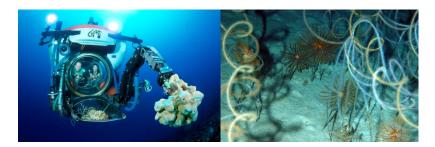
Bonaire Deep Reef Expedition I

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Summary

From 30 May – 1 June 2013 the deep reef of Bonaire, Caribbean Netherlands, was explored with the aid of the "Curasub" submarine of Substation Curaçao. The shallow reefs of the Caribbean are considered a biodiversityhotspot, an area with exceptional diversity of plants, animals and ecosystems (Conservation International 2004; Roberts et al. 2002), yet surprisingly little is known about the flora and fauna of the deeper reefs. Particularly the deep reefs of Bonaire, St. Eustatius and Saba have hardly been explored. This represents a critical knowledge gap for developing future reef policies and management practices. In order to adequately protect the ecosystem and construct sustainable management plans it is essential to document the biodiversity and to gain an understanding of what processes keep it in place. The Dutch Ministry of Economic Affairs (EZ) commissioned IMARES to study the deeper reef of Bonaire as part of the Exclusive Economic Zone (EEZ) management plan for the Dutch Caribbean (Meesters et al. 2010).

Dives were made to depths of 140-250 m. at three locations on the Southern coast of Bonaire: Kralendijk, Cargill, and Statoil.

Distinct depth zonations in substrate features were visible. Coral reef was observed until approximately 45m, then followed a zone of sand mixed with varying amounts of stones. At each site a wide layer of cyanobacteria mats covering sand were found spanning the depths of 45m. to 90m. The depth from 90-100m was typically dominated by sand with occasional small rocks on which fan corals and sponges resided. From 100-150m depth fossil barrier reef and rodolith beds were observed, either in long stretches or in patches within a barren sandscape. By providing hard substrate, these fossil reefs displayed heightened biodiversity in a desert landscape of sand. Below 150m the substrate was generally dominated by fine sand.

The cause of the cyanobacterial mats remains unclear. These mats are generally believed to indicate nutrient enriched (disturbed) environmental conditions, and should therefore be further studied to elucidate the cause.

Trash was observed at all depths.

High biodiversity was observed on the sporadic hard substrate below 100 m., presumably fossil reef. In total 72 species were recorded, of which at least 15 species are new to science (shrimp, sponges, fish). The major focus was on sponges due to their importance in the deep reef in terms of diversity, filtering activities, biomass, and source of pharmaceutical compounds. A species list and picture gallery are provided in this report. This is just of subset of the true biodiversity of Bonaire's deep reef. With the description of new species also comes a better understanding of ecosystems. The discovery of unique species may, furthermore, lead to the discovery of novel applications for human use. These results warrant further investigation and inventory of species in the deep reef, especially in the north sector of the island. Acoustic surveys are proposed to be used in the future to map rocky outcrops and cliffs under water and then explore these "hotspots" with the CuraSub.

Special attention was paid to the invasive Indo-Pacific lionfish. Between the depths of 80-115m schools of 10-15 individual lionfish clustered together were observed, though the density is expected to be higher as they are cryptic species. Lionfish were observed as deep as 165m. There are at present key gaps in knowledge on the size, depth distribution and migration routes of the populations the lionfish within the Dutch Caribbean, which hampers adequate control of this invasive species.

One possibly important archaeological finding was made: an urn that is presumed to be an 18th century Spanish olive urn. Due to permit restrictions this object was not collected.

The Bonaire Deep Reef Expedition I elicited extensive public interest and media coverage in the Dutch, Bonaire and international news (details provided in Appendix C).

1 Introduction

From 30 May – June 1 2013 the deep reef of Bonaire, Caribbean Netherlands, was explored with the aid of the "Curasub" submarine of Substation Curaçao. The shallow reefs of the Caribbean are considered a biodiversityhotspot, an area with exceptional diversity of plants, animals and ecosystems (Roberts et al. 2002). Surprisingly little is however known about the flora and fauna of the deeper reefs. Particularly the deep reefs of Bonaire, St. Eustatius and Saba have hardly been explored. The only previous deepwater submarine research conducted on Bonaire took place in May 2000 during which 24 deep sea dives were conducted with the Johnson-sea-Link II research submersible of Harbor Branch, FLA, USA, down to depths of 900 m, off Curacao, Bonaire and Aruba (Reed and Pomponi 2000). The focus of that expedition was on biomedically- interesting sponges but the biodiversity data of that expedition have not been worked out. The Ministry of Economic Affairs (EZ) commissioned IMARES to study the deeper reef of Bonaire as part of the Exclusive Economic Zone (EEZ) management plan for the Dutch Caribbean.

