

NEKTON

DEEP OCEAN EXPLORATION



NEKTON MISSION I

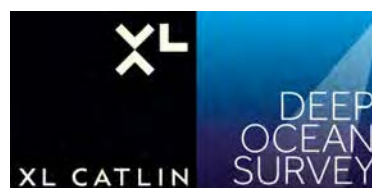
XL Catlin Deep Ocean Survey

17 July - 14 August, Bermuda, NW Atlantic Ocean

CRUISE REPORT

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Nomad and Nemo submersibles on a dive with GUE diver

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Sub *Nemo* moves in to collect a coral sample on Tiger

INTRODUCTION

Bermuda lies in the northwestern Sargasso Sea and is an isolated complex of 150 islands and islets of which only 6 are of a size to be inhabited and are linked by causeways and bridges to form a hook-shaped island chain 34km long (Coates et al., 2013). Despite the main islands only having an area of 53.6km² (Coates et al., 2013) the Bermudan Exclusive Economic Zone (EEZ) has an area of 464,940km² (Government of Bermuda, 2013). The Bermuda Platform is the eroded stump of a large volcano which formed during the Eocene (<45 MYA) and Oligocene (33-34 MYA) and is the largest of four volcanoes which run in a line trending north east and which include the Plantagenet and Challenger Banks and Bowditch Seamount (Coates et al., 2013). Although volcanism ceased in the early Oligocene, the seabed continued to swell into the Miocene forming the Bermuda Rise which is about 1,500km long and 500-1,000km wide (Coates et al., 2013).

Despite its temperate latitude (32.4°N) Bermuda has a subtropical climate and sea surface temperatures (Coates et al., 2013). Temperatures in the open ocean around Bermuda range from ~ 19.2°C (March) to 27.4°C (August) but over the platform there is a wider temperature range of 14-15°C in the winter to 30-31°C in the summer (Coates et al., 2013). Inshore habitats have a markedly lower temperature during winter than those further offshore (Coates et al., 2013). Predominant coastal habitats include: coral reefs (e.g. Coates et al., 2013), seagrass beds (e.g. Manuel et al., 2013), algal-vermetid cup reefs (e.g. Coates et al., 2013), beds of calcareous green algae (e.g. Coates et al., 2013; Manuel et al., 2013), rhodolith beds (Bosellini & Ginsberg, 1971), mangrove forest (e.g. Thomas, 1993), sandy and rocky shores. Deeper-water habitats include extensive rhodolith beds and mesophotic coral-reef ecosystems giving way to rocky substrata with sessile epifaunal communities of sponges, octocorals and black corals (Fricke & Meischner, 1985; Iliffe, 2012; Pinheiro et al., 2016). Zooxanthellate corals are not reported to occur deeper than 70m. This is surprising as light conditions suitable for such corals to occur deeper than 100m depth (Fricke & Meischner, 1985). Fish communities show an increase in abundance, biomass and species richness with depth in Bermuda a pattern opposite to that found in other Caribbean localities such as Curaçao (Pinheiro et al., 2016). Caves also open up on the upper slopes of Bermuda, some of which form extensive systems opening up on the island itself (Hengstum & Scott, 2012; Iliffe, 2012). These host a rich cave fauna of small invertebrates (Iliffe et al., 1983), mainly crustaceans (Sterrer et al., 2004) but also other organisms such as protozoans (Hill, 1986) and archiannelids (Sterrer and Iliffe, 1982). In 2004, of the 86 species of cave fauna described 80 (93%) were endemic to Bermuda (Sterrer et al., 2004) and animals discovered in the last 3-4 decades have been novel at the species, genus, family and even order level.

Sargasso Sea

Further offshore Bermuda is surrounded by the Sargasso Sea, an oligotrophic anticyclonic gyre traditionally associated with free-floating *Sargassum* Weed. The Sargasso Sea is in fact defined by physical oceanographic boundaries, particularly the Gulf Stream to the west, the North Atlantic Current to the north, the Canaries Current to the east and the North Equatorial Current to the south. The Sargasso Sea has been an extremely important area for science being the location of the Bermuda Atlantic Time Series, the longest series of oceanographic measurements in the world started at Station S, just off Bermuda, by Henry Stommel in 1954. In 1988 the Bermuda Atlantic Time series study moved 80km south east of Bermuda to minimise the influence of the island and more parameters were added to the measurements undertaken. Major findings of the BATS study have included:

- Identifying the influence of mesoscale eddies on surface primary production and carbon export to the deep sea
- The identification of an increase in surface CO₂ in the ocean resulting from anthropogenic CO₂ emissions

- The identification of dissolved organic carbon as the major exchangeable pool of carbon in the ocean
- The detection of the influence of major climatic oscillations (El Niño Southern Oscillation and North Atlantic Oscillation) on oceanographic parameters in the BATS region
- New discoveries relating to the diversity of microbial assemblages in the ocean, using genomic approaches, and their role in biogeochemical cycling

As well as important work in the pelagic region, deep-sea science in the Sargasso Sea has been of great importance. The Gay-Head-Bermuda transect was perhaps the most important study of the deep-sea infauna in the Atlantic, sampling animals living in sediments along a transect from 20m depth off the coast of Massachusetts to 1000m depth off Bermuda. The study used an anchor dredge and epibenthic sled to collect sediment samples, which were then passed through a 0.42mm sieve to retain the small animals living in the seafloor. Discoveries included much higher faunal densities and diversity than previously suspected in the deep sea (Hessler & Sanders, 1967), a pattern of decreasing faunal density with increasing depth and distance from land (Sanders, 1965; Hessler & Sanders, 1967; Sanders & Hessler, 1969). Polychaete worms were found to be the most abundant group of animals followed by crustaceans and molluscs, a pattern repeated in many other parts of the deep sea. Species were also found to be distributed in communities zoned by depth along the transect (Sanders & Hessler, 1969; Grassle et al., 1979). Rates of community respiration were also found to decline with depth and were also correlated, to a lesser extent with temperature, oxygen concentrations, community biomass and surface primary production (Smith 1978). Thus, whilst the Challenger Expedition could be viewed as discovering the ubiquity of deep-sea life in the ocean, it was work on the Bermuda - Gay Head transect which gave rise to quantitative ecological studies in the deep sea.

The Sargasso Sea was also the first place biologists attempted to view deep-sea life *in-situ* using a deep-submergence vehicle. William Beebe, an American, had been granted the use of Nonsuch Island in Bermuda in 1928 as a research station which he intended to use to investigate the adjacent sea to a depth of 2 miles. Beebe had already lead an expedition to the Sargasso Sea in the steamer *Arcturus* sampling the associated fauna of floating Sargassum weed as well as the deep-sea pelagic fauna of the area (Beebe, 1926). Beebe wanted to visit the deep sea in person and published his design for a submersible in the New York Times. He subsequently met the engineer Otis Barton who dismissed Beebe's idea for a cylindrical submersible and they agreed to build a bathysphere. This comprised a crude steel sphere, with two small fused-quartz portholes and containing cylinders for oxygen and CO₂ scrubbers, all suspended from a steel cable from a ship on the surface. The vehicle was built in 1929 and between 1930 and in 1934 Beebe and Barton made dives into the pelagic environment as well as down the slopes of the island of Bermuda, reaching a depth of 923m, half a mile down. During the dives they carried out the first *in-situ* observations of deep-sea life as well as making the first live recordings via a cable to broadcast on the radio. Beebe's dives inspired August Piccard who subsequently used the bathyscaphe to dive to 7 miles depth in the Marianas Trench. This in turn lead to the development of more modern scientific submersibles such as *Alvin* which from 1967 to 1972 carried out dives on the Bermuda - Gay head transect (Grassle et al., 1975) and in 1974 on the New England Seamounts.

One of the most notable features of the Sargasso Sea are the floating rafts of *Sargassum* weed comprising two species *Sargassum natans* and *Sargassum fluitans*. Each year the weed initially generates in the northern Gulf of Mexico and then is advected out into the Atlantic, reaching Cape Hatteras by July/August/September and then circulating in the Sargasso Sea anticyclonic gyre further south past Bermuda and to off the Bahamas in the winter (Gower and King, 2011). Remote sensing data suggest that the northern Gulf of Mexico is the nursery area for *Sargassum* weed, a significant consideration given the recent *Deepwater Horizon* disaster that affected that area (Powers et al., 2013). An average biomass of a million tonnes of *Sargassum* was estimated for the Gulf of Mexico and a million tonnes for the Atlantic was estimated from satellite remote sensed data but this may be an underestimate of the total as a result of artefacts relating to use of ocean colour data (Gower & King, 2011).

The community of organisms associated with *Sargassum* is unique and comprises at least 100 species of fish, 145 species of invertebrates and 4 turtles. At least 10 species of fish and invertebrates are endemic to the Sargassum weed communities in the Gulf of Mexico / Atlantic. These include animals such as the Sargassum frogfish *Histrio histrio* which have evolved colouration and shapes that provide camouflage whilst living in the weed (Coston-Clements et al., 1991). The *Sargassum* rafts provide essential fish habitat and are either occupied for the entire life cycle by some species such as the Sargassum frogfish or used as important nursery/foraging habitat by juvenile fish (e.g. dolphin fish; Coston-Clements et al., 1991; Casazza & Ross, 2008; Farrell et al., 2014). These include many important commercial species such as jacks, barracudas, mackerel, tuna, billfish and swordfish (Coston-Clements et al., 1991) as well as reef-associated species found in Bermuda such as chubs (*Kyphosus sectatrix*), sergeant majors (*Abudefduf saxatilis*), seahorses (*Hippocampus* sp.), triggerfish and filefish (Balistidae), porcupine fish (*Diodon* sp.), barracuda (*Sphyrna barracuda*), and sennet (*S. picudilla*; Hallet, 2011). There is also evidence that *Sargassum* rafts form important habitat for neonate and juvenile green, hawksbill, loggerhead and Kemp's ridley turtles (IAC, 2014) and may also be important foraging habitat for seabirds (Moser & Lee, 2012). *Sargassum* weed also sinks to the deep-sea bed and provides food for a community of scavenging invertebrates (Fleury & Drzen, 2013). Understanding trends in *Sargassum* weed biomass, distribution and its associated biota is complicated by marked spatial and temporal variability and the lack of a systematic sampling programme. However, it is clear that a number of threats to the floating communities exist (see Risks) and recent studies of the associated biota suggest a significant change over the last 40 years including a decrease in coverage of the algae by sessile marine animals (Huffard et al., 2014).

The Sargasso Sea is also important as a migration route, foraging ground and spawning ground for iconic, threatened and commercial species including a number of baleen (e.g. humpbacked whales; Kennedy et al., 2014) and toothed whales (e.g. sperm whales; Wong & Whitehead, 2014), turtles (e.g. IAC, 2014) blue fin tuna (Wilson & Block, 2009), marlins (e.g. Luckhurst, 1994; Luckhurst et al., 2006), swordfish, wahoo (Luckhurst & Trott, 2000), dolphinfish (Luckhurst & Trott, 2000; Farrell et al., 2014) European and American freshwater eels (Ginnekan & Maes, 2005; Friedland et al., 2007; Hanel et al., 2014) and sharks (e.g. blue shark; Vandeperre et al., 2014). Commercial fisheries in the Sargasso Sea are thought to have been worth ~ \$171 million in 2005 in terms of economic impact potentially rising to \$1,500, \$2,616, \$4,525 million in 10, 20 and 50 years respectively (Sumaila et al., 2013). Pendleton et al (2014) estimate that fishing revenues from the Sargasso Sea amount to \$99 million per annum, although the contribution to fisheries outside the Sargasso Sea, such as for European eel are not included in this figure. Other ecosystem services provided by the Sargasso Sea are also economically important, for example its contribution to supporting whales which in turn support a whale-watching industry worth nearly half a billion US\$ (Pendleton et al., 2014). It is noteworthy that several important commercial species associated with the Sargasso Sea are in decline as a result of overfishing and/or changes in environmental conditions, the most notable of which are probably the European freshwater eel and blue-fin tuna.

Seamounts

The Sargasso Sea also includes a number of seamounts including both within the Bermudan EEZ and also in the high seas including Plantagenet and Challenger Banks, Bowditch Seamount, the New England Seamount Chain and the Corner Rise Seamounts. Little is known about the seamounts within the Bermuda EEZ. Challenger Bank was investigated by the submersible *GEO* to a depth of nearly 200m in 1983. The greatest depth records for zooxanthellate corals during the submersible expedition were recorded on the Challenger Bank (*Montastraea cavernosa*) and it was also in this location that the azooxanthellate coral *Madrepora carolina* was discovered at between 130 and 140m depth on a Pleistocene terrace (Fricke & Meischner, 1985). Iliffe (2012) shows a photograph taken by a technical diver at 136m depth on Challenger Bank with abundant coral growth along the edges of overhanging rocky shelves or terraces. The photograph has insufficient resolution to identify the species. Bilewitch

(2008) reports that more than half of the collection of octocorals of the Bermuda Natural History Museum identified to species are from deep water (below conventional SCUBA depth). Some species including *Eunicea pinta* and *Muricea elongata* were only found around the Plantagenet and Challenger Banks (Bilewitch, 2008).

