of the dives although animals tended to be concentrated in fissures and holes or along the edges of large boulders. Notable biota included a globe-like glass sponge (Euplectellidae), a hollow, branching glass sponge (*Aphrocallistes* sp.; Fig. 19), a variety of black corals (e.g. *Parantipathes*), octocorals, including bamboo coral (Isididae), Scleractinia (*Stenocyathus vermiformis*), comatulid and stalked crinoids (Crinoidea), sea stars (Asteroidea) and sea urchins (Echinoidea, Echinothuridae). An example of the euplectellid sponge sampled by the *DSV Aurelia* (Specimen ID 014) was inhabited by a pair of ships, a male and a female (Sample ID 016), as is commonly found in these deep-sea sponges. A large bivalve mollusc with a glassy translucent shell was also seen inhabiting overhangs of rock (*Delectopecten* sp.).

Figure 19. Darwin's slimehead, *Gephyroberyx darwini*, filmed at Eagle Island at ~500m depth. Note the glass sponges (top left) which are probably *Aphrocallistes* sp.

Towards the end of the final transect at this depth and location the Triton 3K3 encountered rugged terrain associated with a ridge or spur extending from the atoll shelf. This was the area associated with blocks, pinnacles and gulleys which were inhabited by dense gardens of octocorals but also extensive frameworks of scleractinian coral (Fig. 20). These frameworks can be characterised as coral thickets possibly comprised of *Desmophyllum pertusum* (formerly known as *Lophelia pertusa*) or *Solenosmilia variabilis*. In places the coral framework had accumulated between pinnacles in gulleys probably as a result of the coral breaking off and falling from the rock pinnacles and boulders. The biota in these coral garden and cold-water coral thicket/reef habitat was rich and included numerous large squat lobsters (Galatheidae), mantis shrimps (Stomatopoda) and bright orange octopus (Cephalopoda; see Fig. 20). The discovery of these cold-water coral habitats is unexpected and demonstrates the importance of the Chagos Marine Protected Area for deep-sea conservation (see below).

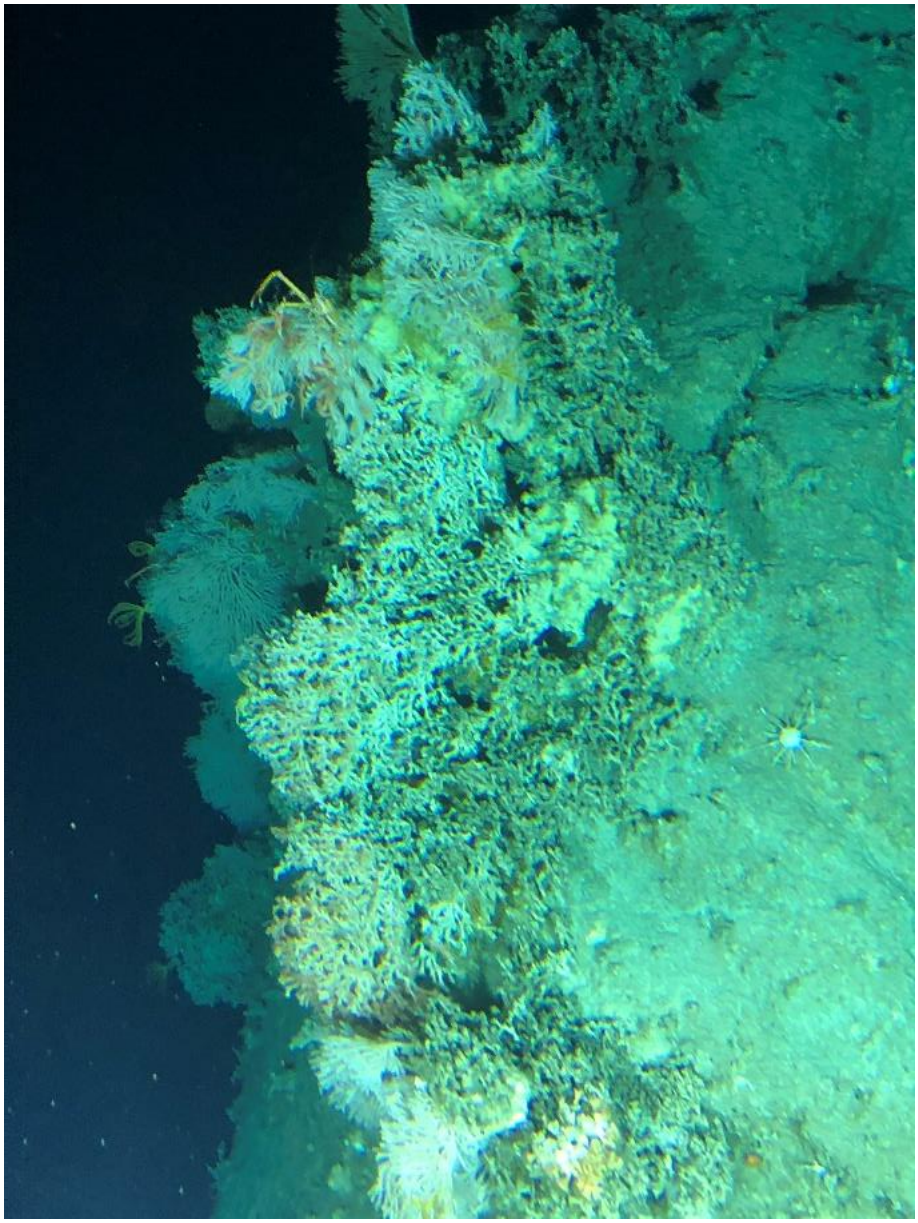

Figure 20. Framework-forming scleractinian coral, possibly *Desmophyllum pertusum*, growing on a rock pinnacle at Peros Banos at 500m depth. Note the coral garden habitat behind and also the large squat lobster on the coral in the top half of the photograph.

250m, 120m, 60m depths

At 250m the terrain was largely fine sediment with a scattering of extremely large boulders (some the size of the submersible or larger). At times sediment was draped thinly on calcareous bedrock and a small area of irregular low-lying chimneys with depressions in the top were observed (later dubbed “alien eggs). Fish fauna was sparse restricted mainly to boar fish (*Antigonia* sp.). Invertebrates were mainly restricted to boulders and included scattered colonies of hydrocorals (Stylasteridae), octocorals and black corals. A yellow encrusting / boring sponge was also evident.

Colonies of large whip corals, probably Ellisellidae (either *Ellisella* or *Viminella*) were frequently encountered on sediment (but probably very thin sediment).

At 120m depth terrain comprised steep rocky slope with a thin drape of sediment in places. The rock provided a highly cryptic habitat with many holes and micro-caves (Fig. 21). Fish present included black-fringed bigeye (*Pristigenys refulgens*), a black and white butterfly fish (possibly *Apolemichthys xanthurus*), groupers (e.g. *Ephinephelus morrhua*; Fig. 21), a variety of snappers (Lutjanidae), slopefish (*Symphysanodon* sp.) and basslets (Antheidae). Vertical rock faces and small caves harbour a rich encrusting fauna as well as pink calcareous encrusting algae. Larger corals including *Cirripathes* and large octocorals are also present.

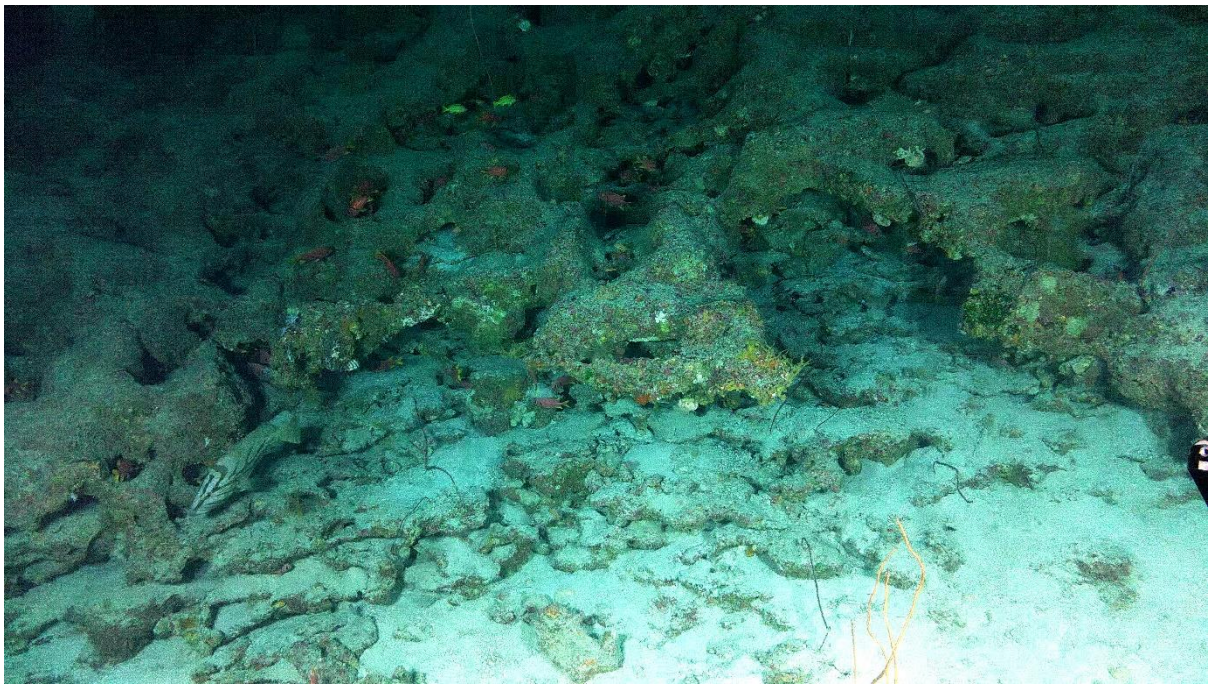

Figure 21. Rocky reef at ~120m showing the highly cryptic rock habitat. The contoured grouper (*Ephinephelus morrhua*) is visible on the left and snappers range amongst the rocks.

At 60m depth there is a mixture of substrata including highly cryptic calcareous rock and sandy areas. Rocky areas are inhabited by an extremely rich biota of octocorals (e.g. Neptheidae), sea fans (Octocorallia, e.g. *Annella reticulata*) and sea whips (Ellisellidae), as well as Scleractinia (e.g. plate corals such as *Pachyseris* and an unidentified robust branching coral). There are a variety of large deep-reef fish including large lyretail groupers (possibly *Variola louti*), blacksaddle coral grouper (*Plectropomus laevis*), emperor angelfish (*Pomacanthus imperator*), and guineafowl puffer (*Arothron meleagris*) as well as many smaller fish species.

2. Eagle Island

500m Depth

The first dive was on an area of vertical carbonate rock walls forming terraces where the horizontal seafloor was draped with sediment. This gave way to an area of deep, heavily bioturbated fine sediment across which very large burrows were distributed. As at Peros Banhos the fauna on the rock was sparse but diverse and comprised a variety of black corals and octocorals. An unusual animal sampled here was a rock sponge (Lithistida; Sample ID 506). On the sediment fast-moving echinothuriid sea urchins were sampled along with a pair of carrier shells (*Xenophora* cf *granulosa*) and both mobile and sessile holothurians. A large decapod crab (*Chaceon* or *Geryon*) was also observed. Fish observed included tinsel fish, rattails (cf *Malacocephalus laevis*) and a gempylid (snake mackerel) thought to be *Thyrsitoides marleyi* (the black snoek). A second dive at this depth took place at the head of a low-relief canyon. The canyon walls were formed by vertical carbonate rock faces which were cracked and fissured and also quite convoluted. Fauna mainly comprised glass sponges (e.g. *Aphrocallistes*), large predatory anemones (Fig. 23) and scattered corals including the scleractinian *Stenocyathus vermiformis* (Fig. 24). Fish usually associated with seamounts were particularly common in this location including spikey oreo, rattails (*M. laevis*), cardinal fish (*Epigonus* sp.) and Darwin's slimehead. Sorcerer eels were also observed (Nettastomatidae) along with the black snoek (*Thyrsitoides* cf *marleyi*; Fig. 22).

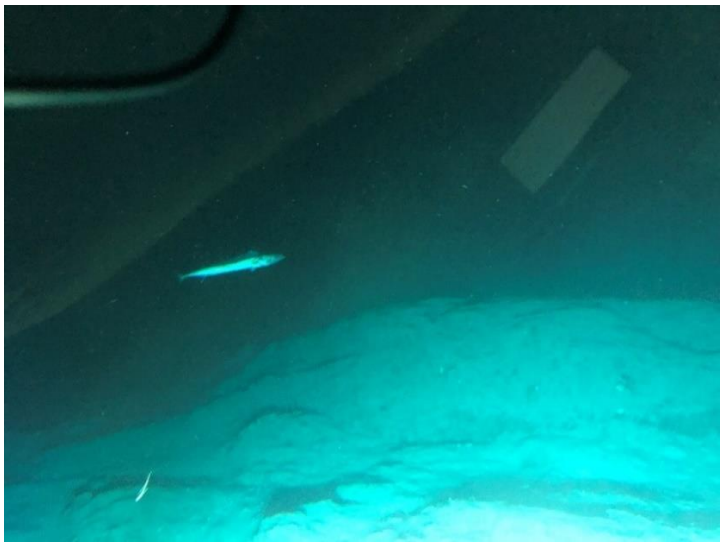

Figure 22. *Thyrsitoides* cf *marleyi* looms out of the darkness at 489m depth off Eagle Island. The fish is probably more than 1.5m long and is from a group of predators typically associated with seamounts.

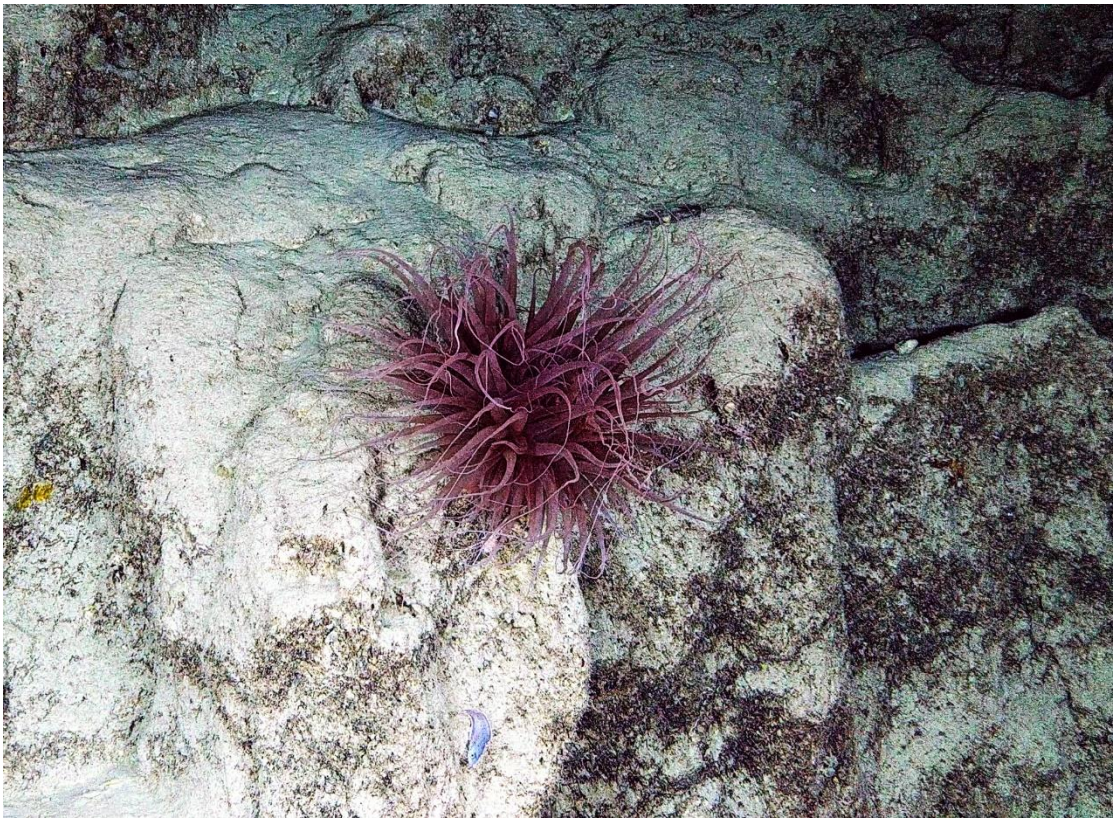

Figure 23. A large predatory sea anemone on a vertical rock face at the head of a deep canyon off Eagle Island. The tentacles are 30-40cm long and some show evidence of having recently stung prey (concertina appearance).