The Convention on Biological Diversity (CBD, 1992) and regional implementations such as the SPAW protocol (Protocol Concerning Specially Protected Areas and Wildlife to the Convention for the Protection and Development of the Marine Environment of the Wider Caribbean Region, adopted in 1990 and entered into force in 2000) urge countries to develop strategies to conserve biological diversity, to sustainably use the components of biological diversity, and to share fairly and equitably the benefits arising out of the utilization of genetic resources. Mesophotic reefs, coral reef environments from 30-150m depth, are recognized as highly biodiverse regions and relatively little studied environments.

The aim of this project was to document the habitat and biodiversity of the deep reef by taking video and still images, as well as collecting samples. This report presents an overview of the results obtained during the survey. For the purposes of this report deep reef is defined as the waters below 50 m.

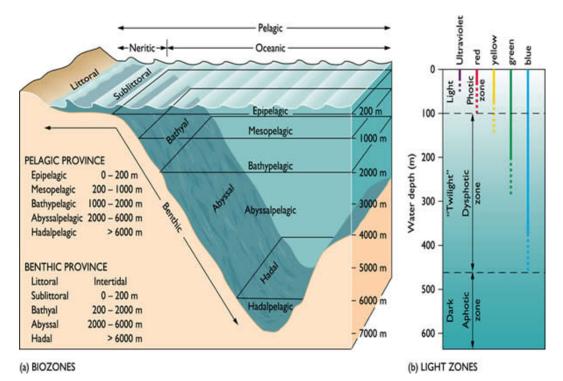


Figure 1. Diagram showing different habitat zones (http://www.marine-conservation.org.uk/marine_ecology.html)

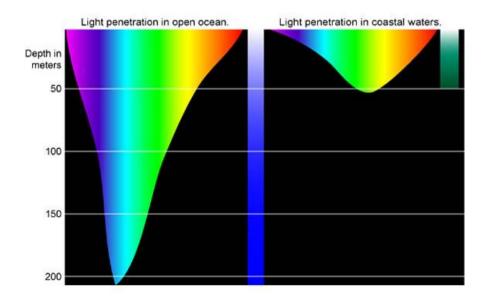


Figure 2. Basic illustration of the depth at which different colors of light penetrate ocean waters. Water absorbs long wavelength light and scatters short wavelength light (Source: Image courtesy of Kyle Carothers, NOAA-OE).

1.1 Ecological relevance of deep reefs

Mesophotic coral ecosystems refer to light-dependent coral communities (including associated communities such as of algae, sponges, and fish) that occur in the deepest half of the photic zone, 30 m to approximately 150 m. At 150-200m is the beginning of the dysphotic zone (or twilight zone) where illumination is too weak for photosynthesis (Figures 1&2). The penetration of light depends on the turbidity of the water and the distance from shore (Figure 2). The waters surrounding Bonaire have a high visibility and therefore the photic zone may reach deeper than is common in other coastal localities.

Depth has been previously shown to be an important environmental factor governing the structure and composition of biological marine communities (Torruco et al. 2003, Syms et al. 2004, Hogg et al. 2010, Becking et al. 2006). Key environmental variables change with depth; each vertical depth zone, for example, has a different degree of light regime, temperature and wave energy, so different species are adapted to such a set of variables thereby leading to different community structures. Compared to shallow reef environments, the composition and intensity of light greatly changes with increasing depth, as well as the influence of waves.

All studies to date have shown mesophotic reef communities to contain diverse benthic communities, including unique depth-endemic species (e.g. Pyle 1996, Lesser et al. 2009, Bongaerts et al. 2010). Photosynthetic reef building corals were found to live in habitats at deeper depths (> 30 m) than previously believed possible. The lack of knowledge about mesophotic coral reef environments has impacted our broader understanding of the ecology, biodiversity, and connectivity of all coral reef communities. Mesophotic coral reefs may serve as important refugia and nursery habitats for crucial fish populations and be potential sources and sinks of shallow coral larvae (Lesser et al. 2009).

1.2 Deep reef refuge hypothesis

Anthropogenic global ocean warming is predicted to cause bleaching of many near-sea-surface coral reefs, placing increased importance on deeper reef habitats to maintain coral reef biodiversity and ecosystem function (Harris et al. 2013). It has been suggested that degraded shallow reefs (<20m) are increasingly reliant on recruitment of larvae from elsewhere, and that brood stocks in other habitats - such as mesophotic reefs (30 – 150 m) for example - could play a key role in in managing coastal seascapes (Slattery et al. 2011).