Threats

Fishing

The human activity with the most obvious historical impact in the North West Atlantic region in which the expedition will take place is fishing. Bermuda perhaps implemented the first fisheries management legislation globally when in 1620 regulations were introduced to limit the minimum size at which turtles could be harvested and also to restrict the harvesting of bait fish for oil (Luckhurst et al., 2003). Fisheries in Bermuda until recently were small-scale, traditionally using pots to catch multiple species, especially groupers and other reef fish (Teh et al., 2014). Until the 1960s commercial fishing was relatively undeveloped and self-regulated through customary tenure (Teh et al., 2014). In the 1960s, in response to “optimistic” reports assessing the fisheries potential of Bermuda, the Government increased efforts to modernise and expand the local fishing industry (Barret, 1991) mainly to supply the tourist industry which expanded from the 1950s peaking at over 3 million visitors in 1980 (Luckhurst et al., 2003). The subsidised expansion of the fishing industry led to the rapid erosion of traditional management through customary tenure and lead to a “tragedy of the commons” situation (Barret, 1991). Catches of groupers dropped from comprising 90% of the catch in pots in the 1950s to 19% of the food fish catch in 1989 (Luckhurst et al., 1993). However, the pot fishermen in Bermuda were able to sustain their activities by gaining market acceptance for a wider range of reef fishes including parrotfish (Burnett-Herkes & Barnes, 1996). Regulation of the fishery was gradually introduced over the course of 20 years from 1972, which gradually reduced the use of pots for reef fishing until a complete ban was introduced in 1990 partly as a result of pressure from other industries (e.g. tourist and SCUBA diving businesses; Barret, 1991). There were efforts to switch the target of fishing to deeper water snappers (Lutjanidae; *Etelis oculatus*; *Pristopomoides macrophthalmus*) and following an FAO demonstration of vertical longlining techniques in Bermuda in 1980, effort was directed at waters between 220 - 350m depth (Luckhurst & Ward, 1996). The fishery collapsed in two years and effort was redirected towards pelagic species (Luckhurst et al., 2003; Teh et al., 2014). Reconstruction of fisheries catches for Bermuda from 1950 to 2010 have identified that official FAO statistics have been deficient in terms of aggregating catch data across species and also in terms of not accounting for subsistence and recreational catches. The overall catch for this period has been estimated at 54,200t, 1.75 times the estimated catch based on FAO figures (Teh et al., 2014). This figure does not include catches by foreign longlining vessels that fished for albacore tuna until the Bermudan EEZ was declared in 1977. In the early 1980s 68 of these vessels were licensed to fish within the Bermudan EEZ, a practice that officially ended in 1994 when licenses to foreign vessels ceased being granted by the Bermudan Government (Teh et al., 2014).

At present many of the targeted larger grouper species around Bermuda have not recovered in abundance post overfishing although populations of other reef fish have stabilised in response to the pot fishing ban (Luckhurst et al., 2003; Smith et al., 2013a, b). The black grouper, *Myctoperca bonaci*, has shown a recovery in numbers but appears to be now subject to increasing levels of illegal fishing which current fisheries monitoring control and surveillance measures appear to be inadequate to prevent (Smith et al., 2013b). Shark catches are apparently increasing and intensive fishing for reef sharks, primarily the Galapagos shark, *Carcharhinus galapagensis*, is a cause for concern (Smith et al., 2013b). The Galapagos shark is thought to give birth around Bermuda (Smith et al., 2013b). Spatial protection measures have been introduced to protect the spawning grounds of groupers. These take the form of two seasonally closed areas located to the south west and north east of the main islands. Fishing is prohibited in these areas between 1st May - 31st August and also in a smaller area, the south western seasonally protected area from 1st September - 29th November.

The north eastern and south western seasonally closed areas were established to protect red hind, *Epinephelus guttatus*, spawning grounds but did not include Nassau grouper, *E. striatus*, whose spawning sites and spawning aggregations were fished out (Luckhurst, 2007). A smaller area in the eastern part of the islands has been designated a closed area from May 1st for two months as a spawning aggregation site for blue-striped grunt (Trott et al., 2009). A number of small sites have been protected from fishing for the purposes of recreational SCUBA diving.

Statistics for catches from recreational fishing remain a significant weakness in understanding of fisheries impacts in Bermuda. Not only have the catches from this activity been historically under-recorded (Teh et al., 2014) but evidence suggests that it is currently largely unregulated and a significant factor in the mortality of some species such as the coney, *Cephalopholis fulva* (Trott & Luckhurst, 2008). The Government of Bermuda has undertaken a survey on non-commercial fishing activities and promised to introduce regulations on the basis of this assessment (Government of Bermuda, 2010). Anecdotally there has also been a suggestion of illegal, unreported and unregulated (IUU) fishing within the Bermudan EEZ (Government of Bermuda, 2014), presumably in offshore waters. However, at present there has been no effort to ascertain whether or not IUU fishing is taking place in Bermudan waters despite the potential use of satellite remote sensing to identify vessel activity within the area. The Bermudan Government and the Fishermen's Association of Bermuda appear to be promulgating the development of an offshore fishery (Government of Bermuda, 2014). Recreational fishing interests such as the Billfish Foundation have also pointed out that offshore fishing, targeting features such as seamounts is an increasing trend in the industry (Government of Bermuda, 2014). However, there is little understanding of the marine ecosystem or fish stock status in the context of a growing fishery and therefore of the likely impacts of such fishing activities in offshore waters in Bermuda (Government of Bermuda, 2014).

Further afield, in the North West Atlantic, many fish stocks have been historically overfished and have gone into decline or been depleted. The large FAO Statistical Area 31, of which Bermuda is a part has seen catches decline from a peak of 2.5 million tonnes in 1984 to 1.3 million tonnes in 2009 (WECAFC, 2014). The decline has mainly been in coastal fishes such as groupers and snappers and in small pelagic species such as herrings, sardines and anchovies (WECAFC, 2014). Offshore iconic species including freshwater eels (e.g. Friedland et al., 2007; Castonguay & Durif, 2015) and blue fin tuna (e.g. Safina & Klinger, 2008) have also notably declined. Although environmental change and other human activities may be partially responsible for such declines in fish populations, especially for eels (Castonguay & Durif, 2015), overexploitation is probably the overriding factor in most cases. Fisheries for deep-sea species in the North West Atlantic have been associated with significant adverse impacts on vulnerable marine ecosystems most notably coral and sponge communities on seamounts and on the continental margin. A Russian fishery on the Corner Rise Seamounts from 1976 to 1996 took ~19,000t of fish from around the seamounts, mainly alfonso but also cardinal fish, barrelfish, black scabbardfish and flint perch (Vinnichenko, 1997). Not only were the target fish stocks, particularly alfonso, depleted, ROV investigations subsequently revealed that the summits of two seamounts, Yakutat and Kükenthal had been effectively denuded of habitat-forming corals and the manganese crust on which they had grown, broken up (Waller et al., 2007). Seamounts that were not apparently fished retained coral communities characterised by high densities of octocorals (Waller et al., 2007).

In response to international concerns raised about observations of similar catastrophic damage to deep-sea coral communities elsewhere in the world the United Nations General Assembly produced several resolutions calling for improved management of deep-sea fisheries (Rogers & Gianni, 2010). This resulted in the FAO producing international guidelines on the management of deep-sea bottom fisheries on the high seas. Regional Fisheries Management Organisations (RFMOs) including the Northwest Atlantic Fisheries Organisation (NAFO) responded with closures of areas to deep-sea bottom fishing on the high seas known to harbour vulnerable marine ecosystems both before and after the guidelines were approved.

Pollution: Oil

Tar balls were found frequently on the beaches of Bermuda where up to 610g m⁻¹ per 6 days has been observed (Ilfiffe & Knap, 1979). The tar originated from onshore and offshore oil production, processing and handling, ship operations and also from natural sources (Peters & Suida, 2014). However, there is evidence that suggests that in the Sargasso Sea oil located at depths of ~250m may originate from natural seepage along the coast of Venezuela (Roquejo & Boehm, 1985). The tar can melt in the sun forming flattened sheets, which become encrusted in sand and other material. If it is washed out to sea it can sink and become buried in subtidal sediments (Ilfiffe & Knap, 1979). Tar balls can be colonised by marine animals such as hydroids but they can also be taken up by marine organisms from grazing zooplankton to predators such as turtles (Peters & Suida, 2014). However, data from neuston tows has shown a dramatic decrease in the occurrence of tar balls in the surface of the Sargasso Sea around Bermuda resulting from stricter controls on the petroleum and shipping industries (Peters & Suida, 2014).

Debris and plastics

Debris, mainly plastic, is an increasing problem in the ocean and one that has recently come to public attention. However, the problem has been reported for some time with Carpenter & Smith (1972) publishing observations of an average of 3,500 pieces of plastic (290g) km⁻² in the Sargasso Sea, mainly in the size range of 0.25-0.5cm diameter. Polyethylene plastic beads, presumably a raw material from plastic manufacturing were collected from the majority of 40 beaches in Bermuda with numbers often reaching 5,000 m⁻¹ and in one case 10,000 m⁻¹ (Gregory, 1983). The plastic pellets were thought to have originated from plastic manufacturing facilities along the US coast and ended up in the Sargasso Sea Gyre.

Sewage contamination

Bermuda lacks a centralised sewage system and domestic waste is generally held in soak away pits adjacent to properties (Smith et al., 2013b). Larger commercial sites use deep bore holes (~40m) for disposal of waste (Smith et al., 2013b). Nutrient enriched groundwater and run-off is an issue for the enclosed bays and harbours around Bermuda as well as karst cave systems, especially in terms of nitrates (Smith et al., 2013b). It is notable that there is no control over direct discharges from many small pleasure craft (Smith et al., 2013b). Nutrient enrichment has caused elevated levels of plankton production but incidents of harmful algal blooms have not occurred (Smith et al., 2013b). Nutrient enrichment can be harmful to reefs in terms of altering the competitive balance between algae and corals as well as introducing favourable conditions for harmful microorganisms. Interestingly, coral cover is still high around Bermuda (39%) compared to many other regions of the Caribbean and by some measures water quality appears to have improved around the islands over recent years (Jackson et al., 2014). This has been explained by strong environmental and fisheries regulations imposed in Bermuda over the last 3 decades (Jackson et al., 2014). Several sewage outfalls are located on the south shore of Bermuda but these are thought to have limited impacts on the marine ecosystems in the vicinity (Smith et al., 2013b).

Tourism and development

Increasing development largely around the international business sector and an increasing tourist industry, especially related to cruise ships, are both potential pressures on the environment in Bermuda. There are currently four channels into Bermuda's ports, North Channel, South Channel, Town Cut and Two Rock Passage. South Channel is the most frequently used and is the route usually taken by container ships (Smith et al., 2013b). Visits by larger cruise vessels have necessitated the more frequent use of North Channel through the centre of the lagoon (Smith et al., 2013). Frequent passage of vessels along North and South Channels potentially resuspends sediment but to date no effect has been demonstrated on the reefs (Smith et al., 2013b). North Channel in particular is narrow and difficult to navigate in windy conditions (Smith et al., 2013b). The increasing size of cruise vessels is creating pressure to increase access to Bermuda's ports (Bermuda National Trust, 2006; Smith et al., 2013).

Any such development may lead to several impacts:

- Direct destruction of coastal habitat for coastal infilling / land creation
- Direct destruction of reefs for widening of channels or the development of new channels
- Increased pollution and sedimentation of reefs adjacent to channels and ports
- Increased potential of vessel groundings
- Impacts on recreational and commercial fisheries resulting from reef degradation
- Reduction in the quality of visitor experience as reefs become more degraded
- Pressure on the land resources of Bermuda if passenger numbers exceed the current 10,000 per day limit

(Bermuda National Trust, 2006; Smith et al., 2013b)

There have been several cruise ship groundings in Bermuda on coral reef habitat including the *Mari Boeing* in 1978 and the *Norwegian Crown* in 2006 (Bermuda National Trust, 2006; Murdoch et al., 2006). In both cases there was severe damage to coral reef with damage from the *Mari Boeing* predicted to take 100 years for recovery (Anderson et al., 2001). Neither of these groundings were associated with oil release although this should be considered a potential threat in the future. During the 1980s there were several groundings of oil tankers which led to improvements in navigation around Bermuda (Smith et al., 2013b). Whether or not an increase in cruise traveller numbers is sustainable in Bermuda is open to question and certainly in need of more thorough investigation and stakeholder consultation (Bermuda National Trust, 2006).

SCUBA can have impact on coral reefs if it is not sufficiently well managed. At present the impacts of SCUBA tourism on Bermuda's reefs has not been investigated. Reef-related tourism is estimated to be worth \$405 million per year with an additional \$36 million in recreational and cultural services (Smith et al., 2013b).

Invasive species

The lionfish, *Pterois volitans*, reached Bermuda in 2000 (Whitfield et al., 2002) and now is present throughout the coral reef system including in mesophotic reefs down to 80m depth (Pinheiro et al., 2016). Lionfish prey on native fish species and have experimentally been shown to reduce recruitment of native fish species (e.g. Bahamas; Albins & Hixon, 2008). In Bermuda they seem to be particularly abundant on outer terrace reefs and the deep fore reef (Smith et al., 2013b). Culling activities are being carried out in an attempt to reduce numbers of this invasive species and efforts are also increasing in deeper water thanks to the increasing presence of technical divers (Smith et al., 2013b).

Climate change

Mass coral bleaching first occurred in Bermuda in 1988 and some level of bleaching has been occurring on Bermudan reefs since 1999 (Smith et al., 2013). Significant bleaching events occurred in 1998, 2000 and 2003 but the 1998 event in Bermuda was nowhere near as severe as elsewhere in the Caribbean (Smith et al., 2013b). A lower occurrence of bleaching may be partially related to the fact that acroporid corals never dominated reefs around Bermuda where massive coral species were most prevalent (Jackson et al., 2014). The latter are more tolerant to elevated sea surface temperatures than branching acroporids (Jackson et al., 2014). Octocorals have also showed bleaching on Bermudan reefs (*Pseudoterogorgia americana*, *Plexaurella nutans* and *Pseudoplexaura porosa*; Smith et al., 2013b). There is evidence to suggest that partially bleached colonies in Bermuda have, to date, generally recovered and there appears to have been no long-term impacts on coral cover (Smith et al., 2013b). It may be that the low winter temperatures help to attenuate bleaching events in Bermuda and reduce mortality compared to the wider Caribbean. Detailed studies following the bleaching and mortality patterns of Bermudan corals have not been undertaken. There is also no information at present on the effects or future effects of ocean acidification on Bermudan reefs. In this context the discovery that northern reefs in Florida are

already in a net erosional state as a result of ocean acidification (Muehllehner et al., 2016) is of particular concern. It is therefore urgent that an assessment of the current rates of reef accretion / erosion are undertaken on Bermuda's reefs especially as cooler waters during winter are likely to have a lower pH than elsewhere in the Caribbean where temperatures are higher.

Disease

Black band disease (BBD) was discovered in Bermuda in 1975 affecting scleractinian corals (Garret & Ducklow, 1975). Subsequently white plague disease (WPD) and Caribbean yellow band disease (CYBD) have been observed on scleractinians and aspergillosis on octocorals (Smith et al., 2013b). 16 species of scleractinians and 12 species of octocorals are affected by at least one disease (Smith et al., 2013b). Patterns in these diseases are not entirely clear, with some showing increases over periods of several years and others showing declines. It is still the case that some of these diseases reduce in occurrence or even disappear during the winter when temperatures are lower (Smith et al., 2013b). More research is required to fully understand the occurrence, levels of coral mortality and other aspects of the epidemiology of these diseases.