Figure 24. A colony of *Stenocyathus vermicularis* growing on a rock face at 489m depth (Specimen ID 594).

250,120, 60m depth

At 250m the seafloor is mainly sediment covered with scattered large boulders. Sediments are heavily bioturbated in places. The most conspicuous biota are an irregular urchin which is pale brown with a flattened shape (Sample ID 346). Darwin's slime head was associated with some large boulders. Moving from 250m to 120m a large area of low-lying chimneys with depressions on the top were encountered. These were more pronounced and numerous than at Peros Banhos and were dubbed "alien eggs" (see below; Fig. 25). These chimneys were found to be rock hard but a part of one was sampled using the Titan T4 manipulator from *Aurelia*. Suspension-feeding animals such as brisingid seastars (Asteroida; Brisingidae) were found located on top of these structures to access a greater flow of water and food. At ~146m dense aggregations of purple heart urchins were found on the sediment, one of these was sampled on a later dive.

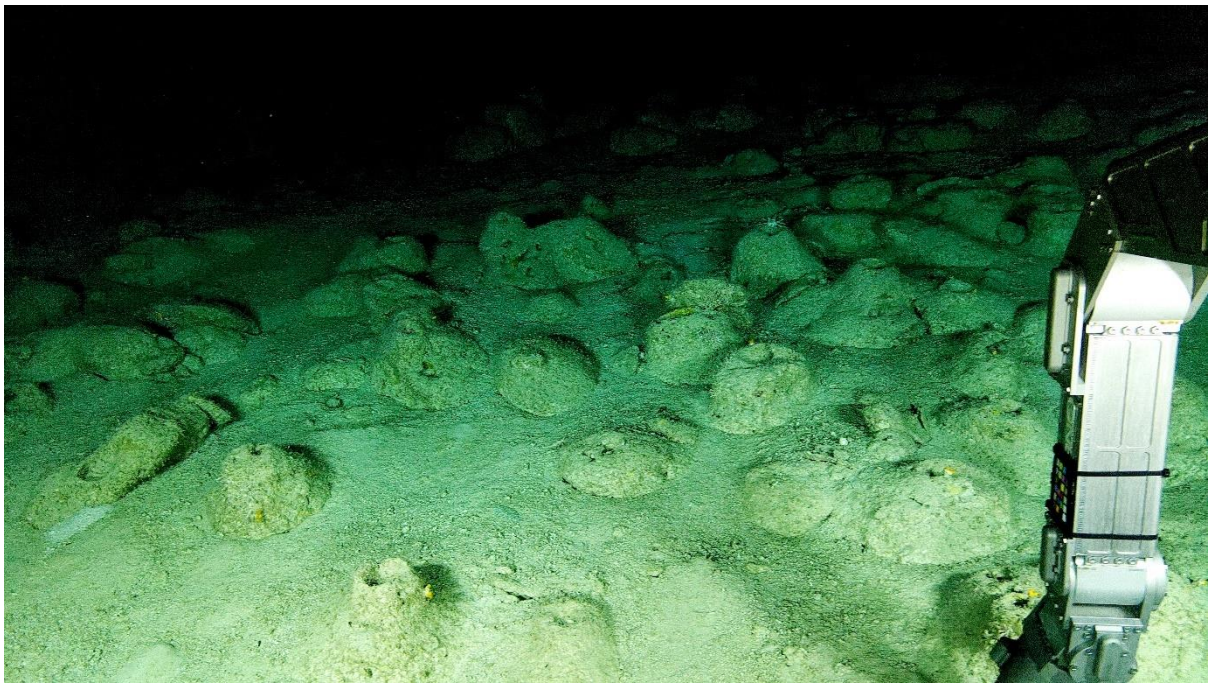

Figure 25. Carbonate chimney structures at Eagle Island with the characteristic depressions in the top. Depth of occurrence varied from ~ 205m to less than 180m.

At 120m the substratum was very similar to that seen at Peros Banhos with highly cryptic rock with a draping of sediment on horizontal surfaces. Rock was colonised by a low-lying animal turf and black corals were the dominant invertebrate fauna especially *Cirripathes* and *Parantipathes*. Large sea cucumbers and seastars were also present. Fish fauna included black-fringed bigeye (*Pristigenys refulgens*),

groupers (e.g. *Ephinephelus morrhua*) and jobfish (Lutjanidae). In places the animal forests are very diverse at this depth.

60m depth is also very similar to Peros Banos with large areas of sand (Fig. 26) and then rocky areas where more invertebrates and fish are apparent (Fig. 27). Large sea fans are present including *Annella reticulata*, plate corals (e.g. *Platyseris*) and mobile invertebrates including large sea cucumbers (Holothuria). Fish include a variety of groupers (e.g. netfin grouper, *Epinephelus miliaris*; possibly golden hind, *Cephalopholis aurantia*) and emperor angelfish (*Pomacanthus imperator*).

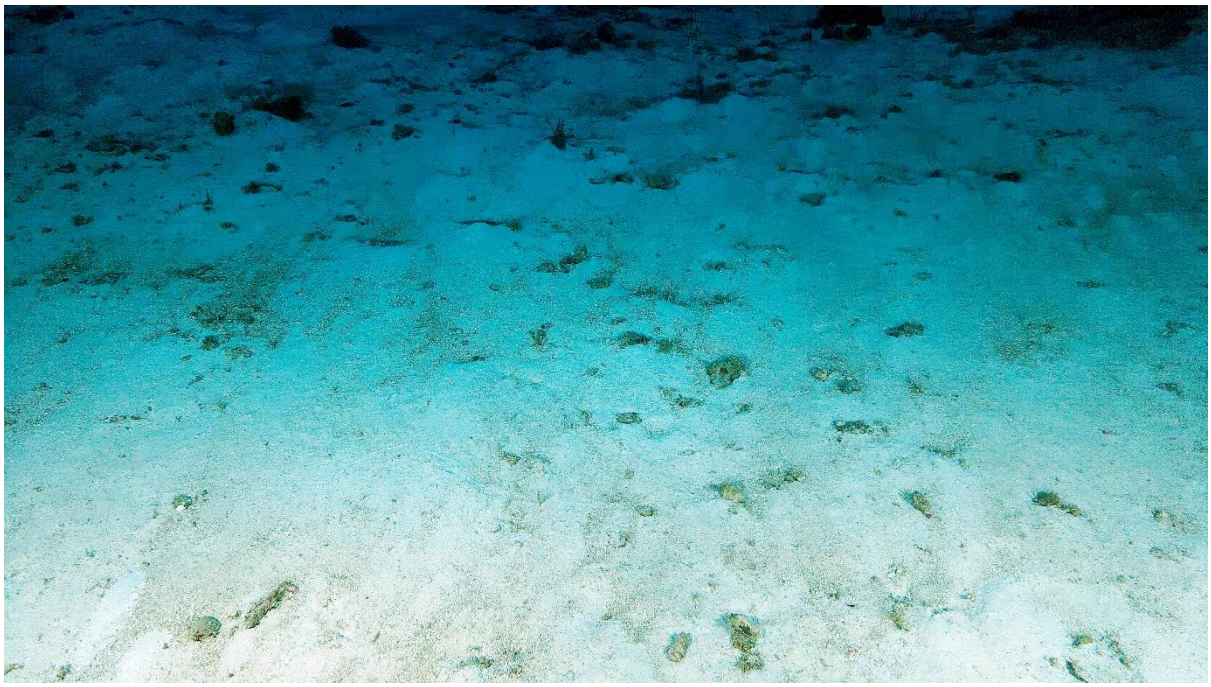

Figure 26. Exposed sand habitat at 60m depth at Eagle Island.

Figure 27. Rocky reef at 60m depth teaming with life including sponges, plate corals and many other invertebrates. Basslet fish (Serranidae) swarm around these types of outcropping at this depth.

Major Findings in Deep Water

Aside from description of habitat and species we consider three findings on this expedition that are of broader significance. These include the discovery of cold-water coral habitat, the discovery of the carbonate chimney structures and accumulation of further evidence for trapping of the deep-scattering layer (DSL) against the seafloor and its importance as a food source for demersal fish and other organisms.

Cold Water Coral Habitat

Over a month's duration in the Maldives during the Nekton Expedition in September/October, 2022, no evidence for cold-water coral habitats were found in any of the sampling locations at depths down to 500m. Azooxanthellate cold-water corals occur frequently in the Chagos Archipelago at depths of 500m. Furthermore, the occurrence of coral framework at Peros Banhos at these depths is a new discovery. Examination of the film and photographs of this framework habitat suggests at least one primary framework-building species (likely *Desmophyllum pertusum* but possibly *Solenosmilia variabilis*; Fig. 20) and perhaps the occurrence of secondary framework building species such as *Madrepora oculata*, recognisable by its zig-zag pattern of growth (see Rogers, 1999). *Desmophyllum pertusum* is typically found in oceanic waters with a temperature between 4º – 12ºC and salinities

between 35-37‰ although it can occur at lower salinities in fjords in Norway (Rogers, 1999). Sufficient dissolved carbonate is also considered as important for the growth of these kinds of coral (Tittensor et al., 2009). The frameworks observed during the Peros Banhos dives were located on the edge of a spur in three-dimensionally complex steep terrain which was mapped using the ship's multibeam. These included both living coral colonies and extensive dead framework with associated species including squat lobsters, crinoids and the sponge *Aphrocallistes*. Patches are sufficiently large to be considered as cold-water coral reef (Rogers, 1999). *Desmophyllum pertusum* is known from two other localities in the Indian Ocean including off Madagascar at 450m depth (12º 39.5'S, 48º 15.6'E) and the island of St Paul (38º 40'S, 77º 38.6'E; 37º 47'S, 77º 43.7'E; 38º 48.8'S, 77º 35.7'E; see Rogers, 1999). Cold water coral reef habitat primarily formed by *Solenosmilia variabilis* has also been documented from the South-West Indian Ridge (Pratt et al., 2019). Given the very complex submarine topography around Peros Banhos it is highly likely that more of this habitat is present and may include even larger reef structures.

Carbonate Chimneys

The carbonate chimneys located both off Eagle Island and Peros Banhos at a similar depth (210 – 150m; Fig. 25) are of unknown origin. Given that they all have a similar structure, that of an irregular cylindrical chimney with a depression at the top, some fused, suggests that a common mechanism has been involved in their construction. Consultation with the literature suggests that in certain conditions chemosynthetic bacteria can form stromatolites, carbonate mound-like structures which also have a depression at the apex. These structures were first described by Himmler et al. (2018) and were observed in the oxygen minimum zone in the northern Arabian Sea where the bacteria *Thioploca* mediate nitrate-driven sulphide oxidation which in turn drives carbonate precipitation. Sulphate-driven anaerobic oxidation of methane is another process that can drive the precipitation of carbonate at methane (hydrocarbon) seeps (Himmler et al., 2018). If these structures are carbonate chimneys formed by chemosynthetic bacteria it begs the question of what the methane source may be at the Chagos Archipelago. Methane can be generated from the biotic degradation of organic carbon buried in ocean sediments globally (Johnson et al., 2015). Alternatively, it can be generated abiotically during high temperature

(>200ºC) serpentinization of ultramafic rocks (Johnson et al., 2015). During the expedition a section of the one of the carbonate chimneys was sampled by the *DSV Aurelia* and this will be taken to the Department of Earth Sciences for analysis to determine whether these are stromatolite structures created through chemosynthesis or if they have been generated through another mechanism.

Trapping of the Deep Scattering Layer

Previous work in the Chagos Archipelago has used biological acoustics to characterise the Deep-Scattering Layer (DSL) a layer of acoustically reflective organisms that aggregate. These organisms include a range of groups of micronekton including fish, squid, crustaceans and gelatinous zooplankton. At dusk these organisms move up to the ocean surface to feed and when the sun rises they dive into deeper water away from sunlight to avoid visual predators. In Chagos these organisms form a shallow and a deep scattering layer (Letessier et al., 2016; see Fig. 28).

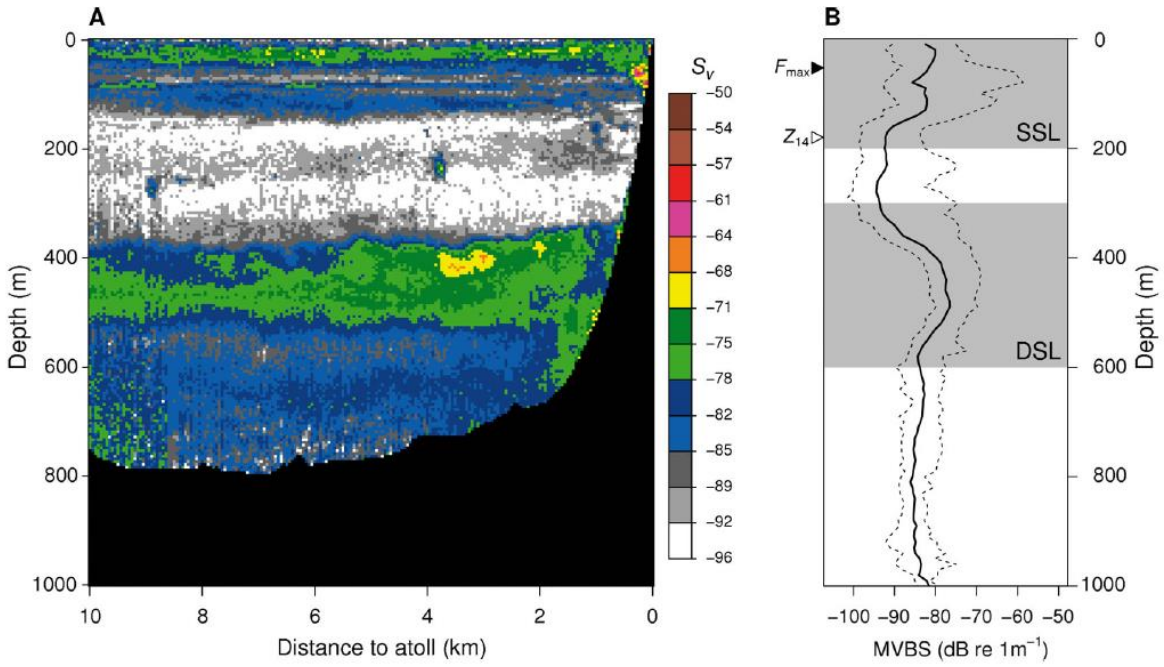

Figure 28. Location of the Shallow Scattering Layer (SSL) and Deep Scattering Layer (DSL) at Peros Banhos, Chagos Archipelago. The SSL is located from the surface to ~ 180m depth and the DSL from 300m to 600m depth (Lestessier et al., 2016).