Many coral and sponge species that are found in the shallow reefs are also found in the upper mesophotic zone, and deep reefs have been assumed to be physically and biologically connected to their shallow-water counterparts and may therefore have the capacity to act as a refuge for endangered corals and sponges from which they could recolonize the shallow reefs and thus increase their resilience (Bridge et 2013). However, as light intensity gradually decreases with increasing depth, the depth of occurrence is limited along the depth gradient for corals and many sponge species that often harbour phototropic symbionts (Figure 3). The evidence to support the idea that mesophotic reef areas act as a viable reproductive source for their relatives in shallow reef areas is also rather scarce. For example, van Oppen et al. (2011) and Bongaerts et al. (2010), refute this hypothesis based on current knowledge, and these authors stress the necessity of further research of the deep reef.

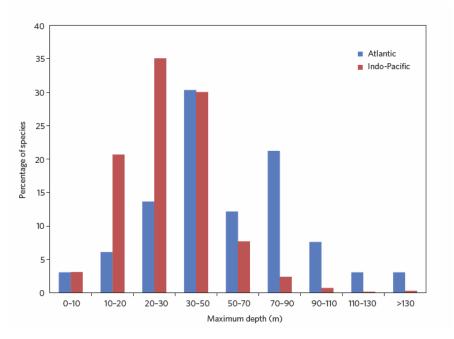


Figure 3. Depth limits of reef-building corals. Maximum recorded depth for corals in the Atlantic and Indo-Pacific are shown. Geographic disparity between oceans may be an artefact of chronic under-exploration of deep-water reefs in the Indo-Pacific (figure from Bridge et al. 2013).

The location and spatial extent of many deep reef habitats is poorly known in general and in particular in the Dutch Caribbean. Furthermore, the extent to which important ecological processes change along depth gradients is not well understood. This represents a critical knowledge gap for developing future reef policies and management practices. In order to adequately protect the ecosystem and construct sustainable management plans it is essential to document the biodiversity and to gain an understanding of what processes keep it in place.

Here we report on three explorative dives down to a depth of 250 m. that were made off the coast of Bonaire.

2 Material and Methods

Three dives were made with the "Curasub" submarine off the SW coast of Bonaire (Table 1, Figure 4 & 5). The dive locations were selected based on available piers where the research vessel "Chapman" could moor. One (Dive 1) was located in Kralendijk harbor. The second station (Dive 2) was located off the pier in front of the airport, and the third station (Dive 3) was in front of the salt pier at Cargill Salt Bonaire. At each station video transects were made from the shallow coral reef down to the maximum depth that could be reached within the available time (maximum 7 hrs). The submarine was followed on the surface by a small boat which kept contact with the submarine through an acoustic system. The position of the submarine was recorded by the follow-boat with a gps device on board.

During the dives digital images were taken of species and specimens were collected of a select number of species of sponges, shrimps, and other interesting fauna. Corals and archeological objects were only photographed and not sampled due to permit restrictions. Data-loggers on the hull of the submarine recorded depth and temperature while diving (Appendix B). Samples were collected using the operational arms of the Curasub and stored on the basket infront of the machine during the dive. Upon reaching the surface, samples were collected from the submarine by divers and transferred to sea water filled buckets which were then transported on board of the "Chapman". Collected specimens were processed as soon as the samples and researchers were on board of the "Chapman".

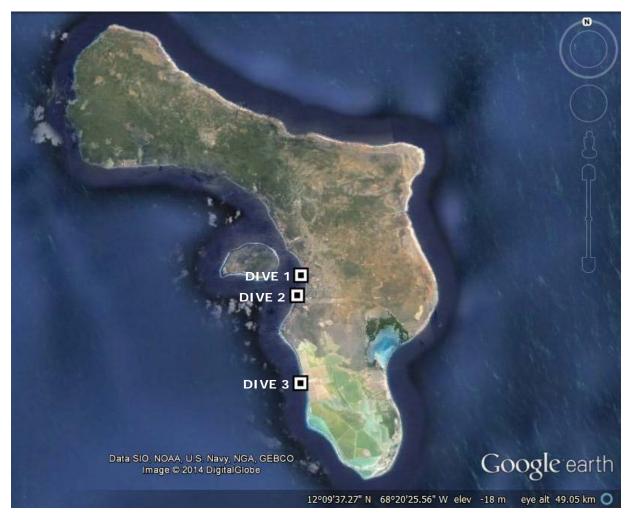


Figure 4. The three dive locations off the South-west coast Bonaire that were explored by the Curasub submersible.

Table 1 Station information of three dives with the "Curasub" submarine off the South coast of Bonaire.