Aims of the Bermuda XL Catlin Deep Ocean Survey

The overall aim of the XL Catlin Deep Ocean Survey is to establish a standardised approach to assessment of ocean biodiversity, including the environmental drivers of patterns of species richness, abundance and biomass and to document human impacts on marine ecosystems including degradation of ecosystem function. As such the survey should provide a contemporary baseline that allows future surveys to measure ecosystem change. Mission 1 is a proof of concept and it is intended to build upon the methods here and to work towards a general acceptance of these approaches amongst the marine biological community. Specifically, for this mission the aims were:

Biodiversity

- To survey the biodiversity of epifaunal communities located in Bermuda, between depths of 15m to 300m.
- To identify the Vulnerable Marine Ecosystems (VMEs) located in Bermuda, including what species they comprise and where they are located.
- To identify the physical and biological drivers of distribution of species and communities on hard substrata in Bermuda.
- To identify the drivers of the biodiversity and abundance of the epibenthic communities of fish and mobile invertebrates in Bermuda.
- To investigate how biogenic-structures influence the biodiversity of benthic communities?
- To investigate the biodiversity and abundance of epifaunal and infaunal communities living on / in soft sediments in Bermuda.
- To estimate the connectivity of benthic communities between Bermuda and the wider Caribbean and between shallow and mesophotic depths in Bermuda (specifically reef-forming corals).
- To assess and document the spatial component of zooplankton communities from 600 m to surface.

Oceanography

- To investigate how the local / regional hydrodynamic regime interacts with seabed topography around the investigated seamounts and island slopes.

Human impacts

- To measure the levels of macronutrients (nitrates, nitrites, phosphate) across the expedition sample sites to identify whether there is evidence of human influence on macronutrient levels (eutrophication)?
- To investigate the distribution of large debris on the slopes of Bermuda and to document any evidence of damage to organisms or other biological effects related to the presence of debris.
- To investigate whether there is evidence of mechanical damage to benthic communities from fishing, dive-related tourism or other human activities.
- Is there evidence that previously impacted benthic communities are recovering?
- To investigate whether conservation and management measures have had a positive effect on the biodiversity, abundance and biomass of marine life.
- To investigate the occurrence of invasive species in Bermuda, specifically lionfish.



Preparing for a sub launch during the Nekton Mission

OVERVIEW OF CRUISE

22 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 19.2536'N 64° 39.4520'W End: 32° 19.2536'N 64° 39.4520'W	01 Spittal	Test of submersible; trial transect, max. depth 121ft.
<i>Nomad:</i>	Start: 32° 19.2536'N 64° 39.4520'W End: 32° 19.2536'N 64° 39.4520'W	01 Spittal	Test of submersible and integration of new equipment, max. depth 182ft.
Tech Divers:	32° 19.2536'N, 64° 39.4526'W	01 Spittal	2 x 60m Transects

23 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 30.148'N 64° 37.245'W	02 North Northeast	Sky News media dive (David Rees)
<i>Nomad:</i>	Start: 32° 30.148'N 64° 37.245'W	02 North Northeast	Sky News media dive (Thomas Moore)
<i>Nemo:</i>	Start: 32° 30.2062'N 64° 37.1064'W End: 32° 29.9540'N 64° 36.8732'W	02 North Northeast	Transect dive
<i>Nomad:</i>	Start: 32° 30.2062'N 64° 37.1064'W End: 32° 29.9540'N 64° 36.9732'W	02 North Northeast	Sampling dive
Tech Divers:	32° 30.1419'N, 64° 37.1641'W	02 North Northeast	4 x 60m transects

24 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 56.725'N, 65° 09.315'W End: 31° 56.3184'N, 65° 09.8863'W	03 Plantagenet Bank	3 x transects at 200m; cameras failed as batteries flat
<i>Nomad:</i>	Start: 31° 56.725'N, 65° 09.315'W End: 31° 56.2425'N, 65° 09.9773'W	03 Plantagenet Bank	Sampling dive
Tech Divers:	31° 55.9338'N, 65° 10.9367'W	03 Plantagenet Bank	2 x 90m transects; algal and rhodolith samples

25 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 31° 56.725'N, 65° 09.315'W End: 31° 56.2106'N, 65° 09.788'W	03 Plantagenet Bank	3 x transects at 300m

<i>Nomad:</i>	Start: 31° 56.725'N, 65° 09.315'W End: 31° 56.2106'N, 65° 09.788'W	03 Plantagenet Bank	Sampling dive
<i>Nemo:</i>	Start: 31° 56.725'N, 65° 09.315'W End: 31° 56.5232'N, 65° 09.4516'W	03 Plantagenet Bank	1 x 150m transect
<i>Nomad:</i>	Start: 31° 56.725'N, 65° 09.315'W End: 31° 56.5232'N, 65° 09.4516'W	03 Plantagenet Bank	Sampling dive
Tech Divers:	31° 56.691'N, 65° 9.4120'W	03 Plantagenet Bank	2 x 90m transects; algal samples; water samples

26 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 18.5905'N, 64° 39.4584'W	01 Spittal	3 x 300m Transects
<i>Nomad:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 18.5905'N, 64° 39.4584'W	01 Spittal	Sampling dive; China Mieville taken down in sub
<i>Nemo:</i>	Start: 32° 19.119'N 64° 39.437'W End: 32° 18.7808'N 64° 39.5398'W	01 Spittal	3 x 200m transects
<i>Nomad:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 18.7808'N, 64° 39.5398'W	01 Spittal	Will West filming
Tech Divers:	32° 19.1378'N, 64° 39.2216'W	01 Spittal	2 x transects at 90m, 30m and 15m

27 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 19.119'N, 64° 39.437'W	01 Spittal	UNESCO Event
<i>Nomad:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 19.119'N, 64° 39.437'W	01 Spittal	UNESCO Event
Tech Divers:	32° 19.1694'N, 64° 39.48'W	01 Spittal	2 x transects at 90m, 30m and 15m

28 July 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 29.021'N 64° 35.637'W End: 32° 28.9833'N 64° 34.8106'W	02 North Northeast	3 x 300m transects
<i>Nomad:</i>	Start: 32° 29.021'N 64° 35.637'W End: 32° 28.9833'N 64° 34.8106'W	02 North Northeast	Dive with Jim Clash
<i>Nemo:</i>	Start: 32° 29.021'N 64° 35.637'W End: 32° 28.5089'N 64° 34.5405'W	02: North Northeast	3 x 200m SVS - one camera failed

Nomad: Start: 32° 29.021'N 64° 35.637'W
End: 32° 28.5089'N 64° 34.5405'W

02: North Northeast Sampling dive

Tech Divers: 32° 28.7959'N, 64° 35.6882'W

02: North Northeast 2 x 90m transects and 3 x 20m transects

29 July 2016

Sub / Divers Position

Site

Activity

Nemo: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.5873'N, 64° 34.5926'W

02: North Northeast 3 x 250m transects

Nomad: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.5873'N, 64° 34.5926'W

02: North Northeast

Afternoon submersible diving cancelled because of bad weather.

Tech Divers: 32° 28.982'N, 64° 35.269'W

02: North Northeast 2 x 90m transects; water samples, algal samples

Tech Divers:

02: North Northeast Recover BEAMS

30 July 2016

Sub / Divers Position

Site

Activity

All day submersible diving cancelled because of bad weather.

Tech Divers: 32° 27.624'N, 64° 36.669'W

02: North Northeast 3 x 20m transects; collected samples of water, coral, algae

31 July 2016

Sub / Divers Position

Site

Activity

Nemo: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.5062'N, 64° 34.3493'W

02: North Northeast 3 x 200m transects; SVS cameras flooded

Nomad: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.3768'N, 64° 34.4715'W

02: North Northeast Sampling dive

Nemo: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.2025'N, 64° 34.3748'W

02 North Northeast 3 x 200m transects; SVS cameras lost on recovery

Nomad: Start: 32° 29.021'N, 64° 35.637'W
End: 32° 28.3472'N, 64° 34.5343'W

02 North Northeast Sampling dive

Tech Divers: 32° 28.9675'N, 64° 35.0719'W

02 North Northeast 2 x 90m transects; sampled algae and coral

1 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 11.168'N 64° 58.360'W End: 32° 11.1776'N 64° 58.5697'W	04 Tiger	3 x 200m and 3 x 150m transects; SVS camera flooded
<i>Nomad:</i>	Start: 32° 11.168'N 64° 58.360'W End: 32° 10.9029'N 64° 58.5663'W	04 Tiger	Sampling dive
Tech Divers:	32° 11.5520'N, 64° 58.1105'W	04 Tiger	4 x 60m Transects; collected water and rock samples

2 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives

<i>Nemo:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives
<i>Nomad:</i>	Start: 32° 11.168'N, 64° 58.360'W End: 32° 11.168'N, 64° 58.360'W	05 Wreck	XL Catlin Guest Dives

Guests: Tom Booth, Paul Brand, Elliot Bundy, Stephen Catlin, Krista Doran, Graham Everard, Greg Hendrick, Myron Hendry, Kelly Lyles, Mike Maran, Mike McGavick, Pete Porrino, Chris Reeves, Paul Ritchie, John Smart, Annie Sousa

Tech divers:	32° 11.168'N, 64° 58.360'W	05 Wreck	6 transects (5 on wreck and one on sand)
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3 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 19.1236'N 64° 39.4992'W	01 Spittal	Sampling dive as SVS cameras not arrived
<i>Nomad:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 19.1236'N 64° 39.4992'W	01 Spittal	Sampling dive
<i>Nemo:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 18.8812'N, 64° 39.6716'W	01 Spittal	Sampling dive as SVS cameras not arrived
<i>Nomad:</i>	Start: 32° 19.119'N, 64° 39.437'W End: 32° 18.8812'N, 64° 39.6716'W	01 Spittal	Sampling dive; Justin Marozzi, BBC.
Tech Divers:	32° 19.198'N, 64° 39.404'W	01 Spittal	3 x 60m transects; water samples, algal samples, rhodoliths

4 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo:</i>	Start: 32° 19.052'N 64° 39.547'W End: 32° 18.6680'N 64° 39.6495'W	01 Spittal	3 x 150m transects; camera not switched on in housing
<i>Nomad:</i>	Start: 32° 19.052'N 64° 39.547'W End: 32° 18.6680'N 64° 39.6495'W	01 Spittal	Sampling dive
<i>Nemo:</i>	Start: 32° 19.052'N 64° 39.547'W End: 32° 18.6938'N 64° 39.5715'W	01 Spittal	3 x 250m transects and 1 x 150m transect
<i>Nomad:</i>	Start: 32° 19.052'N 64° 39.547'W End: 32° 18.6938'N 64° 39.5715'W	01 Spittal	Sampling dive; Oliver Millman, Guardian
Tech Divers:	32° 29.003'N, 64° 35.404'W	02 North Northeast	3 x 90m transects; sampled red algae and sponges
SCUBA Divers:		01: Spittal	Deploy BEAMS

5 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo</i> :	Start: 32° 11.168'N 64° 58.360'W End: 32° 11.2181'N 64° 58.1657'W	04 Tiger	3 x 250m transects
<i>Nomad</i> :	Start: 32° 11.168'N 64° 58.360'W End: 32° 11.2181'N 64° 58.1657'W	04 Tiger	Sampling dive; Nsika Akpan, PBS - interview at 250m.
Tech Divers:	32° 11.613'N 64° 58.039'W	04 Tiger	2 x 90m transects, 2 x 30m transects, 2 x 15m transects; water samples

6 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo</i> :	32° 28.5917'N, 64° 34.4616'W	02: North Northeast	3 x 200m transects
<i>Nomad</i> :	32° 28.5917'N, 64° 34.4616'W	02: North Northeast	Sampling dive
Single dive as a result of poor weather.			
Tech divers:		Nonsuch Island	SVS Calibration

7 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo</i> :	32° 18.9396'N, 64° 39.4059'W	01: Spittal	3 x 150m transects
<i>Nomad</i> :	32° 18.9396'N, 64° 39.4059'W	01: Spittal	Sampling dive
<i>Nemo</i> :	32° 19.1156'N, 64° 39.3894'W	01: Spittal	Sampling dive
<i>Nomad</i> :	32° 19.1156'N, 64° 39.3894'W	01: Spittal	Sampling dive
Tech Divers:	32° 12.214'N, 64° 56.944'W	04 Tiger	2 x 90m transects, 2 x 30m transects, 2 x 15m transects; water, coral and sponge samples

8 August 2016

Sub / Divers	Position	Site	Activity
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All day submersible diving and tech diving cancelled because of bad weather.

9 August 2016

Sub / Divers	Position	Site	Activity
<i>Nemo</i> :	32° 18.9336'N, 64° 39.5909'W	01 Spittal	Test of radio for Sirius event; sampling (Rod Roddenberry)

<i>Nomad:</i>	32° 18.8409'N, 64° 39.5400'W	01 Spittal	Test of radio for Sirius event; sampling (Jen Hegarty)
<i>Nemo:</i>	32° 18.8760'N, 64° 39.4775'W	01 Spittal	Radio Sirius broadcast (John Fugelsang)
<i>Nomad:</i>	32° 18.9688'N, 64° 39.3220'W	01 Spittal	Radio Sirius broadcast
Tech Divers:	32° 04.392'N, 65° 01.068'W	06 Challenger Bank	2 x 90m transects; water samples, coral samples, sponge samples, algal samples. One SVS camera failed as card not present
SCUBA Divers:		Spittal North	BEAMS deployment; coral and algal collections

10 August 2016

Sub / Divers	Position	Site	Activity
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All day submersible diving and tech diving cancelled because of bad weather.

Tech Divers:		Aquarium	SVS Calibration
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11 August 2016

Sub / Divers	Position	Site	Activity
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All day submersible diving and tech diving cancelled because of bad weather.