The DSL was observed at depth during dives to 500m during the Chagos expedition and was notable as a marked increase in the presence of midwater fish including myctophids and other species, squid, crustaceans (shrimp) and gelatinous

zooplankton (e.g. Ctenophora, Scyphozoa) from depths of more than 250m. These animals were observed to be present right to the seafloor at 500m depth coinciding with the presence of predatory fish, typically associated with seamounts, known to feed on micronekton and gelatinous zooplankton. These include spikey oreo and black snoek and likely rattails, coffin fish, tinsel fish and Darwin's slime head. Whilst we have not yet analysed survey data it is likely given our observations that the biomass of fish at the 500m depth stations increases compared to the 250m stations in contradiction to normal expectations of declining biomass with increasing depth. It is likely that the mechanism enabling such an increase in fish biomass is trapping of the DSL against the seafloor of the slopes of the atolls. This mechanism, known as topographic blockage, is known from seamounts but is not usually considered for island ecosystems such as the Chagos Archipelago. Evidence for topographic blockage of the DSL was also seen in the Maldives although this area lacks the biological acoustic data that was gathered by Letessier et al. (2016). The depth zone across which topographic blockage takes place has been termed the "trapping zone" by AD Rogers. It's implications for connection of shallow-water and deep-sea food webs is not yet clear but may influence the presence of large predators at the Chagos Archipelago and elsewhere. Large benthic invertebrates were also observed as likely feeding on DSL organisms including large predatory anemones (Fig. 23) and benthic ctenophores. Another mechanism of shallow to deep connection was the presence of terrestrial vegetation on the deep-sea floor, most notably coconuts and palm fronds.

We note that no coelacanths were observed during deep-water surveys.

Snorkel Surveys

1. Peros Banhos

The seaward reefs of islands (Île Pierre, Île Verte, Île Manon, Grande Soeur and Petite Soeur) along the western edge of Peros Banhos atoll were surveyed by snorkel over three days (16th-18th October 2022), and in most cases, often for the first time (with previous expeditions of the Bertarelli Foundation's Marine Science programme not having focused on these sites in Peros Banhos). The surface support vessel *Northwind* would anchor at 5-7m and snorkellers would invariably survey shallow reefs between the anchor line and reef break. Whilst the sites were in

the lee of islands and the prevailing south-easterly winds during the expedition, these were nonetheless historically exposed shorelines and the state of the reefs reflected this. Overwhelmingly, these reefs (comprising well-defined spurs and grooves) were bare, with plentiful coral rubble in existence, and likely scoured by annual storms (Figs. 29,30).

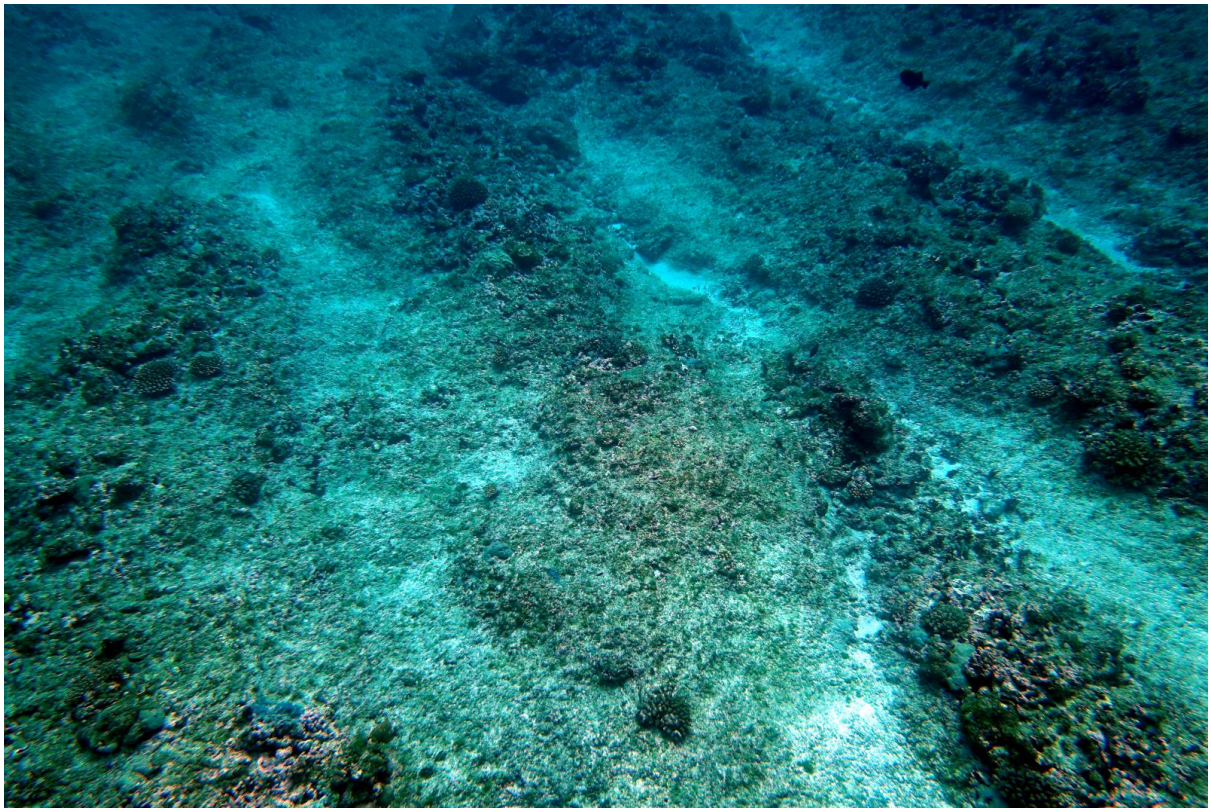

Figure 29. Bare seaward reef at 6m depth at Grande Soeur.

Figure 30. Bare seaward reef at 2-3m depth, showing well defined grooves at Grande Soeur.

Cover and taxonomic diversity of scleractinian corals was comparatively low here, and dominated (where found) by small (5-10cm in diameter) colonies of tabular *Acropora* spp., as well as the more robust members of the *Acropora humilis* species complex (including *A. digitifera*), *Stylophora* spp., *Porites* spp. (primarily *P. lobata* and *P. lutea*), *Platgyra* spp. and *Leptoria* spp. Interestingly, at the northern tip of Grande Soeur (Deployment 05 Dive 2), there were several large stands of *Turbinaria* spp. observed, tens of metres across in places, with numerous other smaller colonies in the environs (Fig. 31).

No *C. chagius* colonies were found at these sites, although colonies have been observed locally in and around Moresby Channel on the northern edge of the atoll (on seaward reefs at Morseby Island and Île de la Passe).

Discarded fishing line was also discovered wrapped around a coral bommie at 6m depth at Île Manon (Deployment 05 Dive 1) and recovered by snorkellers.

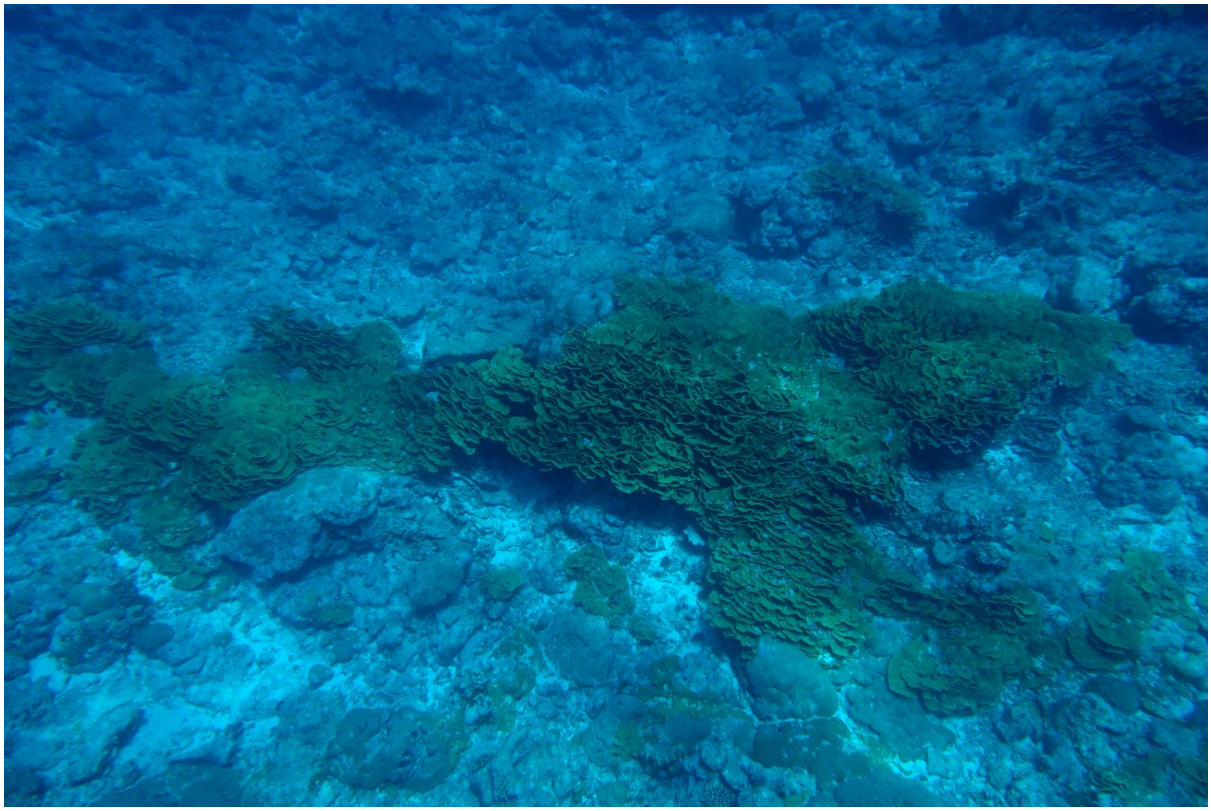

Fig 31. Large stands of *Turbinaria* spp. at the northern tip of Grande Soeur.

2. Eagle Island

The seaward (and lee) side of Eagle Island was the only one of this group of islands (which include Danger and Cow Islands) to be surveyed, and this on one day only (19th October 2022). Similar to the reefs on the western edge of Peros Banhos, those here were characterised by well-defined grooves leading to the shore. However, the substrate was primarily bare rock and judging by the paucity of macroalgal growth, heavily grazed. Whilst coral cover and taxonomic diversity was relatively low here too (likely resulting from the exposed nature of the site), it differed from those reefs in Peros Banhos by being dominated by numerous colonies of *Pocillopora damicornis* and medium- to large-sized tabular *Acropora* spp (Fig. 32).

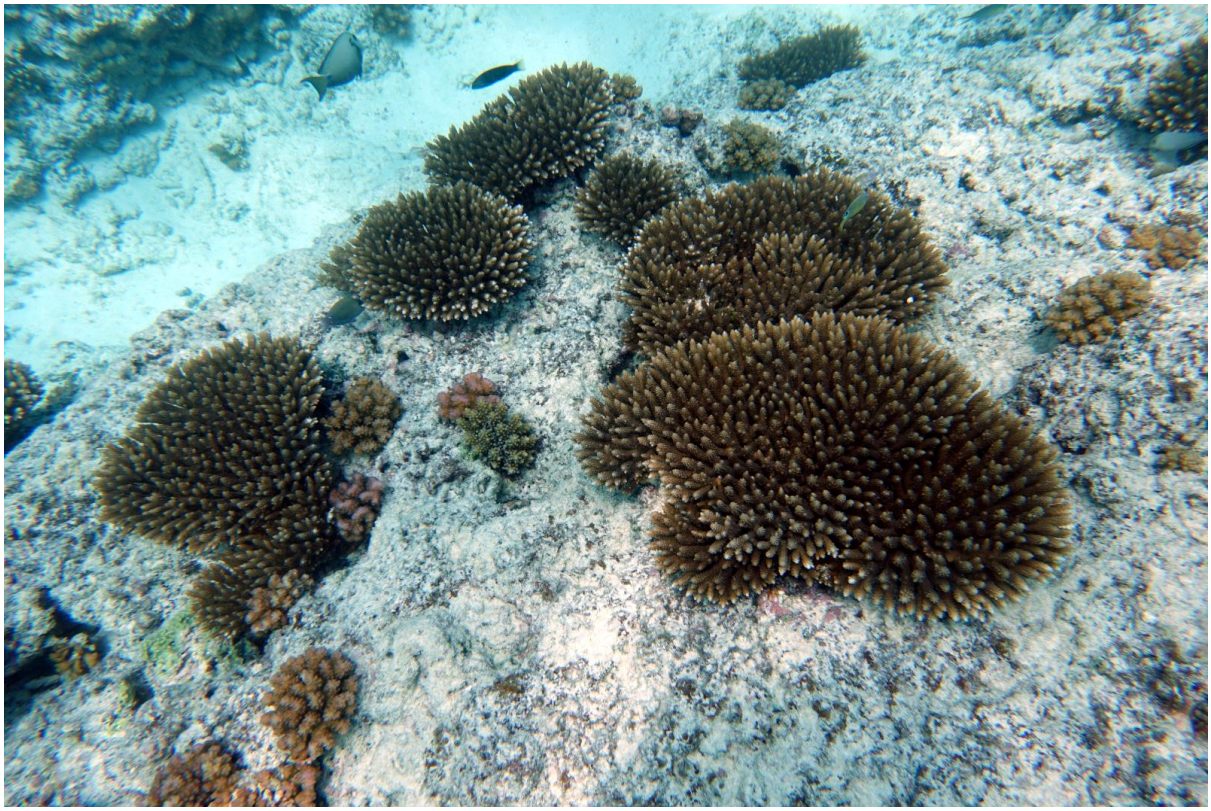

Figure 32. Tabular *Acropora* spp., *Acropora humilis* sp. and *Pocillopora damicornis* colonies at Eagle Island.

Again, no *Ctenella chagius* colonies were recorded at this site.

3. Middle Brother

Middle Brother Island (of the Three Brothers, which also comprise North Brother, South Brother and Resurgence Island) is unique amongst islands in the Chagos Archipelago because of its entire northern shore being protected by a large, enclosed lagoon, with access to small boats only by way of a narrow channel through the reef crest. The lagoon itself is sandy bottomed, with an average depth of between 7-10m, and 14m at its deepest point, and bordered on three sides by reef flats, approximately 1m in depth. Within the lagoon are six coral knolls of different size and character, each bordered by diverse living corals down to the seafloor and topped by a bare substrate crown, with sparse coral colonies and rubble.

The reef flats on the exposed northern edge comprised numerous *Porites* spp. and large (>1m diameter) tabular *Acropora* spp. (Fig. 33), as well as occasional large

colonies of *Platygyra* spp (Fig. 34). However, the presence of large areas of coral rubble suggests that the reefs here are frequently visited by fierce storms.

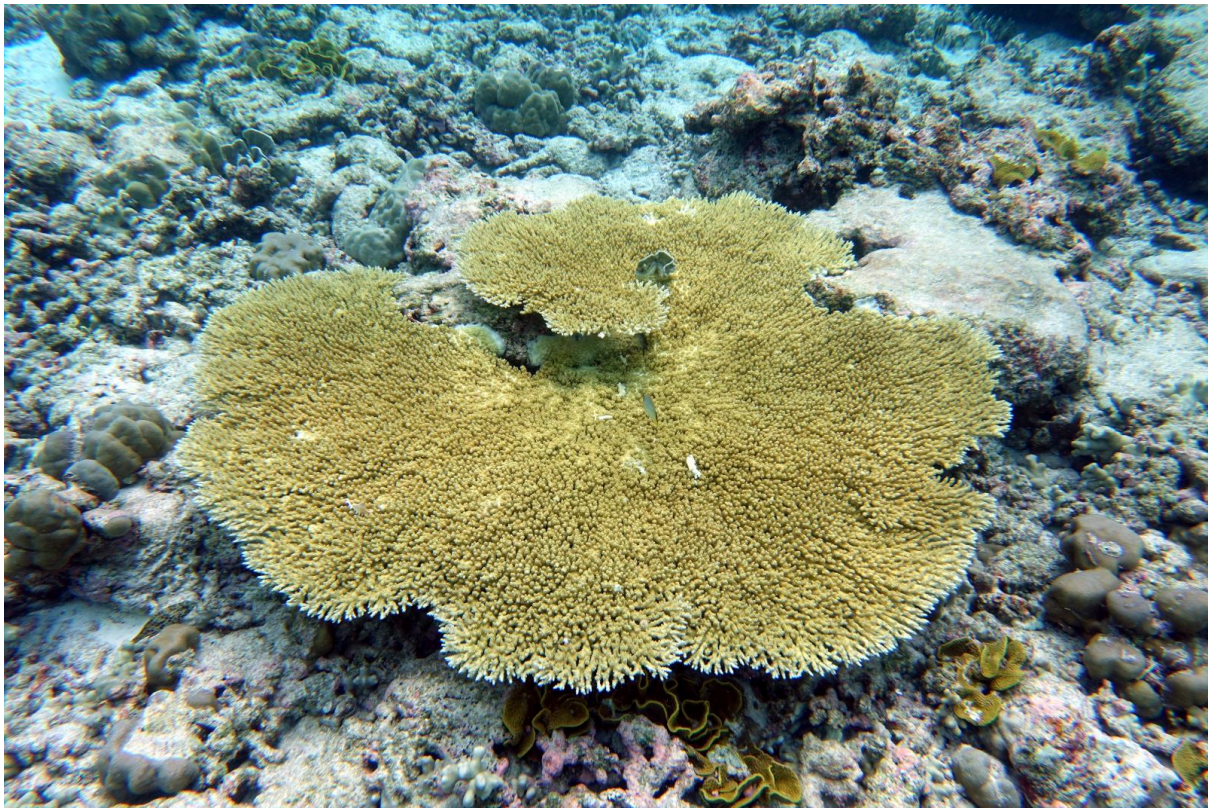

Figure 33. Large (>1m diameter) tabular *Acropora* spp. on reef flats on the northern edge of Middle Brother lagoon.