Station	Date	Island	Location	Latitude	Longitude	Time in	Time out	Max. depth (m)
DIVE 1	30/5/2013	Bonaire	Kralendijk pier	12.14692	-68.2821	9:43	12:53	148
DIVE 2	31/5/2013	Bonaire	Curoil Dock, Boilpier	12.137	-68.286	9:19	14:30	207
DIVE 3	1/6/2013	Bonaire	Cargill pier	12.07996	-68.2938	10:17	15:40	248

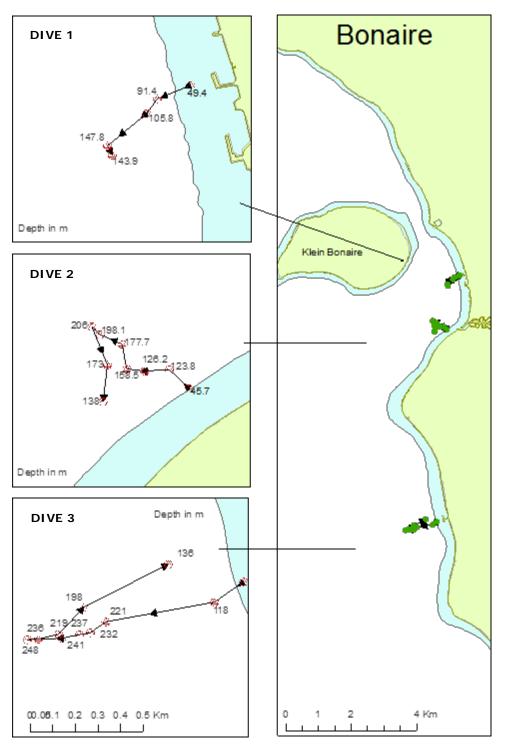


Figure 5. Dive locations and dive route per date. Numbers by arrows indicate depth in meters.

3 General Results

Figure 5 shows the approximate route for each dive including the depths that were recorded for the submarine at each waypoint. The first dive on 30 May in Kralendijk. The maximum depth of the first dive was only 148 m., as the area between Kralendijk en Klein Bonaire is not very deep. The reef in front of the pier is generally degraded and is polluted by cans, plastic, and tires. At 37 m. the reef slope levels off into a sand plane. The sand plane is colonized by garden eels (*Heteroconger longissimus*), but this is soon followed by mats of cyanobacteria that cover large parts of the sandy bottom (Figures 6 & 7).

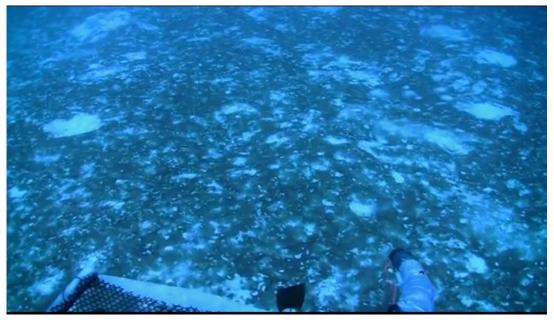


Figure 6. Cyanobacteria mats as seen from the sub during the first dive off Kralendijk.



Figure 7. Close-up of cyanobacteria mat with soft corals (Leptogorgia virgulata).

There is little else living in these cyanobacterial fields and they extend for hundreds of meters covering the whole bottom from approximately the depths of 38m until 60m. The only other organism that was seen within these fields were gorgonians (*Leptogorgia virgulata*). The white patches within the cyanobacteria fields indicate that there is some bioturbation, probably by polychaete worms that live in the sand. Larger white areas within the cyano fields could also be inhabited by sand eels.

A variety of structures under water can attract fish. Lionfish were observed aggregating around and within objects such as large pipes, car tires, or plastic cups (Figure 8; more information on lionfish in Chapter 6).



Figure 8. Off Kralendijk many lionfish were encountered around tires and pipes. This picture was taken at 91m where there were 12 lionfish swimming around two big pipes.

With increasing depth, after the fields with cyanobacteria, the bottom is mostly sandy with occasionally some piles of coral or coralline algae rubble stones that are inhabited by small fish species. Some of these piles are actively constructed by sand tilefish, piskarai, (*Malacanthus plumieri*). At 147m a big rock like structure was encountered, presumably fossil reef, which was covered by sessile species such as small solitary corals and sponges. One of the archeological finds was a large urn (Figure 9) at 144 m, which probably represents an 18th century olive urn (tentative identification by Jay Haviser, Leiden University). Images from this dive can be viewed at <u>http://flic.kr/s/aHsjGBuYt3</u> and in Appendix A.