Tech Divers:	32° 19.234'N, 64° 39.538'W	01 Spittal	Sampling of rhodoliths, algae and coral
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12 August 2016

Sub / Divers	Position	Site	Activity
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<i>Nemo:</i>	32° 10.8554'N, 64° 58.6122'W	04 Tiger	3 x 300m transects, 1 x 200m transect
<i>Nomad:</i>	32° 10.8554'N, 64° 58.6122'W	04 Tiger	Sampling dive
<i>Nemo:</i>	32° 10.8118'N, 64° 58.8748'W	04 Tiger	2 x 200m transects and 2 x 150m transects
<i>Nomad:</i>	32° 10.8118'N, 64° 58.8748'W	04 Tiger	Sampling dive
Tech Divers:	31° 56.994'N, 65° 10.657'W	03 Plantagenet Bank	4 x 60m transects; sampling of water, corals, sponges and algae

13 August 2016

Sub / Divers	Position	Site	Activity
Nemo:	31° 56.5463'N, 65° 09.2938'W	03 Plantagenet Bank	3 x 250m transects and 1 x 200m transect
Nomad:	31° 56.5463'N, 65° 09.2938'W	03 Plantagenet Bank	Sampling dive
Nemo:	31° 56.5463'N, 65° 09.2938'W	03 Plantagenet Bank	2 x 200m transects and 2 x 150m transects
Nomad:	31° 56.5463'N, 65° 09.2938'W	03 Plantagenet Bank	Sampling dive
SCUBA Divers:		04 Tiger	Recover BEAMS

INITIAL SCIENTIFIC REPORTS

Submersible video survey & sample collection methods

Submersibles

Two submersibles, *Nemo* and *Nomad*, were available for the survey, both *Triton 1000-2s* (Fig. 1). Submersibles were stern-launched via an A-Frame with one having to be placed in the water prior to the other being moved to launch position via crane. Submersible surveys were targeted at 150m, 200m, 250m and 300m depth, the maximum depth range of the submersibles. *Nemo* was equipped primarily for video survey whilst *Nomad* (Fig. 1) was equipped for water sampling and for high-resolution filming of operations and marine life.



Figure 1. *Triton 1000-2 Nomad* on the stern deck of the *Baseline Explorer*. The lifting harness attaching the submersible to the A-Frame and winch is shown. The Teledyne Pan and Tilt Camera is visible on the starboard nacelle and the Niskin Bottles on the port.

Nemo

- Four forward facing Deep-Sea Matrix-1 LED Lights (Fig. 2)
- Stereo video system (SVS; Fig. 3) - two GoPro Hero 4 Cameras with ABPAK-401 Extended Batteries in Scout Pro Go Benthic 2 1000m depth-rated housings (Group Binc, Jensen Beach, Florida, USA). Cameras mounted on lower starboard nacelle on aluminium bar, pointed in at an angle of 8° and spaced 80cm apart.
- Hydrolek 6-Function Manipulator Model HLK-CRA6 (Fig. 4)

- Sampling Basket (Fig. 4)
- Downward-pointing camera (Fig. 5) - one GoPro Hero 4 Camera with ABPAK-401 Extended Battery in Scout Pro Go Benthic 2 1000m depth-rated housing (Group Binc, Jensen Beach, Florida, USA) with Teledyne-Bowtech Ocean Lasers (Green) spaced at 25cm (9.75 inches)



Figure 2. Matrix-1 LED lights on the starboard nacelle of Nemo. The lights are adjustable so that position can be changed to suit underwater illumination requirements.



Figure 3. Mounting bar and SVS Go Pro cameras visible beneath the starboard nacelle of *Nemo*.



Figure 4. Hydrolek 6-Function Manipulator and sample basket on the front of *Nemo*.

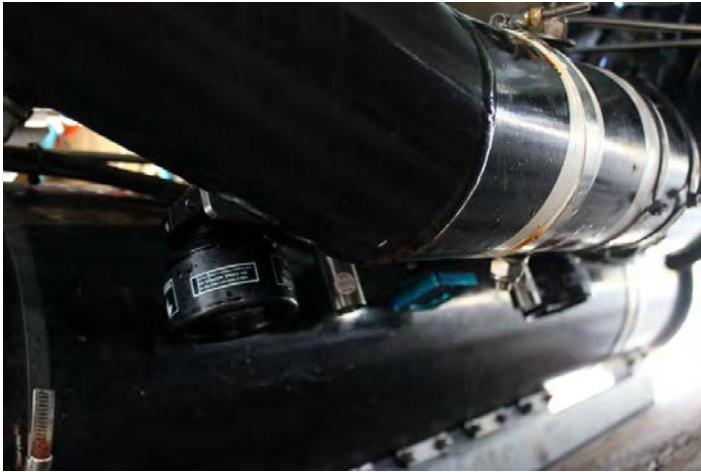


Figure 5. Downward-pointing Go Pro Camera, lasers and lights mounted on the underside, starboard side of *Nemo*.

Nomad

- 500ml Niskin bottles General Oceanics Inc. (Miami, Florida, USA)
- CTD Seabird Electronics Model 49, 1000m-depth rated (Bellevue, Washington, USA) Teledyne Bowtech HD Pro Camera on Sidius Solutions Pan and Tilt with Teledyne-Bowtech Ocean Lasers (Green) spaced at 25cm (9.75 inches; Fig. 6).
- Hydrolek 6-Function Manipulator Model HLK-CRA6
- Sampling basket
- Four forward facing Deep-Sea Matrix-1 LED Lights



Figure 6. Bowtech Teledyne HD Pro Pan and Tilt camera system mounted on upper starboard nacelle of *Nomad*.



Figure 7. General Oceanics Niskin bottle sampling system and Seabird CTD mounted on upper port nacelle of *Nomad*.

Submersible position was estimated from the *Baseline Explorer* using a Linkquest 1500 LC USBL system (Fig. 8). Underwater communications were achieved through 25KHz bandwidth through-water communications systems. Depth in the submersibles was measured via PneumoCal Pneumo Gauges which are calibrated annually in feet of seawater. Electronic transducers were used for estimating depth with calibration from the Pneumo Gauges.



Figure 8. USBL system mounted on the rear of the sphere on Nemo

A total of 77 submersible dives were conducted during the Bermuda mission. Dive objectives primarily focused on the execution of benthic and Stereo Video System (SVS) transect surveys similar in scope to those conducted by the divers. The specific protocols were adapted, however, to conform to the limitations of working with the equipment from inside the submersibles (see below). A total of 65 transects were conducted in which SVS and benthic surveys were performed across a total distance of over 18 km.

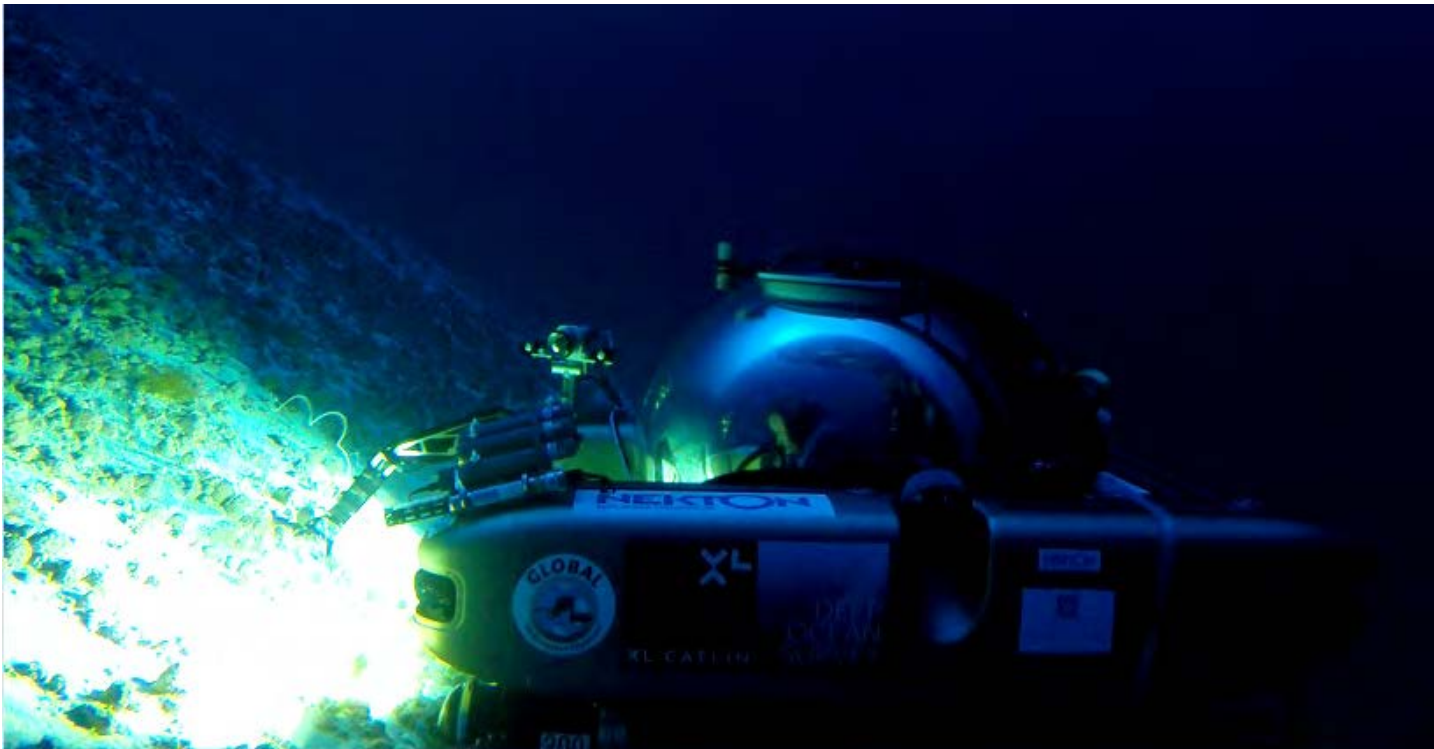
Table 1 Summary of transect surveys performed by submersibles: Number and distance at different depth ranges

Station name	Total		120-150m		180-210m		210-240m		240-270m		270-300m	
	No.	Dis (m)	No.	Dis (m)	No.	Dis (m)	No.	Dis (m)	No.	Dis (m)	No.	Dis (m)
North-Northeast	21	5,774	3	1,180	12	3,447	0	0	3	466	3	682
Spittal	16	5,732	7	2,597	3	967	0	0	3	1,326	3	842
Tiger	14	3,154	6	1,573	5	1,123	3	458	0	0	0	0
Plantagenet	14	3,724	2	198	6	2,136	0	0	3	808	3	582
Total	65	18,384	18	5,549	26	7,672	3	458	9	2,599	9	2,105

In addition to the surveys, sampling for coral, sponges, algae, and water was conducted on several of the dives. Beyond the scientific objectives, dives were also conducted to introduce media and marine policy personnel as well as project sponsors to the marine environment. Table 2 provides a summary of the submersible dives conducted by overall mission objective. Table 3 and Appendix 1 list the organizations and the observers carried by the submersibles and the number of dives and hours conducted by each one.

Table 2. Number of submersible dives conducted by mission objective.

Dive Mission	No. Dives	Duration (hours)	Minimum depth (m)	Maximum depth (m)
Orientation	2	2.8	45	55
Science	32	73.1	37	305
Media	15	33.3	61	305
Exploration	6	13.9	152	304
Policy	2	4.4	159	159
VIP-Promotional	18	10.9	15	248
Training	2	1.6	10	110



Gathering samples for the XL Catlin Survey

Table 3. List of organisations for which representatives were carried during submersible dives and statistics for the dives they conducted.

Observer organisation	No. Dives	Duration (hours)	Minimum depth (m)	Maximum depth (m)
BBC	1	1.5	303	303
Bermuda Aquarium	3	9.2	218	305
Forbes Magazine	1	2.8	305	305
Guardian	1	2.1	152	152
Nekton	11	24.9	10	304
NOAA-NURP	1	1.8	154	154
Univ. Oxford	21	47.0	37	305
PBS News Hour	1	2.8	244	244
Rockefeller Centre	1	1.2	158	158
Rodenberry Entertainment	1	0.9	158	158
Sky News	2	2.6	61	62
Stanford University	1	2.5	199	199
Telegraph	1	0.7	152	152
Trinity College	1	1.6	201	201
Undersea Research	1	2.7	249	249
UNESCO	2	4.4	159	159
Univ. Puerto Rico	1	1.7	167	167
Univ Rhode Is.	2	4.4	158	202
XL Catlin	16	7.9	15	15

Video survey methods

Stereo video surveys (SVS) were undertaken by setting the submersible close to the seabed (4-6 feet) and then moving forward slowly (walking pace) trying to maintain a constant altitude. Most surveys were undertaken on slopes, sometimes near vertical and with erratic rock outcroppings and gulleys. Whilst moving around ridges an attempt was made to maintain a constant depth and altitude although this was not always possible. It was essential to maintain a close distance to the seabed during surveys to ensure

sufficient illumination of the seabed and fish for survey purposes. Replicates of three transects were undertaken during each dive at the survey depth. Each replicate comprised a 20 minute transect (estimated to be 100m distance at 0.2 knots). A 5-minute gap during which the submersible traversed the seabed was used to separate individual depth replicate transects.

The method was significantly limited by the battery life of the GoPro cameras which had to be placed in the housings and switched on whilst the submersible was on deck. For this reason, *Nemo* was usually the second submersible to be launched to maximise survey time. Issues were also encountered with placing the GoPro Cameras into the housings as it was possible to inadvertently switch off the camera during placement. Over tightening of the camera housings also resulted in the eventual impairment of the camera sealing resulting in flooding. Consultation with the manufacturers indicated that sufficient tightening to only seal the O-Ring in the front facing of the camera (visible as a solid black line) was required. Snagging of the Snag Lines with the SVS system during recovery resulted in ripping the entire mounting from the submersible and loss of one of the SVS systems complete with cameras, housings and bar.

Go Pro camera settings were as follows according to SeaGIS protocols:

- Set the camera date and time.
- Turn off the blinking LED (this can cause an annoying reflection off the housing port).
- If using a stereo configuration, the following are recommended:
 - Both cameras should have the same settings
 - 1920 x 1080 video format
 - Medium field of view
 - Frame rate: Initially 60 frames per second was used but in order to conserve batteries this was dropped to 30 frames per second.

It was not possible to use exactly the same camera in the left or right housing for the SVS reducing the accuracy of the calibration. For calibration purposes it was necessary to construct the 1,000mm x 1,000mm calibration cube from SeaGIS.