Close to these reef flats too were large monospecific stands of *Goniopora* spp. and *Heliopora* spp., at the western and eastern edges of the lagoon, respectively, and these harboured distinctly different diversities of fish populations.

Whilst several of the knolls have been the subject of previous expeditions by the Bertarelli Foundation's Marine Science programme, the use of a drone footage to view the entire lagoon from above revealed for the first time in stunning detail, the exact number, size and location of each of the knolls, and these data were used to plan the snorkel surveys much more efficiently than previously. As such, during the two full days (20th and 21st October 2022) spent at Middle Brother, all but one of the knolls were comprehensively surveyed by snorkel.

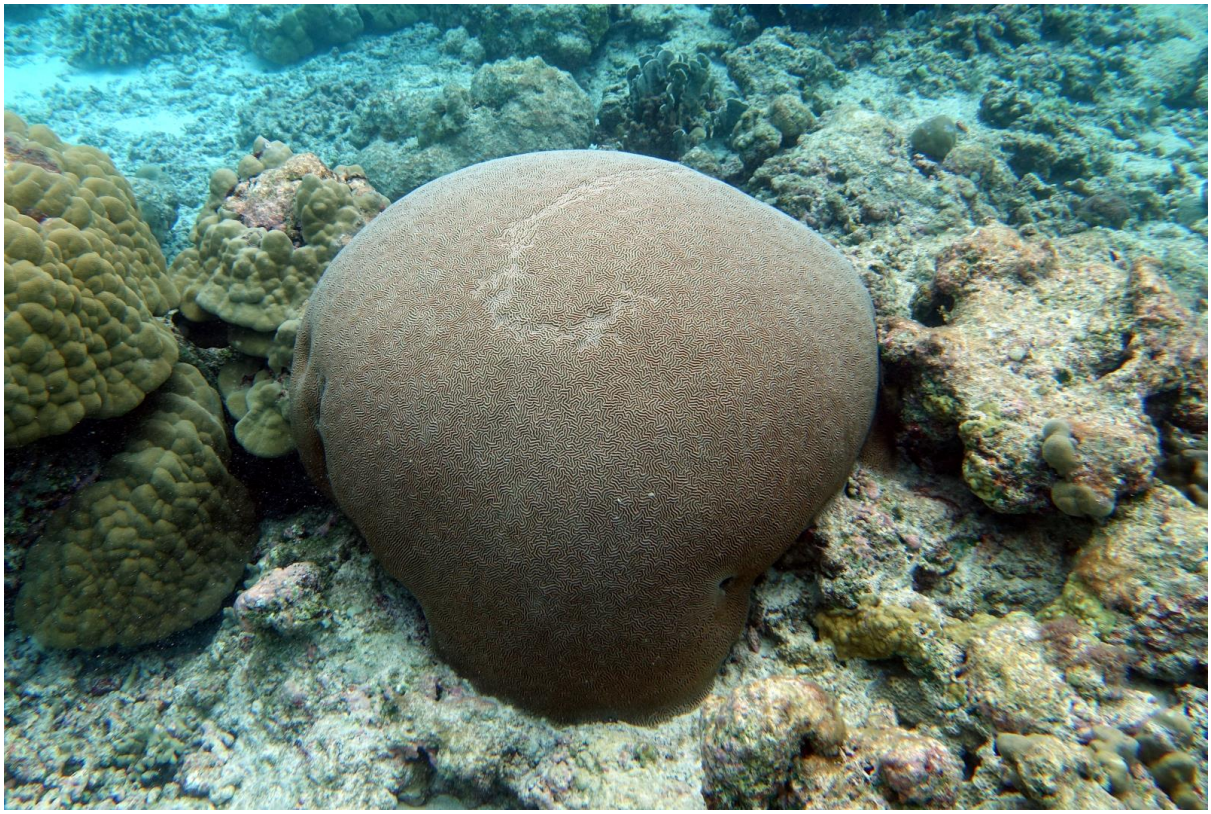

Figure 34. Large (>1m diameter) *Platygyra* spp. on reef flats on the northern edge of Middle Brother lagoon.

These surveys confirmed observations collected on previous expeditionary visits (in 2020 and 2021) that Middle Brother Lagoon is one of the last strongholds of the endemic and critically-endangered coral *Ctenella chagius*, with preliminary footage suggesting that more than thirty healthy colonies (>15cm in diamater) continue to survive here (Fig. 8, 35); it is certainly the most densely clustered population yet known globally.

The number of extant colonies in the lagoon however is likely higher, as there were those that were certainly missed in these counts (and which were observed in previous years on SCUBA) as a result of the limitations of surveying from the surface by snorkel, especially as colonies are usually found in the deeper parts of the lagoon (7-14m). This limitation was exacerbated on the second day (21st) when heavy rains the night before created localised halo- and thermoclines within the lagoon, such that surveying the seafloor was that much more difficult. Previous expeditions have also revealed isolated colonies on reef patches on the lagoon floor, but again, it was not possible to view these by snorkel. The depth of colonies also meant that for the most

part, tissue sampling (so as not to unduly damage the colonies) was not possible by snorkel.

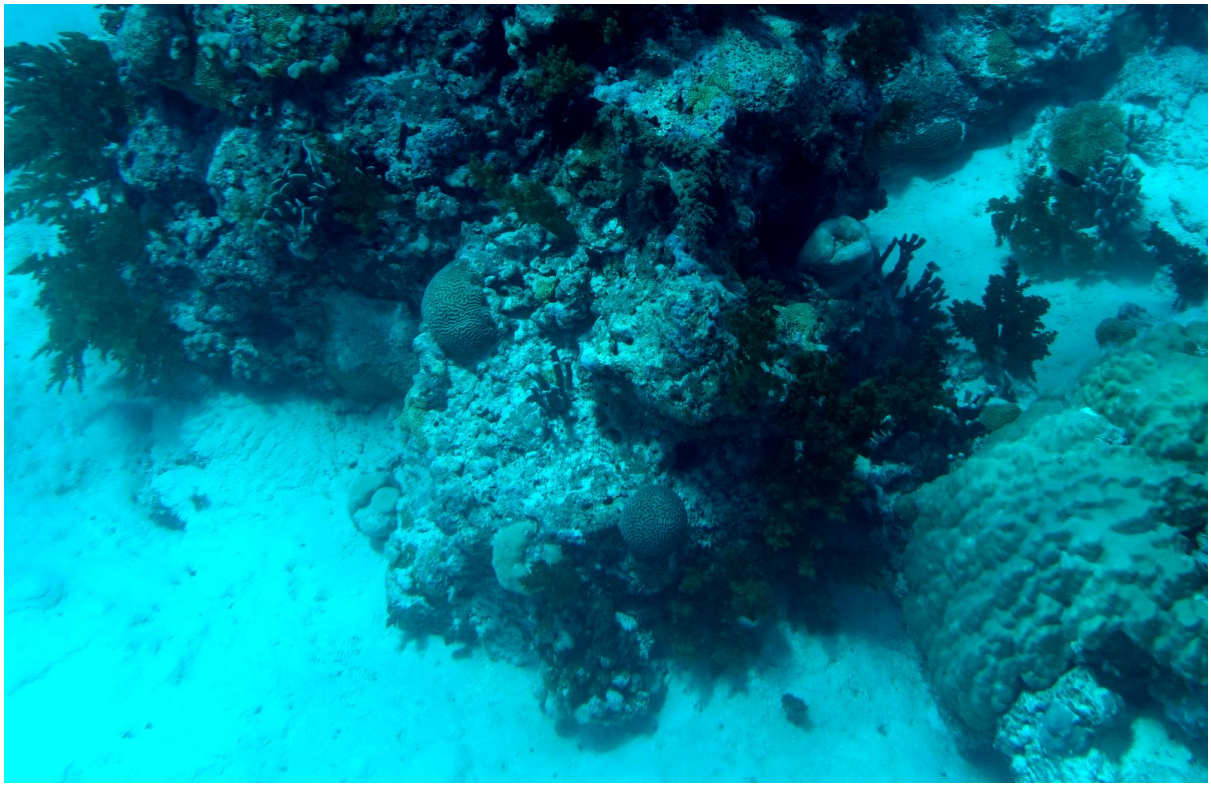

Figure 35. Two colonies of *Ctenella chagius* at 9m depth in Middle Brother Lagoon.

However, surveys of the reef flat at the northern edge of the lagoon revealed a single colony of *C. chagius* (~15cm in diameter) at 1m depth, attached to coral rubble (Fig. 36). The full circumference of the coral was photographed in 360° for 3-D photogrammetry analyses and a tissue sample (5g) taken from the leading edge of the coral and preserved in RNALater on the surface support vessel *Northwind* less than 10 min later (Fig. 9).

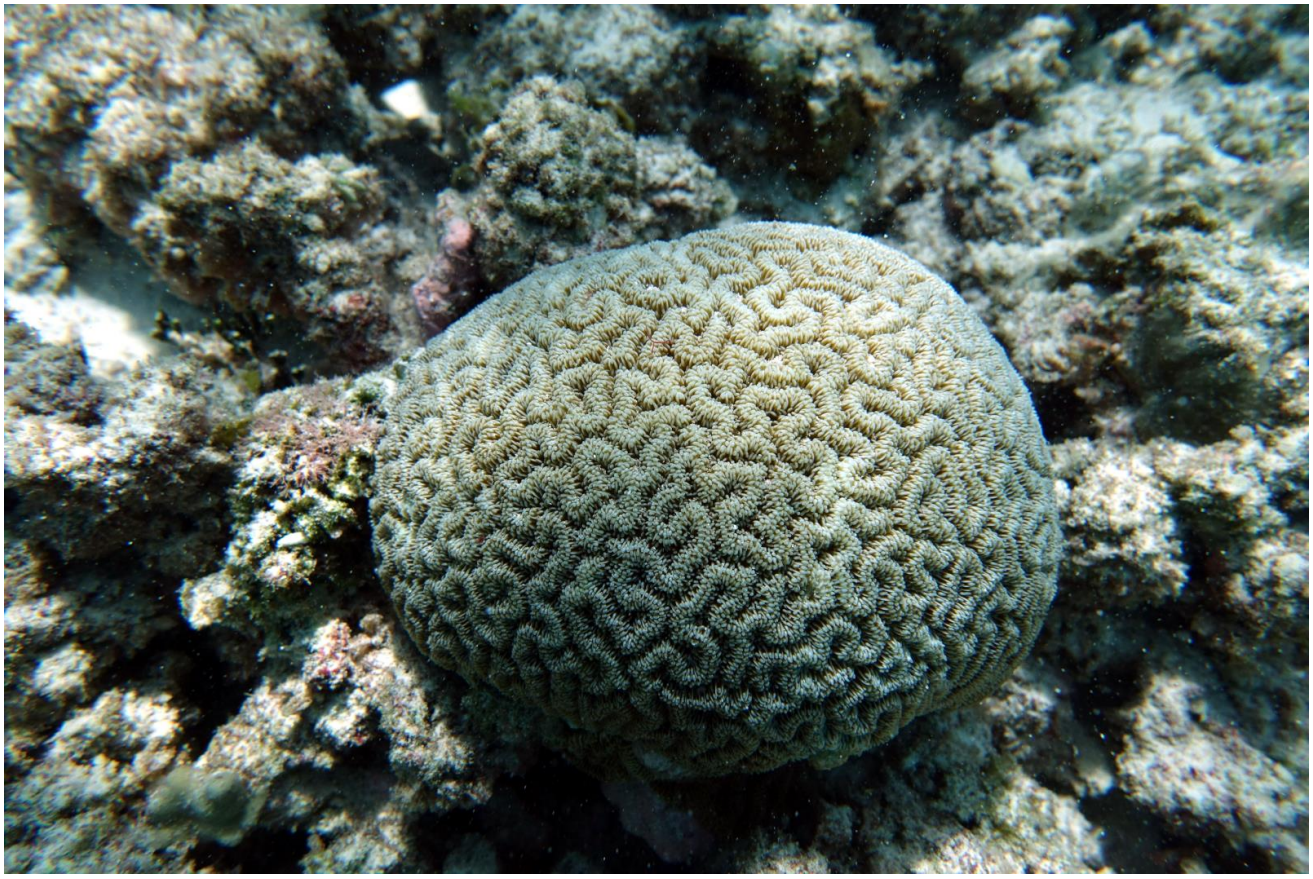

Fig 36. *Ctenella chagius* at 1m depth on reef flats on the northern edge of Middle Brother lagoon, from which tissue samples were collected.

Permission to land on Middle Brother island was also granted and so the snorkel team circumnavigated the shoreline of the island (less than 2km in diameter), checking for discarded fishing gear (of which a gallon drum and attached strobe light were found) and collecting limited plastic waste (filling three large refuse bags).

Seaweeds

A total 152 samples of seaweed were obtained during 10 deployments. The majority of the collections (127) were sampled at the shallow seaward reef slopes during snorkelling surveys. The remainder (24 collections) were obtained from mesophotic habitats by the submersible *Aurelia* 7k3/1.

Shallow water seaweeds were dominated by crustose and or calcified growth forms, indicative of a high grazing pressure by herbivorous fish or herbivores. Crustose coralline algae (CCA; Corallinales, Hapalidiales, Sporolithales) dominated the reef surface close to the reef crest. The cover of crustose coralline algae decreased markedly from ~2 to 5 m depth. Non-calcified crustose seaweed included *Lobophora obscura*, observed at Ile Pierre and Ile Soeur overgrowing living/dead coral colonies,

peyssonnelioid crustose algae (*Peyssonnelia* spp., *Ramicrusta* spp., *Incendia* spp.; Fig. 37) and samples putatively identified as *Hildenbrandia*. Calcified algae were also well represented. Most notable in the lagoon of Middle Brother island large mounds of *Halimeda* spp. were observed closely associated with *Heliopora coerulae* *Tricleocarpa*, *Jania* and *Neomeris* (all three calcified) were occasionally observed (Fig. 38). Frondose seaweed were sparse, with notable exceptions of *Amphisbetema indica*, *Cladophoropsis* sp., *Dictyosphaeria versluysii*, *Dictyurus purpurescens*, *Microdictyon* sp., which were observed during most snorkelling surveys. We hypothesize these species (or their associated bacteria) deter grazers by producing secondary metabolites.