Figure 9. Urn observed on first dive, probably a Spanish 18th century Olive urn (tentative identification by Jay Haviser, Leiden University)

The second dive (Dive 2), on the 31st of May, was in front of the pier that is located at the coast directly west of the airport. The maximum depth of the dive was 207 m. Cyanobacteria fields were present again, this time from 47 to 73m. At the latter depth the submarine had to switch on the outside lights because it was too dark to see clearly. Possibly, light is limiting the deeper expansion of the cyanobacteria. Descending further, many lionfish were observed. The maximum depth that lionfish were observed was 91m. Around 100m depth a large rock wall-formation, presumably a fossil reef, was found that harbored many different taxa. Apart from the many sponge individuals that we sampled we also took some samples of a group of shrimp. All images from this dive can be viewed at http://flic.kr/s/aHsjGzrrSH and in Appendix A.

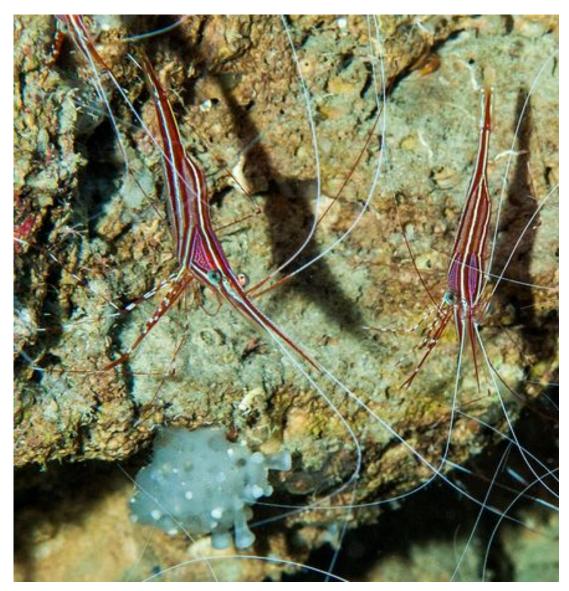


Figure 10. A group of brightly coloured shrimp (Plesionika longicauda) by a vertical wall that was encountered at approximately 100m depth.

The third dive was off the salt pier, with a maximum depth of 248 m. Cyanobacteria fields were first encountered at 37m and were very abundant till light was too low to see without artificial lights. The reef appeared in a much healthier state with much more living corals and less dead parts on the corals. Here was also a double reef with high abundance of barrel sponges (*Xestospongia* cf. *muta*) which appeared after a sand flat at around 40m. On the way down several large gorgonians (soft coral) were observed (Figure 11&14). Lionfish were seen down to 165m. At 218 m. a subsea cliff at least 10m in height was observed. The cliff-face was home to a wide variety of species, mainly sponges, but also worms, tunicates, sea stars, brittle stars, sea urchins, fish, molluscs (Figure 12). All images from this dive can be viewed at http://flic.kr/s/aHsjGzrrSH and some of the most interesting pictures are shown in Appendix A.

During all three dives, trash was observed at all depths, though at lower densities at greater depth. Tires were observed in the shallows, as well as fishing lines and self-made ankers (of stoneblocks) and old cages. Even at great depths, cans or cups were observed.

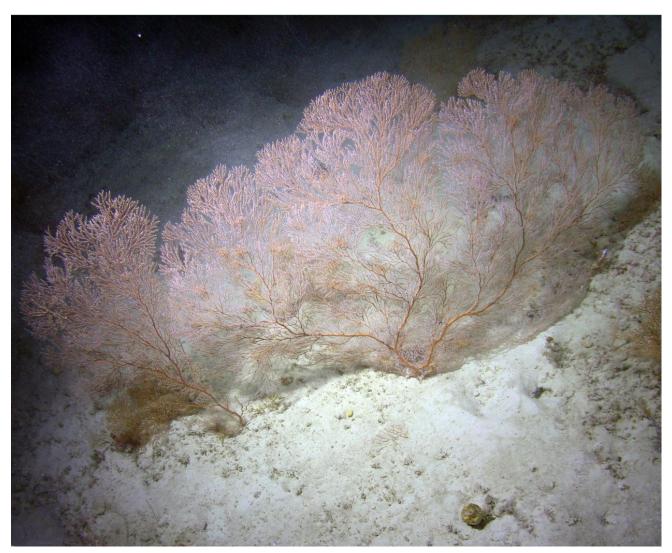


Figure 11. Gorgonian (Nicella guadalupensis).