1,000 x 1,000 mm Calibration Cube Assembly

1. Assemble the faces

Note: protect the targets when assembling the cube - do not rub or scratch them.

Assemble the cube in faces first. On the backside of the angle pieces is scribed a number. All pieces in the first face are labelled 100 through to 103, and the second face 200 through to 203. Arrange the pieces so all common numbers adjoin (see diagram below for a rear view of the front face).

Assemble the faces using the *short* countersunk screws with a washer and nylon locking nut in the locations marked with an 'x' in the diagram on the next page.

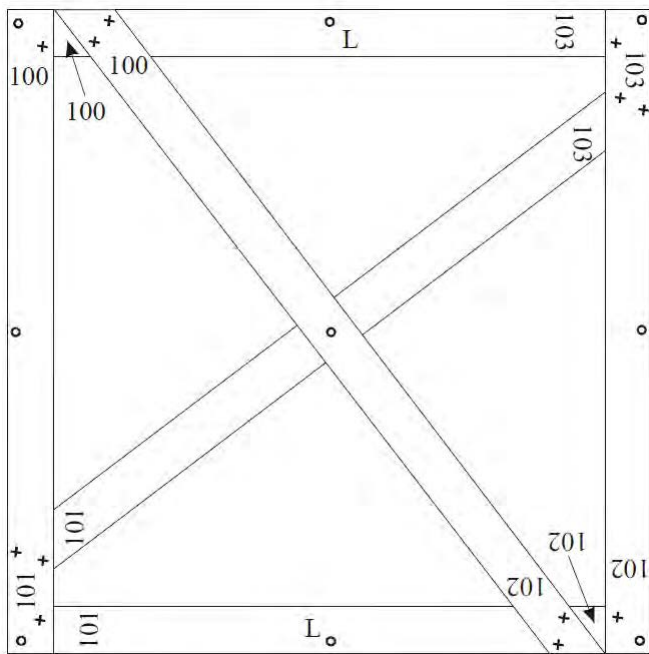


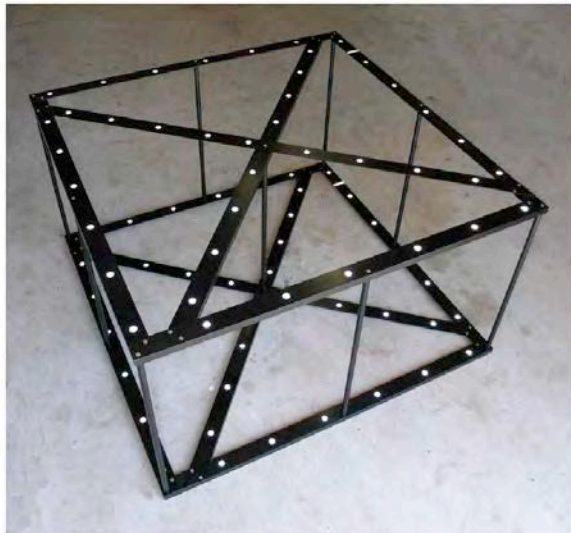
Figure 9. Calibration Cube. M6 x 16 Countersunk socket head screw with washer and locking nut M6 x 25 Countersunk socket head screw into 20mm rod (separating faces); long leg (marked 'L').

2. Connect the faces

Connect the faces to the round legs. There are 9 legs in total attaching at the places marked 'O' in the above diagram. Note that two legs are longer. The longer legs and their attachment locations on the faces are scribed with an 'L'.



Assembly detail



Assembled cube

(Note, not your actual cube, but identical design)

Figure 10. Assembly detail and fully constructed calibration cube.

When assembling the cube, apply a lanoline-based grease to all threads. This will prevent the stainless steel screws binding to the aluminium thread, making it easy to disassemble the cube after use in salt water.

Point numbering

Resection points (front face of cube)

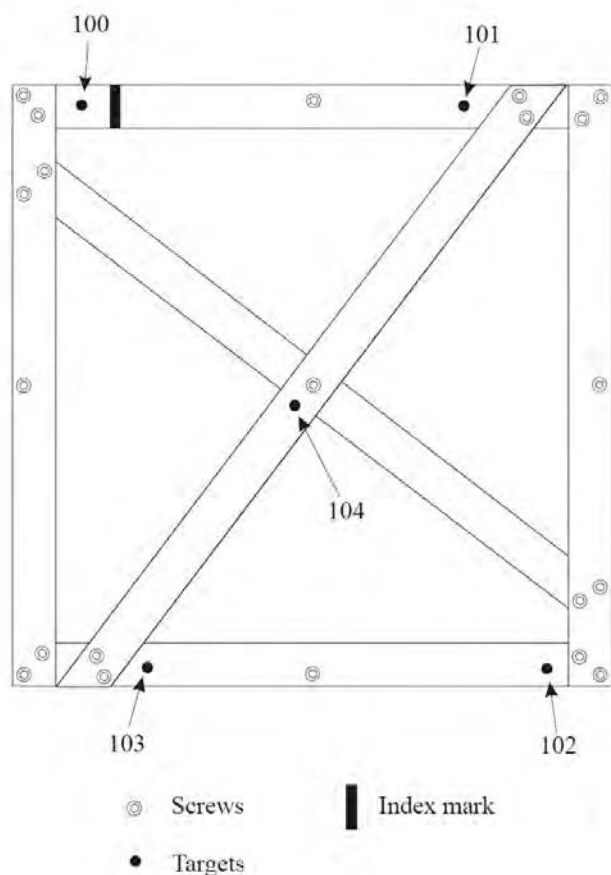


Figure 11. Point numbering for cube assembly.

During surveys it was noted that many fish would swim away from the submersible as it approached, or remain outside of the lighting and swim to the side of the submersible (e.g. some snappers). Other fish might be attracted to the lights of the submersible to feed on smaller species (e.g. saddle bass, *Serranus cf notospilus*, feeding on *Pronotogrammus cf martinicensis*). Occasionally pelagic species such as *Seriola rivolana* or *Caranx lugubris* would swim down to the submersibles from shallow water, 100 feet or more above the depth of transect.

The SVS camera calibration procedure was followed as below:

1. A well-lit location/time (mid-day, shallow water) was chosen.
2. This was a controlled / clear water environment (ideally a pool) preferably a shallow location (~60 feet or less) in clear water with a firm sand bottom. NB - make sure that the white dots on the calibration cube don't get blocked by sand or not visible from sand being stirred up. This proved to be difficult in Bermuda as deeper localities were poorly lit and shallower-water localities suffered from reduced visibility.
3. The calibration cube was positioned directly in front of the SVS camera system on the bottom, such that the square profile was presented to the camera.
4. The distance between the SVS camera and the cube was measured. The cameras were synchronised by using a hand held dive torch and swimming such that the light from the torch was recorded by both cameras. The torch was turned on and off 5 times. This allowed the videos to be lined up to the exact frame based on a frame that has the torch with the light off followed by a frame that has it on.

There was no need to hold the cube in each position for a specific time during calibration. This is because when the calibration is run on the SeaGIS software a frame from the video with the cube in each position is pulled out. Care was taken to ensure that as the cube was held in each position hands did not block any of the white dots on the cube. The cube was positioned at roughly 45 degrees as the cube was angled in each position.

5. Rotation procedure (15 seconds per set point / orientations are relative to the SVS camera view)

- a. Square profile directly in front
 - i. Rotate 30 degrees back
 - ii. Rotate 30 degrees front
 - iii. Return to square - rotate 30 degrees left
 - iv. Return to square - rotate 30 degrees right
- b. Return to square - rotate 90 degrees left to next square profile
 - i. Rotate 30 degrees back
 - ii. Rotate 30 degrees front
 - iii. Return to square - rotate 30 degrees left
 - iv. Return to square - rotate 30 degrees right
- c. Return to square - rotate 90 degrees left to next square profile
 - i. Rotate 30 degrees back
 - ii. Rotate 30 degrees front
 - iii. Return to square - rotate 30 degrees left
 - iv. Return to square - rotate 30 degrees right
- d. Return to square - rotate 90 degrees left to next square profile
 - i. Rotate 30 degrees back
 - ii. Rotate 30 degrees front
 - iii. Return to square - rotate 30 degrees left
 - iv. Return to square - rotate 30 degrees right
- e. Remove cube from field of view

There are no fixed positions that the bar needs to be held in. The idea is that screen grabs of the cube are taken at lots of different angles to check the validity of the calibration.

- f. One diver brings the bar out to same position as the cube was situated in a horizontal position with black ends and white/reflective table (bars) facing cameras
 - i. Hold horizontal for 15 seconds
 - ii. Rotate 45 degrees up and left - hold for 5 seconds
 - iii. In rotated position, rotate forward 45 degrees - hold for 5 seconds
 - iv. In rotated position, rotate bar through horizontal by 180 degrees - hold for 5 seconds
 - v. Rotate bar to vertical position - orthogonal to the ground - hold for 5 seconds
 - vi. Rotate 45 degrees forward - hold for 5 seconds
 - vii. In rotated position, rotate right 45 degrees - hold for 5 seconds
 - viii. In same rotated position, lift the back end up such that the bar is oriented downward on a 45 degree plane - hold for 5 seconds
 - ix. Rotate bar to vertical position, orthogonal to floor and then rotate 45 degrees left - hold for 5 seconds
- g. Complete

Sampling of Fauna

Sampling was undertaken using the Hydrolek 6-Function Manipulator. Ideally specimens growing on rhodoliths were taken as these were easier to place in the sample basket and also the weight of the concretion held the sample in the basket during recovery. In some cases, corals and sponges were collected by holding the manipulator out and piloting the submersible onto the target specimen. Specimens had to be held with the manipulator to prevent them being lost from the basket. Specimens were photographed, measured and preserved as soon as they came aboard. Preservation were either with RNA later, ethanol or formalin depending on the taxa and why they were collected. All details can be found in the sample record book and spreadsheets.

Submersible Initial Results and Further Work

During the cruise 50 SVS transects and 59 benthic transects were undertaken four locations around Bermuda: North Northeast, Spittal, Tiger and Plantagenet by submersible (Fig. 12; Tables 4 and 5).

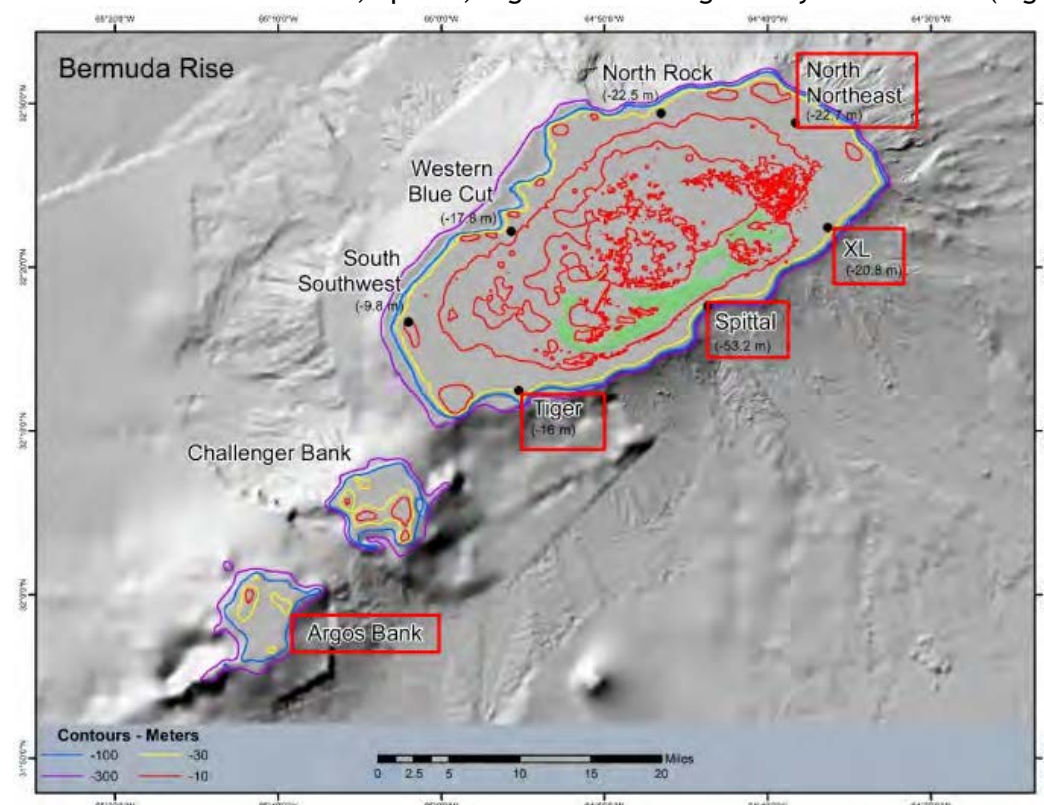


Figure 12. Bathymetric map of Bermuda including Plantagenet Bank. Sampling sites for both submersible and diver surveys are shown outlined in red.

Table 4. Number of SVS transects per site at isobaths obtained by the submersible *Nemo*.

Site / Depth	<150m	150m	200m	250m	300m	Total per site
Spittal	2	4	3	3	3	15
North Northeast	0	0	3	3	3	9
Tiger	1	1	3	3	3	11
Plantagenet	0	6	5	3	3	17
Total per depth	3	11	14	12	12	-
Grand Total	-	-	-	-	-	52

Table 5. Number of benthic transects per site at isobaths obtained by the submersible *Nemo*.

Site / Depth	<150m	150m	200m	250m	300m	Total per site
Spittal	0	7	3	3	3	16
North Northeast	0	0	12	3	3	18
Tiger	1	5	4	2	0	12
Plantagenet	0	6	4	3	3	16
Total per depth	1	18	23	11	9	-
Grand Total	-	-	-	-	-	62

The main reason for loss of survey time during the Mission was bad weather and issues relating to the SVS GoPro system (i.e. flooding, batteries running out, cameras not switched on). Several dives lost one of the SVS cameras meaning that additional video is available for abundance counts of fish only (Tiger 3 x 200m and 3 x 150m; Spittal 3 x 150m North Northeast 3 x 200m).