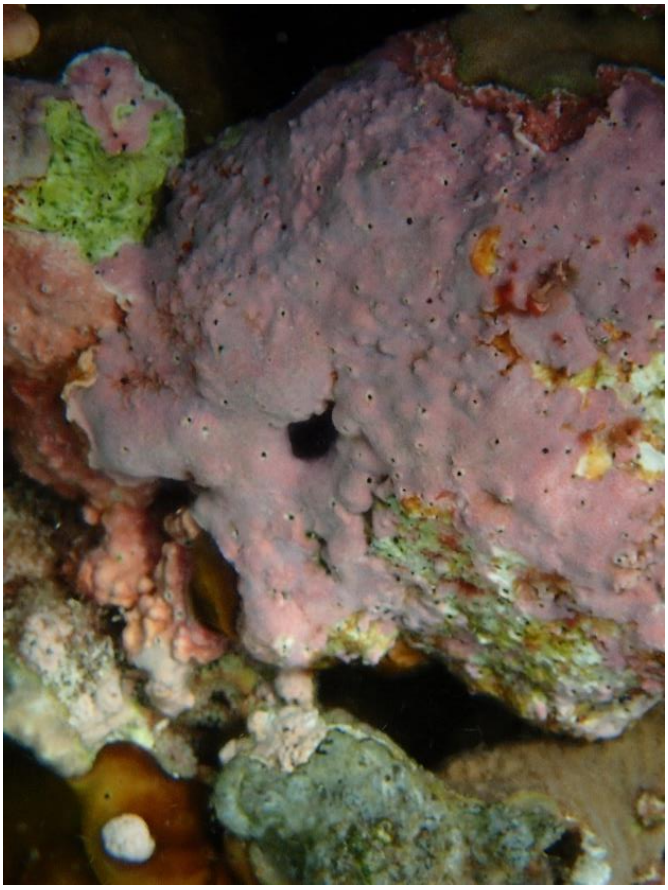

Figure 37. Shallow water Crustose Coralline Algae (CCA) photographed during the current expedition.

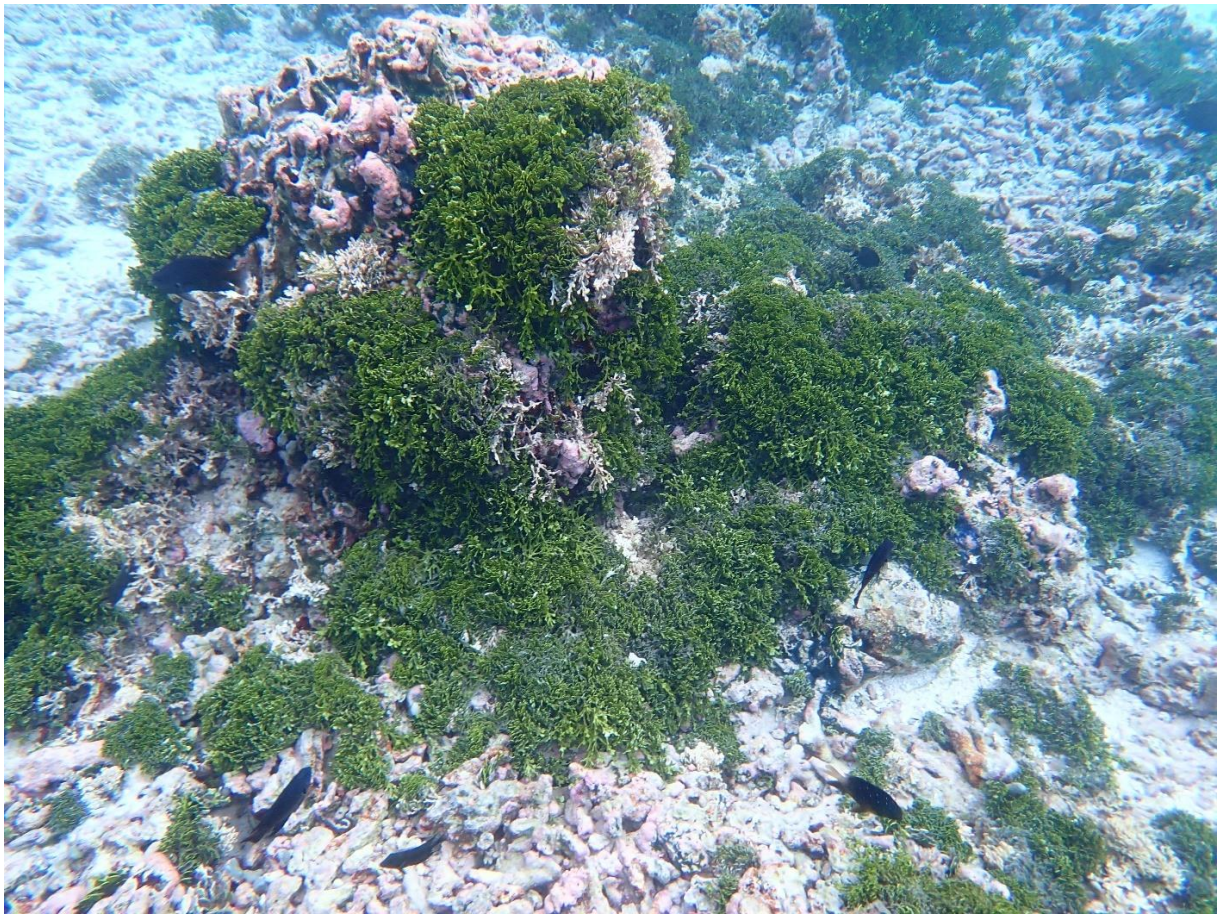

Figure 38. Large mound of *Halimeda* spp.at Brothers lagoon.

The absence or near-absence of common tropical seaweeds from shallow water habitats is most striking. For example, not a single specimen of *Padina*, *Dictyota*, *Gelidiella*, *Gracilaria*, *Hypnea*, or *Sargassum* species was observed during the expedition. Examination of beach cast in Peros Banos, Eagle Island and Middle Brother confirmed their absence in the study area. The absence of 'ubiquitous' tropical species was also noted during seaweed surveys in Rodrigues, another remote island in the Indian Ocean (De Clerck et al. 2004; Schils et al. 2004). However, the absence of common tropical species in the Chagos Archipelago is much more extreme compared to Rodrigues. Schils et al. (2004) discussed the low species richness in Rodrigues as a function of habitat diversity and putative dispersal limitation. As for the Chagos Archipelago both factors are likely also be at play, but low nutrient levels and high grazing levels in these remote atolls also likely restrict seaweed biomass and potentially diversity.

Crustose coralline algae also dominated the seaweed flora in mesophotic habitats. The deepest seaweeds (CCA) were observed at a depth of 126m (Fig. 39). Diversity

and coverage were markedly higher at 60m depth, where crustose coralline algae were often associated with *Halimeda* sp., *Lobophora* sp, *Peyssonnelia* sp. and *Verdigellas* (Fig. 39, 40). Large frondose species, as observed in the Bermuda, Gulf of Mexico and Hawaii were notably absent. Investigations under a dissecting microscope revealed a number of additional algal genera (<1-2 mm in size) collected from rock samples at a depth of 60m.

Figure 39. Deep-water CCA (Sample ID 499)

Figure 40. *Lobophora obscura* photograph in-situ (Specimen ID 120)

Technical Performance of the DSV *Aurelia*

During the mission, the REV Ocean owned and operated Deep Submergence Vehicle (DSV) *Aurelia* completed 30 hours of operations over 8 dives and collected approximately 130 samples (many consisting of multiple species). The Chagos mission was back-to-back with the Nekton “First Descent” mission and is *DSV Aurelia’s* second science mission.

The deployments consisted of:

- 3 x 500m Dives
- 5 x 250m Dives (with shallower 120 and 60m portions)

Details of individual deployments are given below (Table 5).

During these dives, the submersible crew was made up of a Pilot and a Mission Specialist. The Pilot was responsible for the safe operation of the submersible and the Mission Specialist was responsible for cameras, manipulators and data acquisition. A member of the science party occupied the third seat and directed the

science mission. This expedition included “on-mission” training for REV Ocean Pilots. Despite the training requirements the team were able to complete, if not exceed the dive goals.

DSV Aurelia conducted sample acquisition using its Schilling T4 Manipulator which performed well throughout the mission. Care had to be taken to avoid damaging the stereo cameras mounted on the Science Skid while depositing samples in the sampling drawer.

4K video was acquired using the forward-facing Sub-C Rayfin cameras (Fig. 41). These cameras experienced several technical issues including connectivity, latency and power issues. Further investigation with SUBC and Triton is required but it would seem that a custom-built solution may be required for operation of the cameras and data acquisition from them.

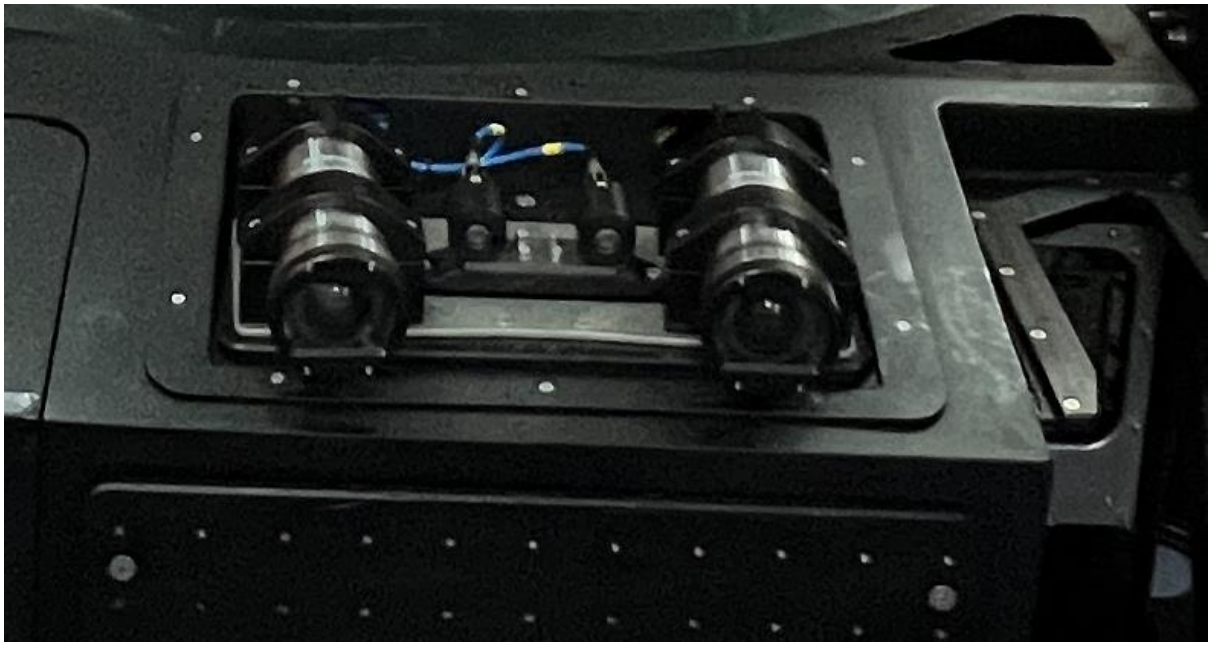

Figure 41. Stereo SubC Rayfin cameras mounted on the science skid.

Earlier dives revealed that the vehicle produced insufficient lighting to acquire good quality 4K footage. *Aurelia’s* downward lights were repositioned to provide forward lighting and this improved the quality of the acquired video (Fig. 42). Footage from the first 4 dives is therefore darker than later dives. A colour balance card was also mounted on the arm enabling future colour correction of video/stills imagery (Fig. 43).

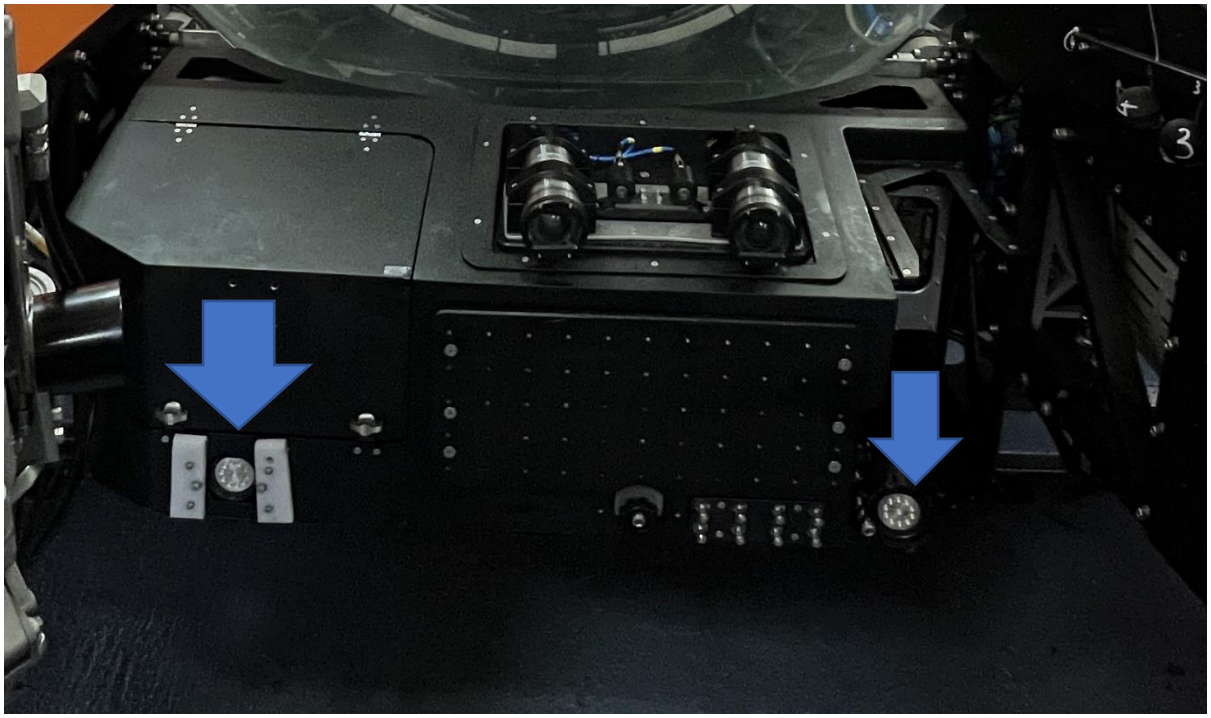

Figure 42. Front of Science Skid showing where the LED Lights were remounted to improve lighting for the cameras.

Figure 43. Titan T4 Arm showing mounted colour correction chart.

Water sampling was conducted using 4 Niskin bottles mounted on the submersible's port MBT (Main Ballast Tank; see Figure 44). These were triggered using the Titan

T4 at target depths determined by the science team. This system worked well with only a single misfired bottle during the mission.

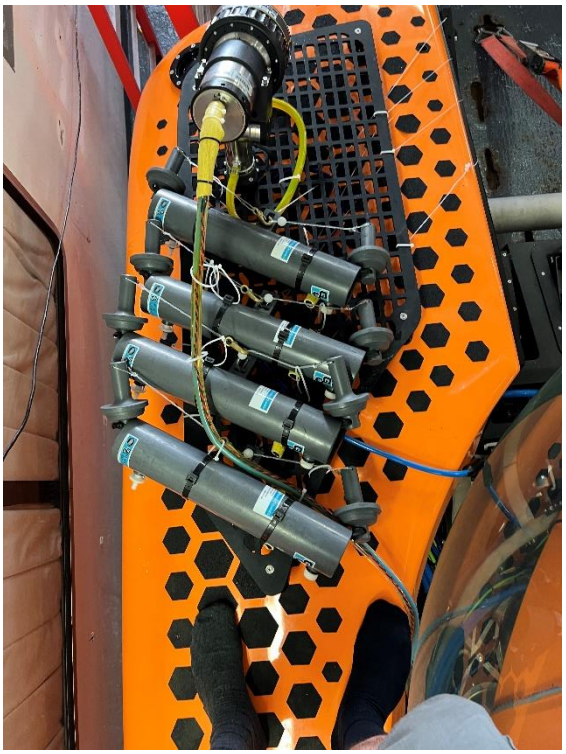

Figure 44. Array of Niskin bottles mounted on the port MBT of the DSV *Aurelia*. These were triggered by using the manipulator to pull the nylon lines visible in the photograph on the upper right. These had a plastic ball attached to the end which the manipulator could grab and pull.