Figure 12. Some of the exiting fauna that was found on the deep cliff off Cargill Salt pier. A. sponge Neopetrosia **new species**, *B. sea anemone* Boloceroides *sp., C brittle star*

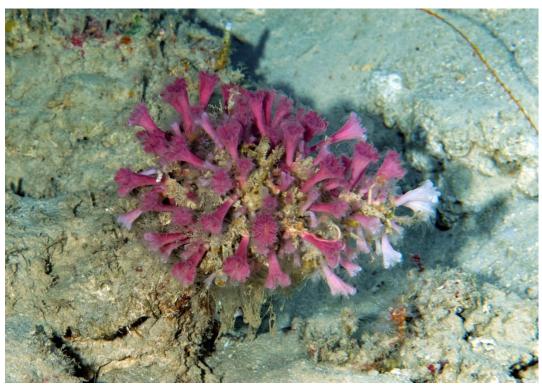


Figure 13. The coral Thalamophyllia riisei



Figure 14. *Gorgonian* Callogorgia gracilis *with white contracted basket sea stars. This species of gorgonian was also host to the new species of crustacean* Pseudocoutierea **new species**

4 Habitat features

4.1 General features

Distinct depth zonations in substrate features were visible. The coral reef was observed until approximately 45m, then followed a zone of sand mixed with varying amounts of stones. Here rubble mounds of Tilefish nests were also observed. Subsequently from approximately 45- 90m depth there was an expansive zone of sand covered by a cyanobacterial mat. The cause of this cyanobacterial mat remains unclear. The only macrofauna observed in this zone were yellow gorgonians (Figure 7) and small groups of garden eels in occasional patches of sand that were not covered by cyanobacteria. The cyanobacterial mats appeared to become thinner when going further away from Kralendijk. The depth from 90-100m was dominated by sand with occasional small rocks on which fan corals and sponges resided. From 100-150m depth fossil barrier reef and rodolith beds (nodules created by algae growth) were observed, either in long stretches or in patches within a barren sandscape. By providing hard substrate, these fossil reefs displayed heightened biodiversity in a desert landscape of sand. Below 150m the substrate was dominated by fine sand. Differences from these general patterns are provided in 2 and are discussed per location below.

Table 2.	Ushitat	donth	profilo	nor	location
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DIVE 1	depth (m)
Coral reef	0-44
Sand with garden eels	45-48
Sand with cyanobacterial mat	49-85
Sand with occasional rock with soft corals	85-103
Fossil reef	104-147

DIVE 2	depth (m)
Coral reef	0-44
Sand & Stone with algae	44-47
Sand with cyanobacterial mat	48-82
Sand and occasional rock	83-87
Sand with fossil reef patches	88-152
Sand and occasional rock	153-207

DIVE 3	depth (m)
Coral reef	0-30 and 40-45
Sand & Stone with algae	30-36
Sand with cyanobacterial mat	37-43
Secondary reef	43-62
Sand with light cyanobacterial mat	63-90
Sand with occasional rock with fancoral	91-131
Sand with fossil reef patches	132-152
Sand and occasional rock	153-172
Sand (fine silt)	173-212
Mound/cliff/fossil reef	220-250

4.2 Fossil reefs



Figure 15. Cliff wall of fossil reef. Many different species such as the new species of sponge Caminus sp. were living on the wall of the large cliff that was found on the third dive.

The fossil barrier reefs were likely formed during previous ice-ages when the sea level was much lower than it is today. During the period 15.000-20.000 years before present, during the last glacial maximum, sea level was situated 100-120m below present level (see Figure 15 for the relative sea level rise since 120.000 years ago) (Peltier 2002, Peltier & Fairbanks 2006). Subsequent tectonics has likely shifted the position of the reef structure (Bandoian et al. 1974, Beets et al. 1984). These fossil reefs provide hard substrate in an expansive sandscape.

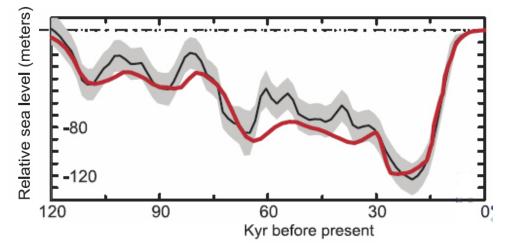


Figure 16 Reconstruction by Waelbroeck et al. (2002) of the relative sea level history from 120 thousand years (Kyr) ago until present based upon calibrated deep sea core derived oxygen isotopic measurements. This reconstruction is denoted by the central black curve and the surrounding error envelope is shown as grey (Figure from Peltier & Fairbanks 2006).

4.3 **Rhodolith formations**

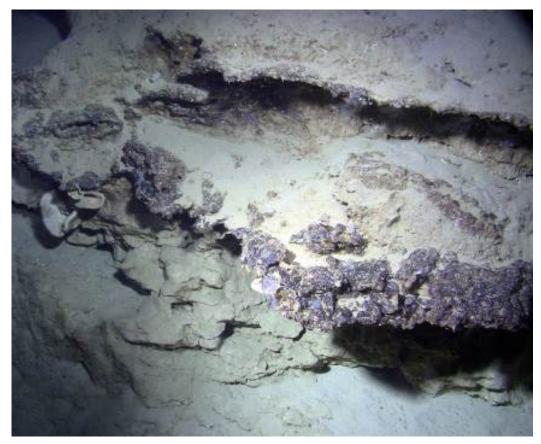


Figure 17. Rhodoliths are structures that are formed by calcareous algae.