The deep-water environments of the Bermuda Platform and associated seamounts generally comprised steep submarine slopes in places near vertical, or even overhanging, to depths of 150m or more followed by slopes of a lower gradient comprising eroded limestone outcroppings and broken rocks draped with slides of coarse sediment (usually very shallow) and eroded rhodoliths. Currents were often noticeable in the submersible including both upwelling and down-welling currents, sometimes during the same dive and within a short time period of each other (~15 minutes). Plantagenet Bank was particularly exposed to currents close to the seabed. Such conditions were likely favourable for coral growth bringing a plentiful supply of food and also reducing sediment cover on the seabed.

Benthic surveys revealed that communities changed rapidly with depth. The 150m depth station was dominated by coral garden habitat dominated by a variety of species of black corals including cf *Antipathes atlantica*, *Antipathes furcata*, *Tanacetipathes hirta*, *Tanacetipathes tanacetum* and *Cirripathes lutkeni*. *Ellisella barbadensis* and cf *Ellisella grandis* were also present but more common

towards the drop off from the Bermuda platform. These corals occurred either on rhodoliths or on outcropping limestone rock. Rhodoliths were heavily colonised by calcareous encrusting red algae and sponges.

This community gave way to one much more dominated by large twisted wire coral (2m or more in length), octocorals and stylasterids. Corals were noticeably more-dense on the ridges formed by limestone outcrops particularly between 200-300m depth. Octocorals included a large *Narella* species, whilst very large twisted wire corals (*Cirripathes* sp.) were also present. Large ophiuroids were frequently observed twisted in the branches of the octocorals, including *Astreopora*. At some localities the soft coral cf *Neospongodes portoricensis* was also observed frequently but never in large numbers. Large megabenthic sponge colonies were infrequently observed during submersible surveys. An irregular bright yellow sponge, forming low-hummocks was seen particularly at depths between 200 and 300m. This species was associated with a tube-forming polychaete worm. At Plantagenet Bank a white sponge forming irregular colonies with thick finger-like projections was also seen. Elsewhere, the same or another species formed bracket-like growths mainly on the apex of ridges. In the deepest surveys a large urchin with curved fine spines was observed, sometimes moving rapidly on the seabed. A large pink-orange seastar with darker orange / red markings was also observed on the seabed in these areas. Less commonly a small dark seastar was also seen.

The fish community at 150m and deeper was dominated by a fish with a pale pink body and yellow dorsal fin and tail. This species resembles *Pronotothorax martinicensis* but does not follow the colour pattern seen in this species elsewhere. We note that the species is variable in colour and may be a species complex and / or the Bermuda specimens may represent a subspecies or endemic species in this group. The species was particularly associated with areas of cryptic habitat such as limestone ridges where crevices or holes were present or on the floors of gulleys or ravines. Usually a few larger (adult?) fish were observed with large numbers (hundreds) of juveniles.

Several predators were typically associated with large numbers of juvenile *Protonotothorax* cf *martinicensis*, especially the saddle bass, *Serranus* cf *notospilus* and also the invasive lionfish *Pterois volitans*. The moray eel, *Gymnothorax madeirensis* was also frequently observed foraging at depths between 200-300m. A butterfly fish characterised by three vertical stripes was also frequently observed at depths from 200-300m. The closest species to this is *Prognathodes guyanensis*, but the Bermudan specimens appear to lack the white borders to the black stripes and are a deeper yellow. *P. guyanensis* inhabits deep waters from 100 - 230m depth and so occurs in a similar habitat to the Bermudan butterfly fish. The long-snouted scorpion fish *Pontinus castor* was also observed on dives at 200m - 300m, usually stationary lying on rock outcrops.

The intention is to analyse all SVS data for fish using Event Measure software to identify, count and estimate the length of the fish observed. Fish occurrence will be compared with other studies on Caribbean shallow-water and mesophotic coral reef ecosystems. Analyses of the factors influencing fish occurrence will be undertaken including depth, habitat, temperature, nutrients etc. This will include consideration of species richness, abundance and biomass. Studies will be cross-referenced with those undertaken by Gretchen Goodbody-Gringley, Bermuda Institute of Oceanographic Sciences.

Benthic communities will be analysed using point-intercept methods to estimate the abundance / cover of key megabenthic species. Identification will be assisted through the use of simultaneously filmed SVS cameras and through identification of specimens of key species collected using the Triton submersibles. Analysis of the drivers of patterns of species richness and abundance will be undertaken versus depth, temperature and other physical factors as available.

DIVER OPERATIONS

Todd Kincaid, Director Project Baseline, Vice-President Global Underwater Explorers

Introduction

Global Underwater Explorers (GUE) sent a team of nine divers, including eight volunteers, to Bermuda in 2016 to support the Mission. The objective of the collaboration was to document the health of the marine environment in support of the *XL Catlin Deep Ocean Survey*. The Mission ran for 27 consecutive days from July 18 to August 14, 2016 during which time the GUE team were supported by the *Baseline Explorer* and dived in conjunction with two 2-person 1000-foot submersibles to document fish populations and benthic conditions along a series of transects at seven general depth levels at five locations around the islands. The target locations included three reefs located along the southwest, southeast and northeast sides of the island and two of the adjacent seamounts, Challenger and Plantagenet Banks (Figure 12). This report provides a summary of the diving activities conducted by the GUE divers and the submersibles during the mission.

Dive Team Activities

Objectives

The primary objective for the GUE dives was to conduct a series of transect surveys at 90m, 60m, 30m, and 15m at each of the five sites. Two types of transects were performed at each depth and at each location. One was a traditional benthic survey in which a video camera and light were oriented perpendicular to the bottom and slowly carried along a 50m survey tape to record the diversity and density of life on the seafloor (benthos). The other was a stereo video survey (SVS) in which two cameras, approximately 1m apart and oriented parallel to the seafloor, were carried along the same 50m line about 1.5m off the bottom following a protocol described by Andradi-Brown (2016). In addition to these transect surveys, the divers also collected a variety of samples and numerical data to support the development of the standardized framework including water, coral, algae, and sponge samples from deep and shallow portions of each site, as well as conductivity, temperature, depth, pH, oxygen, and oxidation-reduction potential measurements at 15-second intervals throughout the water column.

Methods

The divers used the “JJCCR” closed circuit rebreather specifically modified to conform to GUE’s standards. Dilluent gas was mixed to an appropriate trimix (11% oxygen, 75% helium, balance nitrogen) for the maximum depth of the mission dives such that it also served as the primary bailout gas. Each diver also carried sufficient decompression bailout gas to ensure that they could safely ascend to 70 feet, where they were able to receive additional gas from a surface support team. For the dives to 90m, this consisted of one 10L cylinder of trimix (21% oxygen, 35% helium, balance nitrogen) and one 5L cylinder of nitrox (50% oxygen, 50% nitrogen).

Dive team make-up typically involved three divers. One diver operated the SVS and benthic transect cameras. One diver assisted by laying out and reeling up the measuring tape, and the third diver carried a floating tracking device and documented the team activities with still and video cameras. In some cases, the team only consisted of two divers, in which case the assisting diver was required to carry the tracking device. In other cases, the team consisted of four divers, in which case the forth diver was dedicated to video and still media collection.

Dive profiles were generally designed to result in total dive times for the team of approximately 5 hours. Each diver carried a diver propulsion vehicle (DPV) and used the DPVs to navigate to specific target sites at varying depths on each dive. Whenever possible, the divers navigated from the deepest target depth into shallow water during their decompression periods such that additional surveys and sample collection could be conducted on each dive. At the Tiger and Spittal dive sites in particular, the divers were able to navigate from 90m up to 6m following the bottom using the DPVs thereby enabling 90m, 30m, and 15m transects to be conducted on each dive. Generally speaking, the 60m transects were conducted on a dedicated dive.

A surface support team of at least two divers followed the dive team at all times by way of the floating tracking buoy carried by the dive team. The support boat was equipped with two additional 10L cylinders of the trimix decompression gas, two additional 10L cylinders of the nitrox decompression gas, and two 10L cylinders of oxygen.

Results

Thirty-two mission dives were conducted. Twenty-eight of those dives were conducted solely by the GUE divers. Four dives were done with two to three divers from the Nekton team. A total of 313.6 person-hours were logged underwater during the Bermuda mission. Table 6 provides a summary of the dives performed by each of the twelve participating divers.

Table 6. Summary of dives undertaken during the Bermuda mission.

Name	Group	Number of Dives			Mins	Total Dive Time	
		Total	>=300ft	>=200ft		Hrs	Hrs/Dive
Todd Kincaid	GUE	16	7	9	3,302	55.0	3.4
Martin McClellan	GUE	10	6	8	2,525	42.1	4.2
Susan Bird	GUE	10	4	6	2,074	34.6	3.5
Meredith Tanguay	GUE	9	5	7	2,378	39.6	4.4
Kevin Dow	GUE	6	4	5	1,651	27.5	4.6
Graham Blackmore	GUE	8	4	5	1,731	28.9	3.6
JP Bressor	GUE	3	2	2	708	11.8	3.9
Kyungsoo Kim	GUE	8	4	5	1,957	32.6	4.1
SuEun Kim	GUE	6	2	3	1,353	22.6	3.8
Heidi Hirsh	Stanford	7	0	0	463	7.7	1.1
Catherine Head	Oxford	5	0	0	363	6.1	1.2
Melissa Price	Nekton	5	0	0	310	5.2	1.0
Totals		93	38	50	18,815	313.6	3.4

Seventeen of the GUE dives were conducted in which the primary purpose was to conduct SVS and benthic transects surveys. A total of 73 benthic and 67 SVS transect surveys were conducted. Sampling for coral, sponges, algae, and or water was performed on seventeen dives as well, though not necessarily the same dives as those when transects were conducted. In addition to the survey dives, GUE divers also supported scientific dives conducted by some of the collaborating scientists and dives conducted for the specific purpose of filming the submersible operations. The scientific dives were primarily conducted to deploy and retrieve a monitoring station called the BEAMS (*Benthic Ecosystem Acidification Monitoring System*), which delivered important data on how the process of ocean acidification is affecting coral health in Bermuda. Tables 7 and 8 provide a summary of the dive team results.

Table 7. Number of SVS and benthic video transect surveys conducted at each dive site by depth,

Dive site	Total Dives	SVS Transects					Benthic Transects				
		90m	60m	30m	15m	Total	90m	60m	30m	15m	Total
North-Northeast	6	8	4	2	3	17	8	4	2	4	18
Spittal	7	6	4	4	6	20	6	4	6	6	22
Tiger	3	2	4	2	2	10	2	4	2	2	10

Challenger Bank	0	0	0	0	0	0	1	0	0	0	1
Plantagenet Bank	3	4	4	1	0	9	4	4	0	0	8
Wreck-Dredger	1	0	0	0	6	6	0	0	0	5	5
Total	17	20	16	9	17	-	21	16	10	17	-
Grand total	-	-	-	-	-	62	-	-	-	-	64

Table 8. Number of dives conducted for sampling at each site by type of sample collected.

Dive Site	Total Dives	Sampling dives			
		Water	Coral	Algae	Sponge
North-Northeast	6	6	6	4	1
Spittal	4	4	3	4	2
Tiger	3	3	3	3	2
Challenger Bank	1	1	1	1	1
Plantagenet Bank	3	3	1	3	1
Wreck-Dredger	0	0	0	0	0
Total	17	17	14	15	7

NEKTON ALGAE PROJECT

Barcoding the Deep-Water Algal Diversity of the Twilight Zone in Bermuda

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Dr Thea R. Popolizio, Department of Biology, Salem State University, Salem, MA, USA

Prior to the commencement of the expedition, the only collections of attached algae in greater Bermuda made below 60 meters were from the offshore associated seamounts to the southwest of Bermuda, the Challenger and Plantagenet Banks, taken by Navy divers in a lockout submarine or by dredge in the summers of 1960 and 1961. In the first two weeks of the present expedition, BEX tech divers and submersibles have brought 78 algal specimens from 12 sites in waters of 60 or greater meters in depth. One equipment deployment dive from 9-12 meters allowed the collection of six targeted species for specimen preservation including *Wrangelia*, *Spyridia* and an unknown net-forming red alga. Once on deck, algal samples are sorted and processed as follows. Small portions of each specimen are dried on silica gel for DNA analysis and another portion preserved in 10% Formalin in seawater (for anatomical observation), the remainder being pressed onto archival paper as a permanent herbarium voucher and photographed with collection, DNA and BEX numbers. The pressed specimens are dried aboard ship in the engine room in a standard plant press with polyethylene separators.

One of the early interesting findings has been the discovery of a brown meadow of *Sporochnus bolleanus* at depths of 60-125 m. This species has rarely been seen in Bermuda waters shallower than 30 m depths, and the extent of the brown meadows at several deep twilight sites was unknown and unexpected. Many species known from the shallow subtidal in Bermuda are found in these deep water habitats along with *Sporochnus*, including three species of *Caulerpa* and one each of *Dictyota*, *Lobophora*, *Stypopodium*, *Anadyomene*, *Asparagopsis* and *Palisada*. Several targeted species from the 1960s work have been

discovered as of this report, including a few *Codium* spp., a *Botryocladia*, a few *Dasya* spp., *Gloiocladia hassleri*, two *Halymenia* spp., a *Cryptonemia* spp. and *Kallymenia westii* (all of these are the first of the species where DNA collection is taking place). Additionally, several species collected appear either new for Bermuda or simply new to science. These include a *Halymenia*, *Meredithia*, *Agardhiella ramossissima* (tentative first report for the islands), and a *Dasya*, with others possibly being discovered after full DNA analysis. One deep-water species first described from offshore has been collected twice, *Rhododictyon bermudensis*, but not enough material has yet been collected for DNA analysis. Algal red rocks, rhodoliths are being collected and dried for transport and later examination of the coralline algae.

Sponge and Rhodolith Macrofauna and Meiofauna

Professor Nikolaos Schizas, Department of Marine Sciences, University of Puerto Rico

As part of the mission we surveyed deep water (>50 m) sponges and rhodoliths. All collections were made primarily from technical divers and the Triton submersibles with the deepest samples coming from 300 m. Together with the collected sponges and rhodoliths, we also extracted their exterior and interior associated fauna. The associated macrofauna was picked individually by tweezers and the meiofauna was collected by sieve extraction. For the associated meiofauna, we placed sponges and rhodoliths individually on stacked 5 mm and a 0.063 mm sieves and we washed them repeatedly with seawater. Both sponges and rhodoliths were placed in individual containers in a solution of seawater and 5% ethanol for further fauna extraction. Every 24 hours for up to 3 days after the day of collection we processed the samples through the same sieves to extract the cryptic fauna from the crevices. All specimens were placed in 100% ethanol-filled containers for preservation. The majority of the sponges were Demosponges (about 15 specimens) followed by 3 small hexactinellid sponges. All hexactinellid sponges were growing on rhodoliths. For the associated fauna, by far, the most abundant taxon was Copepoda with the most abundant Order being the Harpacticoida copepods.