A Deep-Sea Power and Light pan and tilt Apex 4K camera was mounted on the vehicle but was found to be technically challenging and operator intensive. 4K footage was therefore only acquired using the SUBC Rayfin stereo cameras. The original expedition plan included DSV Aurelia's downward IMENCO HD Camera and lights. However, the downward camera proved wholly inadequate for this mission because of issues with latency, technical integration and poor support. IMENCO have agreed to replace the camera because of the issues encountered.

DSV Aurelia is also fitted with a bio box, a hydraulically operated drawer. This worked well throughout the mission. Because it forms a good seal it not only prevented specimens washing out of the box during recovery of the vehicle but also kept samples in excellent condition. The suction sampler was not available during this mission because of a motor failure. Core tubes were also not used because of a lack of soft substrata encountered during the first dives of the mission.

DSV Aurelia was tracked throughout the dives using a Sonardyne WSM6 USBL beacon. This functioned well and the submersible tracks could be mapped with accuracy against terrain maps gathered by multibeam sonar.

No downtime resulting from technical failures was experienced during the cruise and the only delays arose from schedule and weather.

Overall DSV *Aurelia* performed well and supplied the scientific party with high-quality samples and video.

Date	Dive ID	Max Depth	Launch	Diving	On Surface	Duration
23rd October	38	250	08:50:00	09:02:00	12:47:00	03:57:00
22nd October	38	500	13:00:00	13:07:00	15:36:00	02:36:00
21st October	37	250	10:25:00	10:33:00	13:50:00	03:25:00
20th October	36	500	09:10:00	09:15:00	13:26:00	04:16:00
19th October	35	250	11:10:00	11:15:00	15:30:00	04:20:00
18th October	34	250	09:05:00	09:15:00	12:56:00	03:51:00
17th October	33	250	08:30:00	08:40:00	12:47:00	04:17:00
16th October	32	500	12:45:00	12:59:00	16:08:00	03:23:00

Table 5. Summary of dives undertaken with the DSV *Aurelia*.

Technical Performance of the Triton 3K3 Submersible, Omega

For ease and continuity, Triton submersible 3k3 #006 will be referred to *Omega*, as it was previously referred to in the Nekton Maldives Expedition. *Omega's* sole responsibility was to conduct the deep-sea surveys alongside *DSV Aurelia* while sampling. As mentioned previously, deployments for *Omega* consisted of:

- transects of lengths approximately 250m, measured by the topside team using the Sonardyne tracking software to estimate distance travelled.
- 3 transects were taken at each depth
- depths: 490m, 250m, 120m, 60m

Note that 490m was chosen as the video recording equipment had a pressure rating of 500m so this was a precaution against exceeding this depth limit.

Direction of the transects was along the chosen depth contour and largely determined by the pilot according to the surrounding ocean current. When at all possible, *Omega* executed transects with the cameras and lights facing the contour/wall- in a sideways crabbing motion. Only when the terrain was suitably flat

did *Omega* fly head on, and only if the surrounding currents made sideways transects difficult. This typically can be seen at the depths 490m and 250m. In some cases, because of very large switching currents, it became impossible to continue the transect in the same direction. When this occurred, notes were made on topside and also inside the sphere, and the sub ascended 5m to 10m to continue the transect in the direction of the current. This typically occurred at 60m and 120m.

Because of the nature of the lead acid batteries deployments were planned around a 5-hour window. This allowed for a suitable amount of time to charge batteries following an intensive power-draining dive. The optimum length of time for a transect is about 35 minutes, but because of the estimation of transect length, nature of the topography and current strength, varies considerably.

Typically, the three days of diving on one site looked like the following:

day 1: 3x transects @500m
day 2: 2x transects @250m
2x transects @120m
1x transect @60m
day 2: 1x transect @250m
1x transect @120m
2x transects @60m

During the course of the mission, Omega completed:

- 35 hours of diving
- 9x transects @500m
- 8x transects @250m
- 8x transects @120m
- 7x transects @60m

Social Media

Here statistics to date on social media posts are provided at the time of preparation of the cruise report (27th Ocetober, 2022). Obviously, this only provides a snapshot

of the outreach for the REV Ocean Mesophotic and Deep-Sea Research Cruise and it is likely that expedition and post-expedition posts will reach a wider audience. Presentation of the cruise and preliminary results are already planned for the Pink Flamingo Society Annual Meeting in California (the society for private research vessel owners; November 14th – 15th, 2022) and also for the University of Southampton Marine Conservation Society (December, 2022).

REV Ocean Accounts (Twitter, FB, IG, LinkedIn):

- Audience growth: +25.7%
- Number of published posts: 94
- Impressions: 132,227 +28.3%
- Video views 19,611 +7.7%

Spotlight on Instagram (last 30 days):

- Accounts reached: 14,211 +14%
- Profile visits: 828 +11%
- Net Audience growth: +126
- Published posts: 32
- Impressions: 55,398 +42.3%
- Engagements: +42.6%

Content reach:

Posts: 10.8K (top post based on reach: Coming Soon post – 5,480 accounts reached)

Reels: 7,281 (top reel based on reach: Alex video message – 1,474 accounts reached)

Stories: 1,267 (top story based on reach: PR story – 634 accounts reached)

Videos: 2,427 (top video based on reach: Alex Nekton mission post - 2,341 accounts reached)

Hashtags data (only available on Twitter):

#REVOcean

- Twitter mentions: 121

- Impressions: 345.2K

#Chagos2022

- Twitter mentions: 27
- Impressions: 63.8K

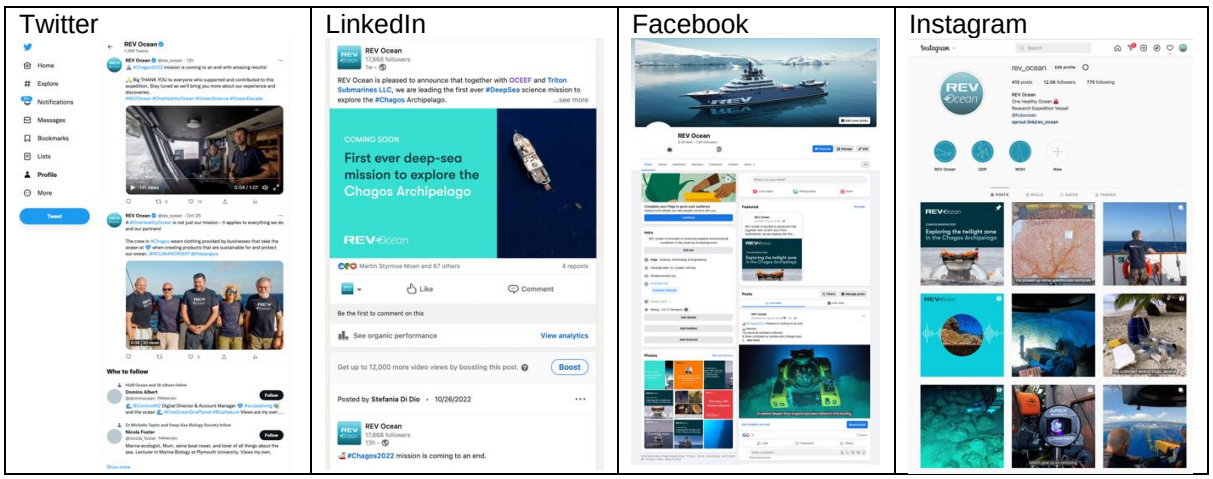

Figure 45. Examples of social media postings during the REV Ocean Mesophotic and Deep-Sea Research Cruise.

Discussion

The REV Ocean Mesophotic and Deep-Sea Research Cruise provided just a snapshot of the biological communities of the deep sea down to upper-bathyal depths (500m) along with their biodiversity and functional ecology. This expedition took place over just eight days and poor weather and sea state restricted it to just two out of three of the original intended sites. Pitt Bank, which is a fully submerged reef/atoll is highly exposed to swell and wind and so was not diveable with submersibles which can only launch in calm conditions. This was unfortunate as the submerged bank, effectively a shallow seamount, would have provided an excellent contrasting site to the westward slopes of the atolls and islands of Peros Banhos and Great Chagos Bank.

Despite the very short duration of the expedition, and limited geographic scope, a diversity of submarine habitats were observed, significantly increasing knowledge of the ecosystems contained within the Chagos Marine Reserve. Moreover, the discovery of cold-water coral reef ecosystems in the Chagos Archipelago is highly

significant. These ecosystems are poorly known from the Indian Ocean and are generally unprotected and subject to destructive bottom trawling on seamounts or continental slopes especially in areas beyond national jurisdiction. In Chagos they are likely to harbour a significant diversity of associated fauna which will enhance the conservation value of the Chagos Marine Reserve extending the significance of its protection to include deep-water ecosystems. They also offer the possibility of palaeoclimatological research through isotopic investigation of cold-water coral skeletons. Given the complexity of the seafloor topography around Peros Banhos we expect that cold-water coral reefs will be more extensive at this location and elsewhere in the Chagos Archipelago. This will require further investigation using deep-submergence technologies (submersibles, Remotely Operated Vehicles, Autonomous Underwater Vehicles) over longer duration expeditions. Other locations should also be investigated for the presence of these coral ecosystems.

The discovery of the carbonate chimneys on the slopes of Peros Banhos and Eagle Island point to the existence of another previously unknown biological habitat, that of methane seeps in the Chagos Archipelago, even if they may be fossil. On current evidence these seem likely to be related to methane seeps possibly of biotic or abiotic origin (Johnson et al., 2015; Himmler et al., 2018). Looking at the geological setting of the Chagos Archipelago located on the Chagos-Laccadives Ridge and abiotic origin of methane would seem to be more likely. If serpentinization of rock is taking place within the ridge, then this gives rise to the possibility of other chemosynthetic environments existing in the Chagos Archipelago and perhaps along the Chagos Laccadive Ridge in general. Confirmation of the nature of these chimneys will require examination of the rock samples collected by a geologist. If they are stromatolite structures associated with methane seeps (Himmler et al., 2018) further investigation will be required to map these structures and to see whether they are all inactive or whether active hydrocarbon seeps are present in the Chagos Marine Reserve. No such ecosystems were detected in the Nekton Maldives Expedition during September and early October.

Despite the limitations of our submersibles for sampling, about 130 samples were collected. These were largely restricted to sessile biota (animals and seaweed living attached to the seafloor) or to slow-moving animals such as seastars. It is important

to recognise, however, that many of the corals, sponges and other structure-forming organisms collected came with associated species so that over 220 species of animals were sampled overall. It is noted that the suction gun on *DSV Aurelia* was not functional meaning that small mobile invertebrates such as shrimp (of which a lot were present at 500m depth) could not be sampled. A range of manipulator tools such as nets or scoops could have also improved sampling success of soft bodied or fragile invertebrates. Also, the size and shape of the sample drawer on *DSV Aurelia* and position and mechanical limiters placed on the Titan T4 arm meant that some larger or longer organisms, most notably whip corals were very difficult to sample. Despite these limitations we are confident that many of the more common organisms seen during surveys on the Chagos expedition and also during the Nekton Maldives expedition have been sampled. These will require taxonomic investigation and it is likely that a proportion of them will be new to science, in some cases at higher than species level (i.e. new genera or even families).

Taken together, these discoveries indicate that the huge expanse of unexplored deep-water ecosystems of the Chagos Archipelago harbour a rich diversity of ecosystems and species. The extraordinary complexity of the deep-sea topography revealed by multibeam bathymetry suggests that the Chagos Marine Reserve is ideally suited to the growth of a range of deep-sea corals and other sessile organisms and alongside them a rich diversity of associated species. Our maps now offer the possibility of further research targeting specific topographic features likely to harbour high biodiversity ecosystems (e.g. ridges, steep cliffs and canyons).

Our investigations of the deep-sea ecosystems of the Chagos Archipelago are also likely to address to some extent questions related to ecosystem connectivity across depths. The reef refugia hypothesis suggests that at least some reef corals and other associated species such as fish may have populations that extend across depth zones from, for example, shallow water to the upper (our 60m survey depth) and even lower mesophotic zone (our 120m survey depth). In the Nekton Maldives Expedition evidence was seen that some fish species had extended depth ranges from 30-40m to 250m or more. In such cases, deep-water populations can help to replenish and support the recovery of shallow populations subject to shocks such as mass coral bleaching and coral mortality, heat waves or overexploitation by illegal

fishing. Our video surveys will help to examine this question alongside genetic investigations of coral population connectivity both across depth zones and across the archipelago.

Evidence that diurnally migrating micronekton are being trapped against the seafloor at 300 – 600m and are acting as a major food source for a variety of mobile (e.g. fish) and sessile (e.g. anemones, benthic ctenophores) predators emphasises the strong links between food webs in the water column and on or close to the seafloor in the Chagos Archipelago and elsewhere. The micronekton of the DSL may be directly preyed on by large predators of conservation importance in the Chagos Archipelago. Alternatively mid-trophic level predators observed during this study that feed on the DSL in the upper bathyal zone may themselves be eaten by higher trophic level predators such as sharks and tuna. Either way, the trapping zone implies a complex interaction between shallow and deep-sea ecosystems which not only has ramifications in terms of ecosystem health within the marine reserve but is also significant in terms of carbon cycling. As such Chagos, as an area of low human influence, offers an ideal setting to investigate the role of trapping zone dynamics in the overall ecology of large predators associated with coral reefs as well as wider ecosystem functions.

Value of research to BIOTA

The identification of cold-water coral reef ecosystems in the Chagos Archipelago significantly adds to the importance of the marine reserve. These ecosystems are poorly known from the Indian Ocean and there is strong evidence that in some regions they have been seriously impacted by deep-sea bottom trawling (e.g. the South West Indian Ridge; Rogers et al., 2017). As such, the Chagos marine reserve can now be said to not only be protecting shallow-water coral reefs but also deep, cold-water coral reefs and their associated fauna. Future management of the Chagos Marine Reserve should consider the further documentation and protection of these biodiverse deep-water ecosystems.

The discovery of the carbonate chimney structures on the seaward slopes of Peros Banhos and Eagle Island are not yet fully understood. If they prove to be associated

with abiotic methane generation along the Chagos-Laccadives Ridge this may suggest the possible existence of further chemosynthetic communities in the region, including within the deeper waters of the Chagos Marine Reserve. This, combined with the exciting submarine topography discovered during the REV Ocean Mesophotic and Deep-Sea Research Cruise points to a rich diversity of as yet undocumented deep-sea ecosystems that are yet to be discovered within the Chagos Marine Reserve. Future research should aim to fill these knowledge gaps as they increase the known value of the Chagos Archipelago as a no-take marine reserve.

Our research has clearly demonstrated strong functional connectivity between shallow-water and deep-water marine ecosystems and from the pelagic to the benthic realm. Marine protected areas are often focused on shallow-water ecosystems such as coral reefs but neglect adjacent deep-sea habitats such as mesophotic and rariphotic reef environments and bathyal slopes. The Chagos Marine Reserve importantly conserved the entire exclusive economic zone (EEZ) of the archipelago with the exception of a small area around Diego Garcia which is subject to local environmental and conservation regulations. It is extremely important that the holistic approach to conservation of ecosystems within the marine reserve is maintained in the future. The implications of our research suggest that consideration of spatial conservation measures globally should aim to conserve the full range of ecosystems within areas as only by doing this is full ecosystem functionality maintained.

The Chagos brain coral, *Ctenella chagius*, is considered to be endemic to the Chagos Archipelago and until recently thought extinct as a result of climate disruption. The data collected during this recent expedition contributes to an ongoing comprehensive survey of the coral, assessing its population size, distribution and genetic diversity. A full description of the coral's life history will form the basis for a Species Recovery Plan (SRP) and its implementation to conserve this iconic species.