Rhodoliths are nodules of coralline algae that typically occur at depths above 150 m, forming large expanses of hard bottom habitat. Rhodolith beds stand with kelp beds, seagrass meadows, and coralline algal reefs as one of the world's four largest macrophyte-dominated benthic communities (Amado-Filho et al 2012). Tropical benthic communities such as coral reefs are well known to be major carbonate producers in coastal areas, but there is growing evidence that communities dominated by crustose coralline algae can also contribute significantly to the CaCO3 cycles of continental shelf ecosystems. Furthermore, rhodoliths are widely recognized as bioengineers that provide structural complexity and relatively stable microhabitats for other species over large extensions, thus resulting in increased biodiversity and benthic primary productivity (Amado-Filho et al 2012), particularly when compared to unconsolidated flat bottom.

5 Biodiversity and new species

In this section we provide a preliminary description of the macrofauna below 100m, with a particular focus on sponges. The list of species is based on observations, images, and a limited number of collected samples from the 3 dives. The list of species in this report is merely a subset of the actual diversity and by no means exhaustive. Cryptic species were not collected and may constitute an important part of the total biodiversity. The identifications made so far are, furthermore, preliminary. The collected specimens have been identified by L.Becking (IMARES) and taxonomists of the Naturalis Biodiversity Center. The molecular lab of Naturalis will generate "DNA-barcodes" to facilitate the identification. A picture gallery of the species is provided in Appendix A.

Three factors appear to govern the diversity and abundance of benthic species. These are a) the morphology of the sea bottom b) the grain size of the sediment, and c) the degree of penetration of light.

5.1 **Discovery of new species**

At least 15 species new to science have been discovered during the Bonaire Deep Reef Expedition. Research in these practically unexplored depths will undoubtedly lead to the discovery of more novel species. For example, as part of the Smithsonian Deep Reef Observation Project in Curacao, Baldwin and Robertson (2013) described a new deepwater blennoid fish species from the Dutch Caribbean. The sea covers over 70% of the earth and it is estimated that between 1-10 million species reside in the sea, of which at least one third is still undescribed (Mora et al. 2011, Appeltans et al. 2013). With the description of new species also comes a better understanding of ecosystems. The discovery of unique species may, furthermore, lead to the discovery of novel applications for human use. For example, the toxins of sponges and octocorals have been explored by natural product chemists for pharmaceutical applications since the 60s, and this exploration has resulted in the discovery of antivirals, anticancer agents, enzyme inhibitors, and antibiotics (Blunt et al. 2013). Previous chemical investigations of the deep reef Caribbean sponges *Plakortis angulospiculatus* and *Plakortis halichondrioides* resulted in the isolation of new bio-active compounds that are of interest to the pharmaceutical industry (anti-inflammatory and cytotoxic activity) (Ankisetty et al. 2010).

5.2 Sponges

Identification: R.W.M. van Soest & L.E. Becking (Naturalis Biodiversity Center/ IMARES)

The major focus was on sponges due to their importance in the shallow and deep reef in terms of diversity, filtering activities, biomass, and source of pharmaceutical compounds (van Soest et al. 2012). Marine sponges are dominant players in the reefs and harbour an impressive internal diversity with respect to their associated microorganisms and biochemicals with pharmaceutical potential, and may, therefore, provide the basis for evaluation of sustainable development of this resource in the Dutch Caribbean (Schippers et al. 2012). Sponges are a source of nutrition for fish, turtles, echinoderms; they provide refuge for a diversity of micro-and macro-organisms (e.g. Westinga & Hoetjes 1981, Erwin & Thacker 2008); and they may be a significant source of dissolved organic matter on coral reefs (de Goeij et al. 2013). The Caribbean shallow-water sponge fauna is among the better known of the world's sponge fauna, however our knowledge of deep water sponges of the Caribbean is relatively limited (Hogg et al. 2010)

50 sponge specimens were collected, which represent at least 30 species, of which 11 are new to science. An additional 4 species were observed but not collected, bringing the total number of observed sponge species in Bonaire at 34 species (Table 3). The assemblage of sponges below 100 m. is dominated by glass sponges (Hexactinallida) and stony sponges (Lithistida). In the deep reef of the Bahamas, Van Soest & Stentoft (1988) distinguished three distinct depth zones of sponge assemblages: a zone from 110-137 m. with few sponges and *Spongosorites siliquaria* and *Ciocalypta porrecta* as characteristic species, a zone from 137-172 m. rich in

species and numbers, and a third zone below 208 m. in which *Vetulina stalactites* is the dominant sponge. Though the deep reef sponge fauna in Bonaire is similar to Barbados, these zones were not observed in Bonaire.