The second most abundant taxon was the polychaetes which had the biggest biomass of the associated fauna. Other taxa in relatively high numbers were tanaids, amphipods, isopods. Less commonly we found penaeid shrimps, squat lobsters, nudibranchs, bryozoans and nematodes. Preliminary estimates on the number of meiofaunal samples suggest that over 1000 specimens were collected. Most specimens are still within sediments and need to be extracted under the microscope.

Coral connectivity and the microbiome

Dr Catherine Head, Department of Zoology, University of Oxford

Porites astreoides and *Montastraea cavernosa*, two species of hard coral (Scleractinia), were collected from shallow (15-20m) and mesophotic reefs (40-60m) by the Project Baseline tech divers. All specimens were preserved in ethanol and a subsample was preserved in RNA later. These specimens will be used to address three hypotheses, which will contribute to the management of Bermuda's reefs and the wider Caribbean:

1. Establishing the genetic connectivity of these species and their symbiotic zooxanthellae vertically in the water column using new RAD-seq techniques. If genetic connectivity is found between shallow and mesophotic reefs, this would suggest that deeper reefs could offer the potential to act as a refugia and potential source reef for coral recruits for shallow corals in Bermuda. This is of great interest to coral reef managers because shallow reefs are under heavy pressure from direct human impacts and climate change induced impacts, such as increases in sea surface temperature. Therefore establishing if mesophotic reefs may act as a refugia for shallow areas and protecting them could be key to the conservation of coral reefs.

2. We will also investigate the connectivity of these corals and their symbiotic zooxanthellae spatially with specimens from reefs in the Caribbean (12 locations around the Porto Rico and US Virgin Islands from Prof Nick Schizas). Identifying the connectivity between Bermuda and the Caribbean will allow us to understand where potential sources of coral recruits are geographically situated. This will build on genetic micro-satellite work carried out on *Montastraea cavernosa* in the Caribbean by extending the geographical range of our connectivity knowledge and using the most up-to-date genetic techniques (RAD-seq). Whilst *Porites astreoides* has not yet been studied in Bermuda.
3. Next generation sequencing will be used to identify the diversity of the microbial community associated with *Porites astreoides* and *Montastraea cavernosa* and how this changes with depth. We know that the microbial community is important in maintaining the health of the colony and preventing coral disease but very little is known about the corals' microbial community with depth. This could be key to understanding how deeper corals function

Seawater Chemistry

Heidi Hirsh, Earth System Science, Stanford University

Discrete Water Sampling

Discrete water samples were collected by technical divers and via Niskin bottles on the *Nomad* submersible. Technical divers collected water samples in soft sided water bottles. This bladder-like bottle made it easy for divers to bring multiple empty bottles on their dives without buoyancy or space constraints.

To take a water sample the diver would unscrew the cap and swish the bottle back and forth in the water column until it was full. Each had a 1L holding capacity and this method allowed divers to collect about 700mL of water for each sample. Each water sample was subsampled immediately after the divers/submersible returned to the *Baseline Explorer* as follows (Table 9)



Figure 13. Collapsible 1L Water Bottle

Table 9. Analyses that water samples were collected for and methods of preservation.

Volume	Analysis	Container	Preservation
125 mL	Total Alkalinity (TA)	Glass Serum Bottle	250 µL HgCl ₂
30 mL	Dissolved Inorganic Carbon (DIC)	Glass Serum Bottle	100 µL HgCl ₂
30 mL	Carbon-13	Glass Serum Bottle	100 µL HgCl ₂
15 mL	Phosphate	Plastic Centrifuge Tube	Frozen
50 mL	Nitrate	Plastic Centrifuge Tube	Frozen

The technical divers collected **59** water samples. See below for a summary of the sites and depths sampled (all depths are in feet; Table 10)

Table 10. Summary of water samples collected by Technical Divers along with depths (Feet).

NNE	Spittal	Plantagenet	Tiger	Challenger
103	22	30	30	21
131	22	50	49	33
199	30	52	49	49
199	30	70	49	149
300	48	70	66	158
	50	100	98	209
	56	133	98	230
	60	185	131	249
	68	210	134	295
	100	300	157	
	110	302	164	
	137		197	
	154		230	
	200		295	
	200		295	
	200			
	205			
	305			
	307			

24 total water samples were collected via the six 500mL Niskin Bottles aboard the *Nomad* Submersible (Table 11):

Table 11. Water samples taken by submersible giving depths (feet) and locations.

Site	Date	Depths (ft)
NNE	31 July 2016	660, 630, 517, 415, 315, 255
Spittal	3 August 2016	1000, 800, 600, 400, 200, surface
Spittal	7 August 2016	550, 500, 450, 400, 350, 300
Tiger	12 August 2016	600, 500, 400, 300, 200, 100

Additional Data Collected

Nomad was equipped with an **SBE 49 FastCAT CTD Sensor** to measure conductivity (salinity), temperature, and depth on each submersible dive.

Link: <http://www.seabird.com/sbe49-fastcat-ctd>

The technical divers were equipped with **YSI EXO1 Multiparameter Sonde**. The main purpose of this instrument was to record temperature and salinity profiles in order to calibrate the discrete water samples collected by the technical divers.

Link <https://www.ysi.com/exo1>

BEAMS Deployment

The BEAMS (Benthic Ecosystem and Acidification Measurement System) uses a boundary layer flux approach to monitor coral reef calcification dynamics. It is the first fully autonomous system capable of measuring reef Net Community Calcification (NCC) in undisturbed, natural conditions on timescales of minutes. Deployment of the BEAMS can provide unprecedented resolution for reef metabolic measurements - coral reef production (photosynthesis/respiration) and calcification/dissolution - and will help towards gaining a deeper understanding about what controls these processes. BEAMS is capable of simultaneously measuring benthic Net Community Production (NCP) and Net Community Calcification (NCC) by quantifying mean gradients of pH and oxygen as well as current velocity and using these measurements to calculate benthic fluxes of O₂ (NCP) and total alkalinity (NCC).

We completed three deployments of the BEAMS. The first deployment was unsuccessful because the instrumentation was retrieved after less than 24 hours (Table 12).

Table 12. Deployment details for the BEAMS system.

Site	Deployment	Retrieval	Days	Depth (ft)
NNE	28 July 2016	29 July 2016	1	60
Spittal	04 August 2016	07 August 2016	3	22
Cathedral	09 August 2016	13 August 2016	4	36

BEAMS Data: Discrete water samples were collected upon deployment and retrieval for calibration during data processing. An ADP (Acoustic Doppler Profiler) was used to measure current velocity. Chemistry data was recorded by a SeapHox Ocean CT(D)-pH-DO Sensor.

Link: <http://www.seabird.com/seaphox>

Photosynthetically active radiation (PAR) was also measured and recorded. Benthic photo transects were completed to characterize the benthic environment around the BEAMS. Eight 10m transects were completed (N, NE, E, SE, S, SW, W, NW) around the central post of the PVC frame at each site.

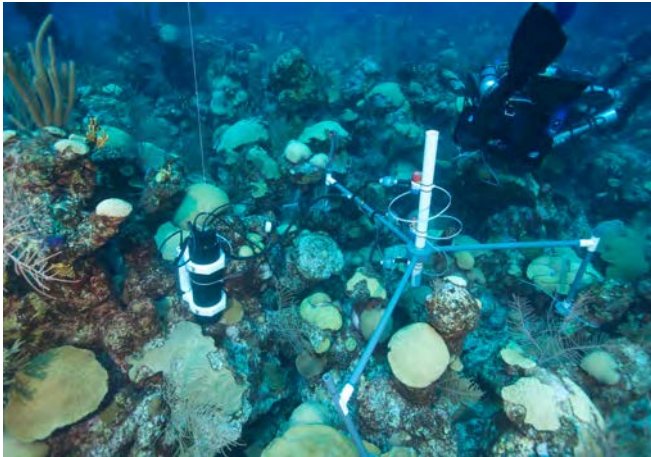


Figure 14. BEAMS being deployed at North Northeast.

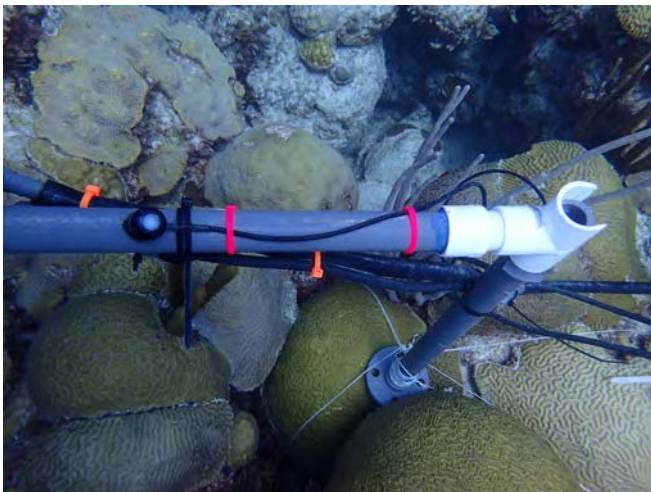


Figure 15. PAR Sensor deployed on PVC frame.



Figure 16. ADP deployed at Spittal.

Credit for BEAMS

The development of the BEAMS was the result of a project funded by the National Science Foundation in collaboration with Wade McGillis (Columbia University), Todd Martz (Scripps Institution of Oceanography (SIO), University of California San Diego (UCSD)), Jennifer Smith (SIO, UCSD), and Nichole Price (Bigelow Laboratory for Ocean Sciences). Thank you to Yui Takeshita and Ken Caldeira's Lab at the Carnegie Institution for Science for providing the instrumentation and expertise to make these deployments possible. For more information on the system please see Yui Takeshita's recent publication in the Journal of Geophysical Research, "Assessment of net community production and calcification of a coral reef using a boundary layer approach."

Link: <http://onlinelibrary.wiley.com/doi/10.1002/2016JC011886/full>

Environmental DNA

Dr Catherine Head, Department of Zoology, University of Oxford

Environmental DNA (eDNA) is the DNA from all organisms released into the water column in a host of ways including in their faeces, sperm, eggs and skin. These traces of DNA have the potential to be used to monitor the diversity of organisms that inhabit an area. In the marine environment initial studies, show promise for future non-invasive assessments and monitoring of marine biodiversity and resources. Around Bermuda we will use eDNA sampling to quantify the diversity of the microbiome, invertebrates, such as zooplankton, and vertebrates, and to identify these species.

Water samples have been taken across a depth profile (surface water, 15m, 90m and 200m) from 5 sites - North-northeast, Spittal, Tiger, Plantagenet Bank and Challenger Bank, allowing for rare vertical and horizontal comparisons of diversity around Bermuda. One litre of water was filtered using a vacuum pump system with a Whatmann 0.7 micron filter of 47 mm diameter. Filter papers were removed with forceps and placed onto clean lab grade aluminium foil and wrapped. The filters were then frozen immediately at -20°C until the end of the expedition. After the expedition the filters were returned to the UK on dry ice and stored at -80°C.

The next step is to extract the DNA from the preserved filters and use next generation sequencing to 'read' the DNA. The DNA reads can then be compared to global databases of DNA sequences to establish the number and identity of organisms in the samples from their DNA. The results will then be crosschecked with visual survey records taken by the subs and tech divers. Taxonomically very few scientists would be able to identify the invertebrate component of biodiversity, particularly the sessile fraction, creating a bottleneck to establishing the true biodiversity of Bermuda, but this can be overcome using eDNA sampling and next generation sequencing.

Environment data on the carbonate cycle (collected by Heidi Hirsh), such as pH, carbon levels and sea temperature will be used to investigate if there is any correlation between species richness, particularly in the microbial realm, and environmental conditions. For instance, the presence of certain microbes is thought to be connected with the level of the carbon and nitrogen in the water column. This will help us unravel differences in community structure across sites and reefs across trophic groups. In addition, we also have water samples available to us from locations in the Atlantic (Florida Keys) and Indian Ocean (Chagos Archipelago) that will allow for rare spatial and temporal comparisons.

COMMUNICATIONS

Introduction

The communications target markets across traditional and social media are primarily divided into the following geographical regions:

- Primary: UK, North America, Bermuda (as host country)
- Secondary: countries known for links to underwater exploration and high levels of active marine conservation, e.g. Philippines, Indonesia, Belize, South Africa
- Tertiary: responding as opportunities present, or as emerging storylines dictate, e.g. an Australian broadcast picking up / syndicating a Sky broadcast.

Our audiences have been further divided as follows, to maximise interest exposure and storyline opportunities for the rich content being generated by the Mission Communications and Content team:

- General adventure and science storylines: these are typified by the visits from media to the Mission in Bermuda, which is vital for engaging media / storytelling which speaks to a broader public. This is also part of our profile establishing strategy, which introduces the XL Catlin Deep ocean Survey and Nekton to the world.
- More specific science storylines which include:
 - Initial findings and imagery released in real time from mission
 - Invasive lionfish and new technology for the reduction of populations
 - Discovery of new species of Ctenophores, a model group for studying regeneration and which could lead to 'medical breakthroughs'
- Industry specific storylines which include:
 - Educational outreach, which profiles our animated 360 video and schools pack
 - Oceans risk story for insurance and financial press
- Bermuda / in-country storylines to engage host country in mission, which include:
 - Arrival in country
 - Findings released from mission at mid point
 - Departure from country

Media reporting from mothership

Nekton invited selected media to join the mothership for one day and experience a dive in one of the two Triton submersibles.