Conclusions

The REV Ocean Mesophotic and Deep-Sea Research Cruise represented a brief snapshot of the deep-sea ecosystems present with the Chagos Marine Reserve down to 500m depth. In just eight days we documented the biodiversity of mesophotic, rariphotic and upper bathyal communities including the presence of cold-water coral reefs, potential chemosynthetic ecosystems and a community of predators feeding on trapped deep scattering layer micronekton. Our research has clearly demonstrated that the holistic approach of adopting the entire EEZ as a no-take marine reserve has conserved poorly known deep-sea marine ecosystems in the context of the Indian Ocean, some of which are threatened by human activities such as deep-sea bottom trawling. It has also identified strong functional connectivity between shallow water and deep-sea ecosystems that have been suspected in the scientific literature but hitherto undocumented apart from at seamounts.

As is usual the findings of this expedition raise a range of new questions. One immediate priority is further documenting the extent and distribution of cold-water coral reef ecosystems in the Chagos Archipelago. This will require the use of deep-submergence technology to document both the occurrence of these corals, the extent of living coral colonies and associated fauna. It will also require the measurement of environmental parameters so that the further distribution of these communities can be modelled within the Chagos Archipelago and beyond. This will also offer the possibility of modelling the effects of future climate disruption on habitat suitability for these corals to predict whether their distribution is likely to change with ocean warming and acidification as well as changes in other parameters (for an example see Tittensor et al., 2010).

Multibeam mapping off Peros Banhos, Eagle Island and Pitt Bank revealed complex submarine topography including ridges, spurs and canyons. Such topographic features are well known to harbour high biodiversity in deep-sea ecosystems and can also be associated with hotspots of biological activity as rich foraging grounds for predators. Again, investigation of these submarine features using deep-submergence technology is a priority and is certain to reveal new and exciting biodiversity protected within the Chagos Marine Reserve. Likewise further mapping will likely reveal where else such complex topography exists within the Chagos

Marine Reserve and may help to further prioritise and target future deep-sea research.

Understanding the linkages between different parts of the ecosystems protected within the Chagos Marine Reserve are important for a number of reasons. First, in the case of the deep-reef-refugia hypothesis understanding the full vertical distribution of species and the connectivity of populations across the depth gradient are important to understand the resilience of coral reef communities versus environmental shocks such as mass coral bleaching, severe storms or incidents of illegal fishing. The results of both analysis of video survey data and genetic connectivity studies arising from this expedition will be highly informative in terms of the management of the Chagos Marine Reserve. Findings related to vertical connectivity of food webs of deep-sea and shallow-water ecosystems emphasise the importance of the holistic approach to the adoption of large-scale marine protected areas. As such the Chagos Marine Reserve provides for an excellent case study to really understand and demonstrate why the adoption of large-scale spatial conservation measures are important and how they should be applied elsewhere including within EEZs and in Areas Beyond National Jurisdiction. This reinforces the philosophy behind the Convention of Biological Diversity's EBSA concept (Ecologically and Biologically Sensitive Areas) which examine the conservation importance of regions based on both their biodiversity and ecological function (CBD, 2009). Future research in the Chagos Marine Reserve should aim at increasing understanding of the extent and importance of food web connectivity between shallow and deep-sea ecosystems especially in the context of large predators but also in terms of maintenance of biodiversity and ecosystem health of the reserve in general. Such studies will require a combination of biological acoustics, tagging studies, dietary studies and deep-water observations. The results will not only be relevant to the Chagos Marine Reserve but will increase the case for establishment of large marine reserves that protect multiple connected ecosystems elsewhere.

Our major issue with *Ctenella* is the paucity in knowledge regarding its biology and ecology, both of which are essential if any kind of intervention to reverse the threat to this species is to occur. Therefore, continued research is necessary to gather the necessary information that will allow us to formulate an appropriate and measured

Species Recovery Plan; surveys of the region by SCUBA (and with contributions by third parties, such as researchers and citizen scientists) will provide estimates of population sizes, distributions and ranges; tagged and monitored colonies around Diego Garcia will confirm measurements of rates of coral health, mortality and growth, and spawning times; genomic analyses of tissue samples will contribute to a variety of questions regarding the biology, ecology, evolutionary history, connectivity and resilience of the species. It is hoped that studies of the life history and reproductive strategies of *Ctenella* will be used in concert with these data, to assess the feasibility of various mitigation measures to intervene and conserve a repository of both genetic information and viable biological materials for future generations. If feasible, these will be initiated as part of the practical conservation activities considered in future work.

Whilst implementation of the Convention of Biological Diversity (CBD) has not currently been extended to BIOT, research here still contributes to the UK's obligations to the convention. This research also addresses targets defined by Sustainable Development Goal 14 (Life Below Water), in particular Targets 14.2 (Protect and restore ecosystems) and 14.8 (Increase scientific knowledge, research and technology for ocean health). It also aligns with DEFRA's "25 Year Environment Plan", specifically under "Thriving Plants and Wildlife", by "reversing the loss of marine biodiversity and, where practicable, restoring it". Finally, BIOT Administration has identified eleven conservation and environmental priorities. This ongoing research addresses three of these: understanding and mitigating against the effects of global climate change where possible; understanding more about BIOT's unique terrestrial environment; and studying our key species and habitats to ensure we are providing the best protection and stewardship.

Finally, we point out that this project contributes to the UN Decade of Ocean Science for Sustainable Development in several ways. First, the project contributes directly to the Challenger 150 UN Decade of Ocean Science approved project on documenting deep-sea biodiversity (Howell et al., 2019a,b). This project has an Indian Ocean research steering group of which AD Rogers is a member. The results from both video surveys and subsequent analysis of the samples collected during this expedition will both contribute to this project. Secondly, the UN Decade of Ocean Science strongly emphasises the training of early career scientists (ECRs). This

expedition has trained four Ph.D. students in the use of submersibles for deep-sea science as well as about the broader elements of deep-sea ecology. Future deep-sea expeditions to the Chagos Archipelago should be encouraged to adopt similar approaches to training of ECRs.

Data sharing

During this expedition we have adopted a policy of digitising all activities including scientific fieldwork (e.g. placement of cameras inside submersibles), processing of samples, personal video and photographs and social media. This has generated a very large collection of data given the length of the expedition. It will take some time to process these data as well as to work up scientific material from the cruise so outputs in terms of science will likely continue for several years.

The data collected during this expedition will be hosted at Ocean Hub (https://www.hubocean.earth/) and also distributed to relevant publicly accessible databases such as the Ocean Biodiversity Information System (OBIS; https://obis.org/) with the agreement of the British Indian Ocean Territory Administration. REV Ocean has a data policy that all data from research cruises should be released normally within 12 months of the end of the expedition and exceptionally after 24 months. The exception to this is where data release conflicts with national security or may jeopardise the conservation of threatened species. All data will be made available to the administration of British Indian Ocean Territory as well as to other scientists involved in work at the Chagos Archipelago on request at any time within practical considerations of copy and transport/transmission.

References

Alexander, J. B., Bunce, M., White, N., Wilkinson, S. P., Adam, A. A. S., Berry, T., Stat, M., Thomas, L., Newman, S. J., Dugal, L., Richards, Z. T. (2020) 'Development of a multi-assay approach for monitoring coral diversity using eDNA metabarcoding'. *Coral Reefs* 39: 159-171.

Andradi-Brown, D.A., Dinesan, Z., Head, C.E.I., Tickler, D.M., Rowlands, G., Rogers, A.D. (2019) The Chagos Archipelago. In: Loya Y, Puglise KA, Bridge T (Eds.) *Mesophotic Coral Ecosystems*. Springer-Nature, Cham, Switzerland, pp 215-229.

Baird, A. H., Madin, J. S., Álvarez-Noriega, M., Fontoura, L., Kerry, J. T., Kuo, C. Y., Precoda, K., Torres-Pulliza, D., Woods, R. M., Zawada, K. J. A. & Hughes, T. P. (2018) 'A decline in bleaching suggests that depth can provide a refuge from global warming in most coral taxa'. *Marine Ecology Progress Series* 603: 257-264.

Baker, E. K., Puglise, K. A. & Harris, P. T. (2016) *Mesophotic coral ecosystems — A lifeboat for coral reefs?* Arendal, Nairobi: The United Nations Environment Programme and GRID. Available.

Baldwin, E.A. (1975) *Joint Services Expedition Danger Island 1975*. MOD Publication, London, UK

Bongaerts, P., Ridgway, T., Sampayo, E. M. & Hoegh-Guldberg, O. (2010) 'Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs'. *Coral Reefs* 29: 309-327.

Bridge, T. & Guinotte, J. (2013) *Mesophotic Coral Reef Ecosystems in the Great Barrier Reef World Heritage Area: their potential distribution and possible role as refugia from disturbance.* Research Publication no.109, Great Barrier Reef Marine Park Authority, Townsville. Available.

Bridge, T. C. L., Hughes, T. P., Guinotte, J. M. & Bongaerts, P. (2013) 'COMMENTARY: Call to protect all coral reefs'. *Nature Climate Change* 3: 528-530.

CBD (2009) Azores Scientific Criteria And Guidance for identifying ecologically or biologically significant marine areas and designing representative networks of marine protected areas in open ocean waters and deep sea habitats. Secretariat of the Convention on Biological Diversity, Québec, Canada, 11pp.

Cooper, C.F. (1909) The Percy Sladen Trust Expedition to the Indian Ocean in 1905, under the leadership of Mr. J. Stanley Gardiner. No. XVII-Antipatharia. *Transactions of the Linnean Society of London 2nd Series: Zoology* 12: 301–321.

Cowie, R.H., Bouchet, P., Fontaine, B., 2022. The Sixth Mass Extinction: fact, fiction or speculation? *Biological Reviews* 97: 640-663.

De Clerck, O., Coppejans, E., Schils, T., Verbruggen, H., Leliaert, F., De Vriese, T. & Marie, D. 2004. The marine red algae of Rodrigues (Mauritius, Indian Ocean). *Journal of Natural History* 38: 3021-3057.

Gardiner, J.S. (1905) The Percy Sladen Expedition in H.M.S. Sealark. The Chagos Archipelago. *Nature* 72: 571–572.

Gardiner, J.S., Cooper, C.F. (1907) The Percy Sladen Trust Expedition to the Indian Ocean in 1905, under the leadership of Mr. J. Stanley Gardiner. No. I.-Description of the Expedition. *Transactions of the Linnean Society of London 2nd Series: Zoology* 12: 1–56.

Glynn, P. W. (1996) 'Coral reef bleaching: Facts, hypotheses and implications'. *Global Change Biology* 2: 495-509.

Griffiths, J.D. (1981) *Chagos: The 1978/79 Expedition*. MOD Publication, London, UK

Hays, G.C., Koldewey, H.J., Andrzejaczek, S., Attrill, M.J., Barley, S., Bayley, D.T.I., Benkwitt, C.E., Block, B., Schallert, R.J., Carlisle, A.B., Carr, P., Taylor K. Chapple, T.K., Collins, C., Diaz, C., Dunn, N., Dunbar, R.B., Eager, D.S., Engel, J., Embling, C.B., Esteban, N., Ferretti, F., Foster, N.L., Freeman, R., Gollock, M., Graham, N.A.J., Harris, J.L., Head, C.E.I., Hosegood, P., Howell, K.L., Hussey, N.E., Jacoby, D.M.P., Jones, R., Pilly, S.S., Lange, I.D., Letessier, T.B., Levy, E., Lindhart, M., McDevitt-Irwin, J.M., Meekan, M., Meeuwig, J.J., Micheli, F., Mogg, A.O.M., Mortimer, J.A., Mucciarone, D.A., Nicoll, M.A.,Nuno, A., Perry, C.T., Preston, S.G., Rattray, A.J., Robinson, E., Roche, R.C., Schiele, M., Sheehan, E.V., Sheppard, A., Sheppard, C., Smith, A.L., Soule, B., Spalding, M., Stevens, G.M.W., Steyaert, M., Stiffel, S., Taylor, B.M., Tickler, D., Trevail, A.M., Trueba, P., Turner, J., Votier, S., Wilson, B., Williams, G.J., Williamson, B.J., Williamson, M.J., Wood, H., Curnick, D.J. (2020) A review of a decade of lessons from one of the world's largest MPAs: 1 conservation gains and key challenges. *Marine Biology* 167: 159.

Himmler, T., Smrzka, D., Zwicker, J., Kasten, S., Shapiro, R.S., Bohrmann, G., Peckmann, J. (2018) Stromatolites below the photic zone in the northern Arabian Sea formed by calcifying chemotrophic microbial mats. *Geology* 46: 339-342.

Hoegh-Guldberg, O., Hughes, L., McIntyre, S., Lindenmayer, D. B., Parmesan, C., Possingham, H. P. & Thomas, C. D. (2008) 'Assisted Colonization and Rapid Climate Change'. *Science* 321: 345-346.

Hosegood, P.J., Nimmo-Smith, W.A.M., Proud, R., Adams, K., Brierley, A.S. (2019) Internal lee waves and baroclinic bores over a tropical seamount shark 'hotspot'. Progress in Oceanography 172: 34-50.

Howell, K.L., Hilário, A., Allcock, A.L., Bailey, D., Baker, M., Clark, M.R., Colaço, A., Copley, J., Cordes, E., Danovaro, R., Dissanayake, A., Escobar-Briones, E.G., Garrote, P.E., Gallagher, A., Gates, A.R., Gaudron, S.M., German, C., Gjerde, K., Higgs, N.D., Le Bris, N., Levin, L.A., Manea, E., McClain, C.R., Menot, L., Mestre, N.C., Metaxas, A., Milligan, R.J., Muthumbi, A.W., Narayanaswamy, B.E., Ramalho, S., Ramirez-Llodra, E., Robson, L., Rogers, A.D., Sellanes, J., Sigwart, J.D., Sink, K.J., Snelgrove, P.V.R., Stefanoudis, P.V., Sumida, P.Y., Taylor, M.L., Thurber, A.R., Vieira, R., Watanabe, H.K., Woodall, L.C., Xavier, J.R. (2020a,) Blueprint for a decade to study deep-sea life. *Nature Ecology and Evolution* https://doi.org/10.1038/s41559-020-01352-5.

Howell, K.L., Hilário, A., Allcock, A.L., Bailey, D., Baker, M., Clark, M.R., Colaço, A., Copley, J., Cordes, E., Danovaro, R., Dissanayake, A., Escobar-Briones, E.G., Garrote, P.E., Gallagher, A., Gates, A.R., Gaudron, S.M., German, C., Gjerde, K., Higgs, N.D., Le Bris, N., Levin, L.A., Manea, E., McClain, C.R., Menot, L., Mestre, N.C., Metaxas, A., Milligan, R.J., Muthumbi, A.W., Narayanaswamy, B.E., Ramalho, S., Ramirez-Llodra, E., Robson, L., Rogers, A.D., Sellanes, J., Sigwart, J.D., Sink, K.J., Snelgrove, P.V.R., Stefanoudis, P.V., Sumida, P.Y., Taylor, M.L., Thurber, A.R., Vieira, R., Watanabe, H.K., Woodall, L.C., Xavier, J.R. (2020b) A blueprint for an inclusive, global deep-sea Ocean Decade field programme. *Frontiers in Marine Science* **7**:584861.

Johnson, J.E., Mienert, J., Plaza-Faverola, A., Vadakkepuliyambatta, S., Knies, J., Bünz, S., Andreassen, K., Ferré, B. (2015) Abiotic methane from ultraslow-spreading ridges can charge Arctic gas hydrates. *Geology* 43: 371–374.

Laverick, J. H., Andradi-Brown, D. A. & Rogers, A. D. (2017) 'Using light-dependent scleractinia to define the upper boundary of mesophotic coral ecosystems on the reefs of Utila, Honduras'. *PLoS ONE* 12: e0183075.

Laverick, J.H., Piango, S., Andradi-Brown, D., Exton, D.A., Bongaerts, P., Bridge, T.C.L., Lesser, M.P., Pyle, R.L., Slattery, M., Wagner, D., Rogers, A.D. (2018) To what extent do mesophotic coral ecosystems and shallow reefs share species of conservation interest? A systematic review. *Environmental Evidence* **7**: 15

Lesser, M. P., Slattery, M. & Leichter, J. J. (2009) 'Ecology of mesophotic coral reefs'. *Journal of Experimental Marine Biology and Ecology* 375: 1-8.

Letessier, T.B., Cox, M.J., Meeuwig, J.J., Boersch-Supan, P.H., Brierley, A.S. (2016) Enhanced pelagic biomass around coral atolls. *Marine Ecology Progress Series* 546: 271-276.

Muir, P., Wallace, C., Bridge, T. C. & Bongaerts, P. (2015) 'Diverse staghorn coral fauna on the mesophotic reefs of north-east Australia'. *PLoS ONE* 10: e0117933.

Muir, P. R., Marshall, P. A., Abdulla, A. & Aguirre, J. D. (2017) 'Species identity and depth predict bleaching severity in reef-building corals: shall the deep inherit the reef?'. *Proceedings of the Royal Society B: Biological Sciences* 284: 20171551.

Nguyen, B. N., Shen, E. W., Seemann, J., Correa, A. M. S., O'Donnell, J. L., Altieri, A. H., Knowlton, N., Crandall, K. A., Egan, S. P., McMillan, W. O. & Leray, M. (2020) 'Environmental DNA survey captures patterns of fish and invertebrate diversity across a tropical seascape'. *Scientific Reports* 10: 6729.

Nichols, P. K., Timmers, M. & Marko, P. B. (2022) 'Hide 'n seq: Direct versus indirect metabarcoding of coral reef cryptic communities'. *Environmental DNA*, 4 (1), pp. 93-107.

Pratt, N., Chen, T., Li, T., Wilson, D.J., van de Flierdt, T., Little, S.H., Taylor, M.L., Robinson, L.F., Rogers, A.D., Santodomingo, N. (2019) Temporal distribution and diversity of cold-water corals in the southwest Indian Ocean over the past 25,000 years. *Deep-Sea Research I* 149: 103049.

Puglise, K. A., Hinderstein, L. M., Marr, J. C. A., Dowgiallo, M. J. & Martinez, F. A. (2009) *Mesophotic Coral Ecosystems Research Strategy: International Workshop to Prioritize Research and Management Needs for Mesophotic Coral Ecosystems, Jupiter, Florida, 12-15 July 2008. Silver Spring, MD: NOAA National Centers for Coastal Ocean Science, Center for Sponsored Coastal*

Ocean Research, and Office of Ocean Exploration and Research, NOAA Undersea Research Program. NOAA Technical Memorandum NOS NCCOS 98 and OAR OER 2. 24 pp.

Ridgway, T., Hoegh-Guldberg, O. & Zitek, A. (2002) 'Reef recovery in disturbed coral reef ecosystems', *Proceedings of the 9th International Coral Reef Symposium*. Bali, pp. 1117-1121.

Rogers, A.D. (1999) The biology of *Lophelia pertusa* (Linnaeus 1758) and other deep-water reef-forming corals and impacts from human activities. *International Review of Hydrobiology* 84: 315-406.

Rogers, A.D., Alvheim, O., Bemanaja, E., Benivary, D., Boersch-Supan, P., Bornman, T.G., Cedras, R., Du Plessis, N., Gotheil, S., Høines, A., Kemp, K., Kristiansen, J., Letessier, T., Mangar, V., Mazungula, N., Mørk, T., Pinet, P., Pollard, R., Read, J., Sonnekus, T. (2017) Pelagic communities of the South West Indian Ocean seamounts: R/V *Dr Fridtjof Nansen* Cruise 2009-410. *Deep-Sea Research II* 136: 5-35.

Schils, T., Coppejans, E., Verbruggen, H., De Clerck, O. & Leliaert, F. 2004. The marine flora of Rodrigues (Republic of Mauritius, Indian Ocean): an island with low habitat diversity or one in the process of colonization? *Journal of Natural History* 38: 3059-3076.

Sheppard, C.R.C. (1981) Report on the scientific work undertaken on the 1978/79 Joint Sevices Expediton to the Chagos Archipelago, British Indian Ocean Territories. In: Griffiths JD (ed) *Chagos: The 1978/79 Expedition*. MOD Publication, London, UK, pp 1–52.

Sheppard, C.R.C., Bowen, B., Carr, P., Chen, C.A., Clubbe, C., Craig, M., Eble, J., Fitzsimmons, N., Gaither, M., Gan, C.-H., Gollock, M., Guzman, N., Graham, N.A.J., Harris, A., Jones, R., Keshavmurthy, S., Koldeway, H., Lundin, C.G., Mortimer, J.A., Obura, D., Pfeiffer, M., Price, A.R.G., Purkis, S., Raines, P., Riegl, B., Rogers A., Schleyer, M., Seaward, M.R.D., Sheppard, A.L.S., Tamelander, J., Turner, J.R., Visram, S., Vogler, C., Vogt, S., Yang, J.M.-C., Yang, S.-Y., Yesson, C. (2012) Reefs and islands of the Chagos Archipelago, Indian Ocean: Why it is the world's largest no-take marine protected area. *Aquatic Conservation: Marine and Freshwater Research* 22: 232-261.

Sinniger, F., Morita, M. & Harii, S. (2013) '"Locally extinct" coral species Seriatopora hystrix found at upper mesophotic depths in Okinawa'. *Coral Reefs* 32: 153-153.

Slattery, M., Lesser, M. P., Brazeau, D., Stokes, M. D. & Leichter, J. J. (2011) 'Connectivity and stability of mesophotic coral reefs'. *Journal of Experimental Marine Biology and Ecology* 408: 32-41.

Smith, T. B., Blondeau, J., Nemeth, R. S., Pittman, S. J., Calnan, J. M., Kadison, E. & Gass, J. (2010) 'Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, US Virgin Islands'. *Coral Reefs* 29: 289-308.

Spalding, H. L., Amado-Filho, G. M., Bahia, R. G., Ballantine, D. L., Fredericq, S., Leichter, J. J., Nelson, W. A., Slattery, M. & Tsuda, R. T. 2019. Macroalgae. *Mesophotic Coral Ecosystems.* pp. 507-536.

Stat, M., Huggett, M. J., Bernasconi, R., DiBattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S. & Bunce, M. (2017) 'Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment'. *Scientific Reports* 7: 12240.

Stefanoudis, P. V., Gress, E., Pitt, J. M., Smith, S. R., Kincaid, T., Rivers, M., Andradi-Brown, D. A., Rowlands, G., Woodall, L. C. & Rogers, A. D. (2019a) 'Depth-dependent structuring of reef fish assemblages from the shallows to the rariphotic zone'. *Frontiers in Marine Science* 6: 307.

Stefanoudis, P.V., Rivers, M., Smith, S.R., Schneider, C.W., Wagner, D., Ford, H., Rogers, A.D., Woodall, L.C. (2019b) Low connectivity between shallow, mesophotic and rariphotic zone benthos. *Royal Society Open Science* 6: 190958.

Taberlet, P., Coissac, E., Hajibabaei, M. & Rieseberg, L. H. (2012) 'Environmental DNA'. *Molecular Ecology* 21: 1789-1793.

Tickler, D.M., Letessier, T.B., Koldewey, H.J., Meeuwig, J.J. (2017) Drivers of abundance and spatial 1332 distribution of reef-associated sharks in an isolated atoll reef system. *PloS One* 12: e0177374.

Tittensor, D.P., Baco-Taylor, A.R., Brewin, P., Clark, M.R., Consalvey, M., Hall-Spencer, J., Rowden, A.A., Schlacher, T., Stocks, K., Rogers, A.D. (2009) Predicting global habitat suitability for stony corals on seamounts. *Journal of Biogeography* 36: 1111-1128.

Tittensor, D.P., Baco, A.R., Hall-Spencer, J.M., Orr, J.C., Rogers, A.D. (2010) Seamounts as refugia from ocean acidification for cold-water stony corals. *Marine Ecology* 31 (Suppl.1): 212-225.

Winterbottom, R., Emery, A.R., Holm, E. (1989) An Annoted Checklist of the Fishes of the Chagos Archipelago Central Indian Ocean. *Royal Ontario Museum* 145: 1-228.

Yesson, C., Clark, M.R., Taylor, M., Rogers, A.D. (2011) The global distribution of seamounts based on 30-second bathymetry data. *Deep Sea Research I* 58: 442-453.

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Appendix I Dive Recoding Sheets

Submersible Dive Record

Deployment Number:
Submersible:
Location:
Site:
Date:
Intended depth (m):
Dive objectives: 1. 2. 3. 4. 5.

Note: Switch on cameras before transects if in Omega

Name of scribe:
Pilot / co-pilot / other:
Time in water (GMT):
Start position (Dec. Deg):
Time leaving surface (GMT):
Time of arrival at seafloor (GMT):
Depth at seafloor:
Time leaving seafloor:
Depth leaving seafloor:
Time at surface:
Technical or other issues with dive:

Dive Observations / Events Deployment Number:

Event / Observation No.	Time (GMT)	Depth (m)	Description (taxon sampled, organisms / phenomena observed)

Dive Observations / Events Deployment Number:

Event / Observation No.	Time (GMT)	Depth (m)	Description (taxon sampled, organisms / phenomena observed)

Note: Standard dives will only require one observation sheet.

REVOcean		Dive Number:	VHF Ch.
		Date:	
		Dive Site:	
Cruise: RV *Odyssey* 005 Chagos		Submarine: Triton 3K3/6	
Deployment number		Dive Log	
Pre-Dive / Maintenance Logs	Check	Latitude/Longitude	
"Remove Before Dive" Tags (x4)	Removed	Target depth	
Surface Officer		Conditions on surface	
1. Pilot & Equipment	kg	Hatch secured	
2. Pax 1 & Equipment	kg	Arrival to Max Depth	
3. Pax 2 & Equipment	kg	Cleared to surface	
Soft ballast in Sphere	kg	On surface	
Payload (350kg Max)	kg	Heading to maintain on surface	
All times are in UTC		Hatch open	

Science equipment	Supplier	Present / absent
Cameras		
1. Mid-bow stereo cameras	Teledyne	
2. Downward stereo cameras	Teledyne	
3. Stern pointing camera		
4. Pan and tilt 4k camera	Teledyne	
5. Additional camera 1		
6. Additional camera 2		
Sampling equipment		
Manipulator	Hydrolek	
Manipulator modifications		
Biobox	Triton	
Tracking		
Additional Comms		
Other equipment		
1.		
2.		
3.		
4.		

COMMUNICATIONS LOG				
DIVE No. SHEET No.		DATE		DIVE SITE
TIME	DEPTH (M)	Event No.	Description	Position

COMMUNICATIONS LOG				
DIVE No. SHEET No.		DATE		DIVE SITE
TIME	DEPTH (M)	Event No.	Description	Position
Additional notes				

REV Ocean		Dive Number:	VHF Ch.
		Date:	
		Dive Site:	
Cruise: RV *Odyssey* 005 Chagos		Submarine: 7K3/1 Aurelia	
Deployment number			
Pre-Dive / Maintenance Logs	Check	Latitude/Longitude	
"Remove Before Dive" Tags (x4)	Removed	Target depth	
Surface Officer		Conditions on surface	
1. Pilot & Equipment	kg	Hatch secured	
2. Pax 1 & Equipment	kg	Arrival to Max Depth	
3. Pax 2 & Equipment	kg	Cleared to surface	
Soft ballast in Sphere	kg	On surface	
Payload (350kg Max)	kg	Heading to maintain on surface	
All times are in UTC		Hatch open	

Science equipment	Supplier	Present / absent
Cameras		
1. Mid-bow stereo cameras		
2. Downward pointing bowtech		
3. Stern pointing camera		
4. Pan and tilt 4k camera		
5. Additional camera 1		
6. Additional camera 2		
Lasers		Camera
1.		
2.		
Sampling equipment		
Manipulator		
Manipulator modifications		
Niskin bottles		
1.		
2.		
3.		
4.		
5.		
6.		
Slurp gun		
1.		
2.		
3.		
4.		
5.		
6.		
Corers		
Smooth corers		
1.		
2.		

3.		
4.		
5.		
6.		
AACs		
1.		
2.		
3.		
4.		
Biobox		
D-Samplers		
1.		
2.		
Configuration		
CTD		
Multibeam		
Tracking		
Additional Comms		
Other equipment		
1.		
2.		
3.		
4.		

COMMUNICATIONS LOG				
DIVE No. SHEET No.		DATE		DIVE SITE
TIME	DEPTH (M)	Event No.	Description	Position

COMMUNICATIONS LOG

DIVE No. SHEET No.		DATE		DIVE SITE
TIME	DEPTH (M)	Event No.	Description	Position

Additional notes

Appendix II Treatment of Submersible Samples

Table 1 WHERE DOES THIS SAMPLE GO?

NOTE = 5 x volume of RNALater/ 100% ethanol to tissue in vial. If sample too bulky for 2/5 ml tubes, use 25ml. RNALater and ethanol subsamples left in cold room for 24hrs then moved to -80 freezer.

What is it?	What preservation?	Where does it go?
SCLERACTINIA (hard coral) – genetics subsamples	E100	1 cm^3 2ml or 5ml cryovial or 15ml (D) vials if too big
	RNALater	1 cm^3 or 15ml (C) vials if too big > 12-24hrs in cold room > -80 freezer
SCLERACTINIA (hard coral) - rest of specimen	Formalin > 70% ethanol	Specimen into > formalin > for ~7-14 days then transfer to 70% ethanol
STYLASTERID - genetics subsample	E100	1 cm^3 2ml or 5ml cryovial > Ethanol cryobox (B) or 15ml (D) vials if too big
	RNALater	1 cm^3 > RNALater cryobox (A) > or 15ml (C) vials if too big > 12-24hrs in cold room > -80 freezer
STYLASTERID – rest of specimen	E96%	In container in 96% ethanol
OCTOCORAL - genetics subsample	E100	A few branchlets with polyps > 2ml or 5ml cryovial > Ethanol cryobox (B)
	RNALater	A few branchlets with polyps > RNALater cryobox (A) > 12-24hrs in cold room > -80 freezer
OCTOCORAL – rest of specimen	Formalin > 70% ethanol	First 30 specimens > Formalin > for ~1 day (NOT LONGER) > 70% ethanol All extra > 100% Ethanol
SEA CUCUMBERS - genetics subsample	E100	1 cm^3 muscle tissue >2ml or 5ml cryovial > Ethanol cryobox (B)
	RNALater	1 cm^3 muscle tissue > RNALater cryobox (A) > 12-24hrs in cold room > -80 freezer
SEA CUCUMBERS – rest of specimen	E100	Suitable-sized container
URCHIN - genetics subsample	E100	1 cm^3 gonad tissue >2ml or 5ml cryovial > Ethanol cryobox (B)
	RNALater	1 cm^3 gonad tissue > RNALater cryobox (A) > 12-24hrs in cold room > -80 freezer
URCHIN – rest of sample	E96%	Suitable sized container
SPONGE - genetics subsample	E100	3cm^3 > in 15ml (D) ethanol vials> into SPONGE tube holder

	Drying	$3cm^3$ > drying area of wet lab with label
	-80	$3cm^3$ > sandwich bag > -80 freezer box marked “SPONGE”
SPONGE – rest of specimen	-20	Into sandwich bag / thermosealed bag > -20 freezer
CRUSTACEAN	E100	If small, individual or individuals in 100% ETOH. Otherwise excise muscle tissue from a limb.
CRUSTACEAN – Rest of specimen	E96%	Suitable container.
Other invertebrates (MOLLUSCS, POLYCHAETES, PLATYHELMINTHES, NEMERTEANS)	E100	If small and multiple specimens, entire specimen in E100. If large then excise up to 1cm3 of muscle / body tissue and preserve in E100.