Resulting in:

- 15 media dives
- Journalists represented: Sky, Telegraph, BBC Radio 4, Guardian, Sirius XM, PBS Newshour, New Scientist, Forbes

Resulting coverage

MEDIA OUTLET	RESULTING COVERAGE
Sky News	The crew came out to Bermuda and dedicated a week of themed reporting around our work, called ‘Oceans Week’. This resulted in three online video features, repurposed and used for 14 on air broadcasts. http://news.sky.com/story/scientists-health-check-of-the-worlds-oceans-10511839
The Guardian	Print feature and online multimedia feature. https://www.theguardian.com/environment/2016/aug/17/ocean-research-marine-life-bermuda-coral-reefs-nekton-triton-vessel
Daily Telegraph	Published online and as a spread in the Saturday Magazine http://www.telegraph.co.uk/news/2016/10/08/the-divers-plunging-1000ft-underwater-to-uncover-the-secrets-of/
New Scientist	Print and online story https://www.newscientist.com/article/mg23130872-900-plunging-deep-beneath-the-sea-in-a-tiny-sub-to-map-the-ocean/
PBS	Broadcast feature on lionfish invasion http://www.pbs.org/newshour/updates/robot-lionfish-invasive-species-rise-nekton/
BBC Radio 4 From Our Own Correspondent	Part of a segment of stories for the 13 August edition http://www.bbc.co.uk/programmes/b07mvygc
The National	http://www.thenational.ae/arts-life/the-review/nekton-an-international-scientific-mission-plumbing-the-depths-to-help-save-our-oceans
Forbes	Part of three online series published: http://www.forbes.com/sites/jimclash/2016/07/30/nekton-deep-ocean-science-thrills-writer-1000-feet-below-atlantics-surface/#207f3440650c http://www.forbes.com/sites/jimclash/2016/08/05/guinness-world-record-intrepid-interview-conducted-1000-feet-deep-in-nekton-sub-near-bermuda/#7dff2ab63b0c http://www.forbes.com/sites/jimclash/2016/08/06/nekton-chief-scientist-alex-rogers-speculates-on-new-bio-finds-1000-feet-deep-near-bermuda/#3b4f198728e7 http://www.forbes.com/sites/jimclash/2016/08/07/nekton-mission-director-oliver-steeds-gave-up-his-career-as-a-journalist-to-help-save-the-oceans/#72ef13c65f4e
Sirius XM	“Deep Dive Radio” was aired live on Tuesday, August 9, at 2:00 pm ET on SiriusXM Insight channel 121. The show was also available on SiriusXM On Demand for subscribers listening via smartphones and other connected devices or online at www.siriusxm.com . It replayed August 9 at 4:00 pm; Wednesday, August 10 at 3:00 pm; and Thursday, August 11 at 4:00 pm. http://blog.siriusxm.com/2016/08/08/siriusxm-takes-broadcasting-under-the-sea/

Social media snapshot

The communities are very new and outreach commenced the week before the mission deployed. The response to the Mission has been phenomenal, and we have experienced a quick rise in followers and engagement, particularly where our video-based updates are used. (Stats from immediate post mission).



It is also worth noting our high levels of engagement on Facebook - we average around 40% engagement rates on our posts, with the charity industry average standing at 7.94%

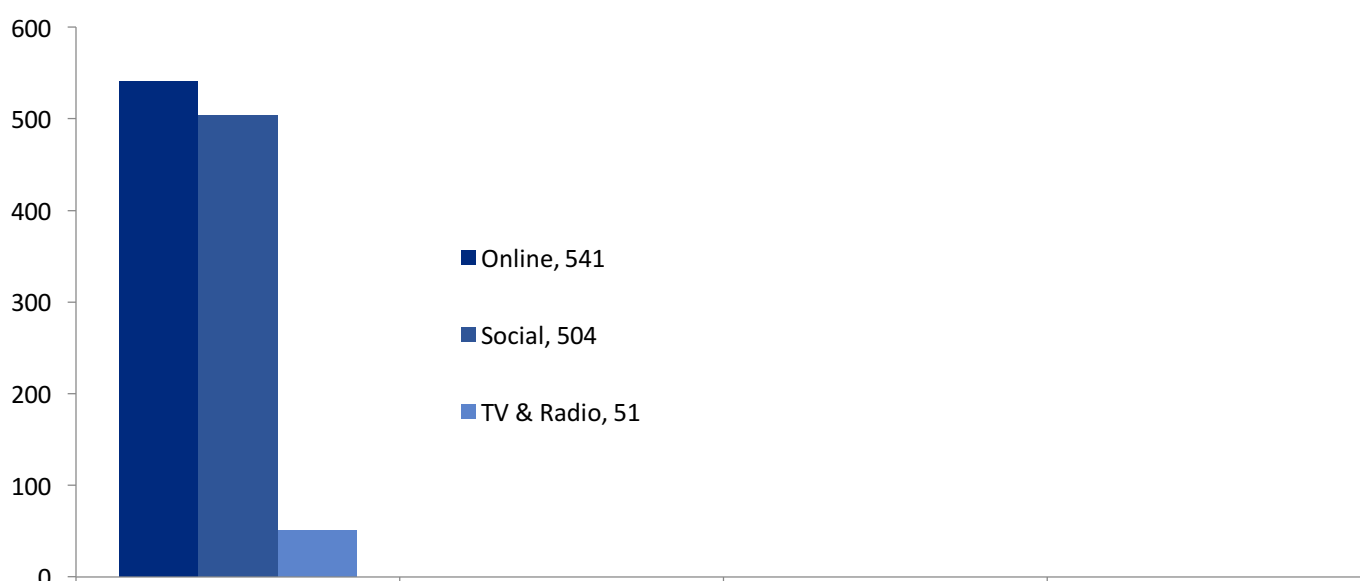
All activities were supported by the launch of the Nekton Mission website: www.nektonmission.org

Within the site are designated science, technology and education sections. As and when robust scientific conclusions can be formulated, they will be posted on the website at: <https://nektonmission.org/the-science>

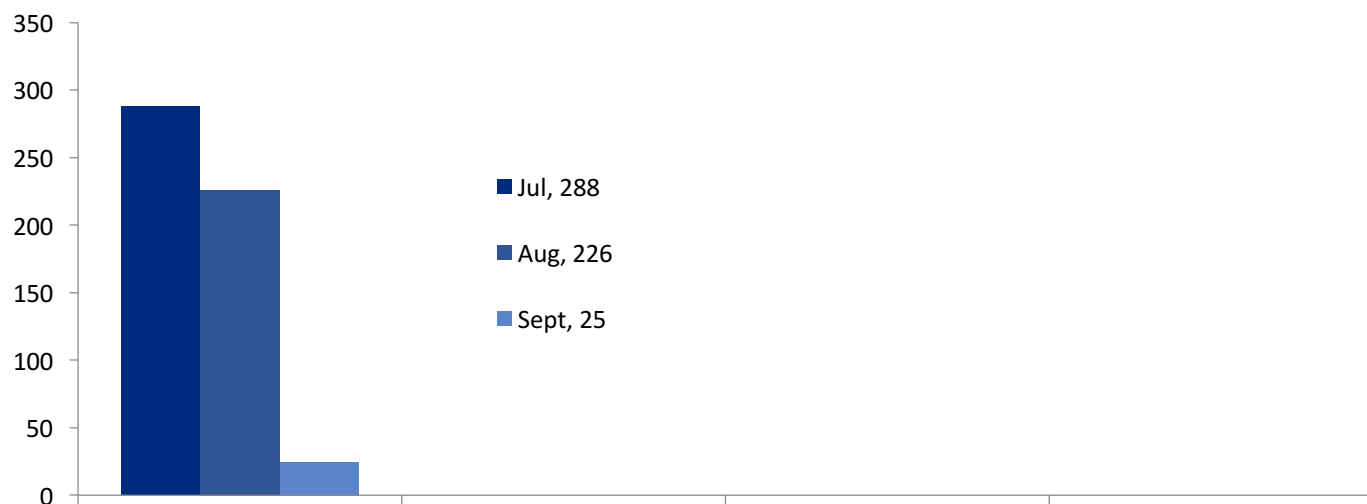
A snapshot of coverage results so far

TO NOTE: Meltwater tracking tool (www.meltwater.com) only monitors online, broadcast and social. Figures for print coverage are not included.

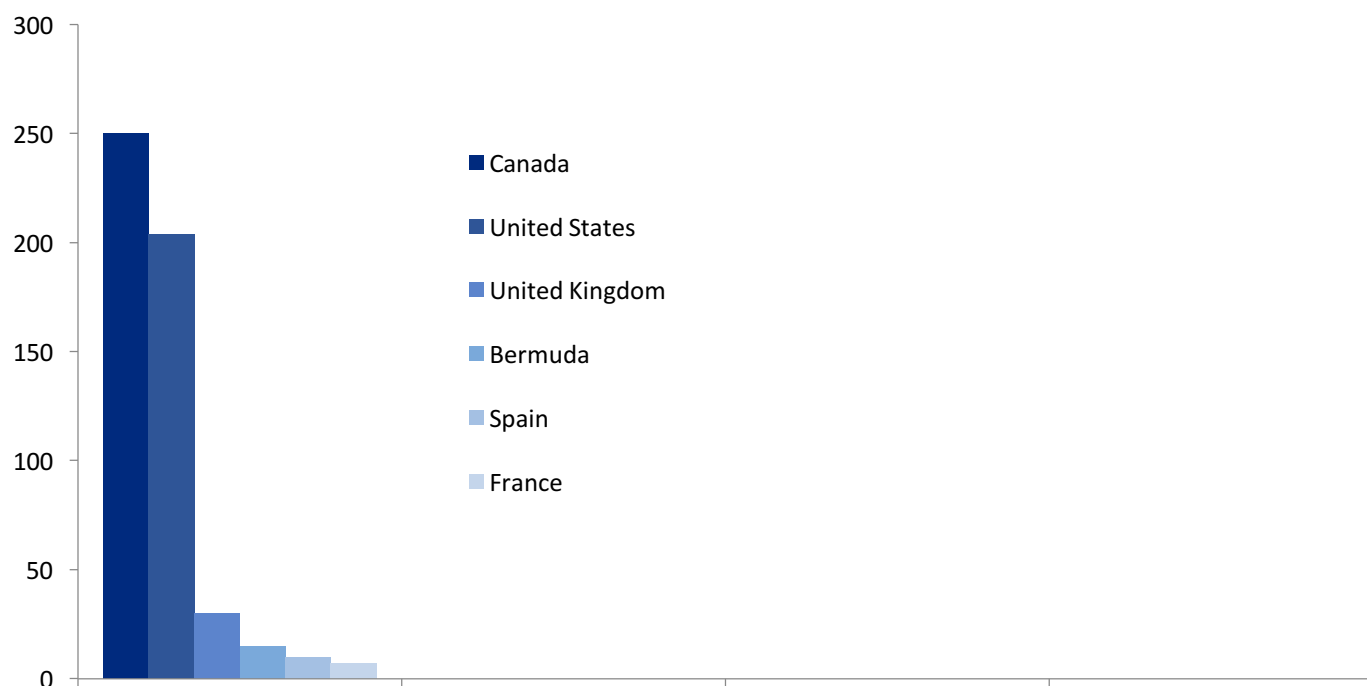
Coverage Volume: From 6 July 2016 to 10 October 2016



Digital coverage spread: During and immediately post mission



Coverage location: During mission



Potential global audience reach during mission:

Online opportunities to view: **682 million**

Broadcast opportunities to view: **70 million**

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APPENDIX 1

List of individuals carried by the submersibles and statistics for the dives they conducted

Observer / Organisation	No. Dives	Duration (hrs)	Min Depth (m)	Max Depth (m)
Alex Rogers / Univ. Oxford	19	41.6	37	305
Oliver Steeds / Nekton	7	14.1	10	304
Robbie Smith / Bermuda Aquarium	2	6.3	218	305
Will West / Nekton	2	6.0	200	203
John Fugelsang / Radio Corres.	1	4.4	304	304
Thea Popolizio / Univ. Rhode Is.	2	4.4	158	202
Douglas Batchelor / Baird & Warner Real Estate	1	4.1	201	248
Chris Flooker / Bermuda Aquarium	1	2.9	303	303
Jim Clash / Forbes Magazine	1	2.8	305	305
Catherine Head / Univ. Oxford	1	2.8	200	200
Nsikan Akpan / PBS News Hour	1	2.8	244	244
Joe McInnis / Undersea Research	1	2.7	249	249
Melissa Price / Nekton	1	2.7	303	303

China Mieville / Writer	1	2.6	303	303
Lucy Woodall / Univ. Oxford	1	2.6	200	200
Heidi Hirsh / Stanford University	1	2.5	199	199
Gayle Batchelor / Baird & Warner Real Estate	1	2.5	248	248
Fanny Douvere / UNESCO	1	2.3	159	159
Greg Foote / Nekton	1	2.2	250	250
Philip Renaud / UNESCO	1	2.2	159	159
Oliver Millman / Guardian	1	2.1	152	152
Aviva Ruthkin / New Scientist	1	2.0	200	200
David Rees / Sky News	1	1.9	62	62
Karen Kohanovich / NOAA-NURP	1	1.8	154	154
Nikolaos Schizas / Univ. Puerto Rico	1	1.7	167	167
Craig Schneider / Trinity College	1	1.6	201	201
Justin Marozzi / BBC	1	1.5	303	303
Jen Hegarty / Rockefeller Centre	1	1.2	158	158
Kelvin Magee / Triton	1	0.9	110	110
Rod Rodenberry / Rodenberry Entertainment	1	0.9	158	158
Chris Reeves / XL Catlin	1	0.8	15	15
India Sturges / Telegraph	1	0.7	152	152
Stephen Catlin / XL Catlin	1	0.7	15	15
Thomas Moore / Sky News	1	0.6	61	61
Paul Brand / XL Catlin	1	0.6	15	15
Mike Maran / XL Catlin	1	0.6	15	15
Elliot Bundy / XL Catlin	1	0.6	15	15
Annie Sousa / XL Catlin	1	0.5	15	15
Graham Everard / XL Catlin	1	0.5	15	15
Paul Ritchie / XL Catlin	1	0.5	15	15
Pete Porrino / XL Catlin	1	0.5	15	15
Myron Hendry / XL Catlin	1	0.4	15	15
Krista Doran / XL Catlin	1	0.4	15	15
Greg Hendrick / XL Catlin	1	0.4	15	15
Tom Booth / XL Catlin	1	0.4	15	15
John Smart / XL Catlin	1	0.4	15	15
Kelly Lyles / XL Catlin	1	0.3	15	15
Mike McGavick / XL Catlin	1	0.3	15	15



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