L.E. Becking, E.H.W.G. Meesters, and R.W.M. van Soest are preparing a manuscript for publication in a scientific peer-reviewed journal on the sponge diversity of Bonaire's deep reef, including a description of the new species.

Class	Order	Family	Species
Demospongiae	Agelasida	Agelasidae	Agelas flabelliformis
	Astrophorida	Geodiidae	Caminus new species
	Astrophorida	Pachastrellidae	Characella aspera
	Astrophorida	Pachastrellidae	Characella new species
	Astrophorida	Pachastrellidae	Pachastrella sp. aff. abyssi
	Halichondrida	Heteroxyidae	Parahigginsia new species
	Halichondrida	Axinellidae	Phakellia folium
	Halichondrida	Halichondriidae	Spongosorites ruetzleri
	Halichondrida	Halichondriidae	Topsentia pseudoporrecta
	Haplosclerida	Phloeodictyidae	Calyx new species
	Haplosclerida	Phloeodictyidae	Siphonodictyon viridescens
	Haplosclerida	Petrosiidae	Neopetrosia new species 1
	Haplosclerida	Petrosiidae	Neopetrosia new species 2
	Homosclerophorida	Plakinidae	Plakinastrella new species
	Lithistida	Scleritodermidae	Aciculites cribrophora
	Lithistida	Corallistidae	Corallistes typus
	Lithistida	Neopeltidae	Daedalopelta nodosa
	Lithistida	Theonellidae	Discodermia dissoluta
	Lithistida	Theonellidae	Discodermia new species
	Lithistida	Siphonidiidae	Gastrophanella implexa
	Lithistida	Azoricidae	Leiodermatium lynceus
	Lithistida	Neopeltidae	Neopelta perfecta
	Lithistida	Theonellidae	Theonella atlantica
	Poecilosclerida	Acarnidae	Acarnus new species
	Poecilosclerida	Microcionidae	Antho (Acarnia) new species
	Dendroceratida	Darwinellidae	<i>Aplysilla</i> sp
	Poecilosclerida	Hamacanthidae	<i>Hamacantha</i> sp.
	Haplosclerida	Chalinidae	Haliclona sp.
Hexactinellida	Hexactinosida	Tretodictyidae	Cyrtaulon sigsbeei
	Hexactinosida	Dactylocalycidae	Dactylocalyx pumiceus
	Hexactinosida	Euretidae	Verrucocoeloidea new species
Unidentified			Encrusting 1 (unidentidied)
			Encrusting 2 (unidentidied)
			Encrusting 3 (unidentidied)
			Encrusting 4 (unidentidied)

Table 3 List of observed and collected sponges species

5.3 Crustaceans

Identification: C. Fransen (Naturalis Biodiversity Center)

Crustacean fauna associated with sponges and gorgonians was collected. Approximately 20 specimens were collected, which represent at least 7 species (Table 3). One new species of shrimp (genus *Pseudocoutierea*), associated with gorgonians, was found. To date there are six species described within this genus *Pseudocoutierea* in the Atlantic, the majority are associated with Antipatharia (black coral) and Gorgonaria (gorgonians, seafans).

Order	Family	Species
Decopoda	Palaemonidae	Pseudocoutierea new species
		Majid crab
Decopoda	Pandalidae	Plesionika longicauda
Decopoda	Disciadidae	Discias vernbergi
Decopoda	Hippolytidae	Lysmata aff. olavoi
Decopoda	Palaemonidae	Periclimenes pandionis

5.4 **Fish**

Identification: C. Baldwin (Smithsonian Institution, USA)

The majority of the observed fish were small (<15cm in length) and associated with the sediment, hard substrate or tilefish nests. Notable exceptions were amberjacks that were attracted to the sub by the light. For information on lionfish, see Chapter 6.

Table 4 List of observed and collected fish species

Order	Family	Species
Anguilliformes	Muraenidae	Gymnothorax moringa
Perciformes	Apogonidae	Apogon affinis
Perciformes	Apogonidae	Apogon pillionatus
Perciformes	Apogonidae	Apogon pseudomaculatus
Perciformes	Gobiidae	Antilligobius nikkiae
Perciformes	Gobiidae	Gobiidae 1 new species
Perciformes	Gobiidae	Gobiidae 2 new species
Perciformes	Grammatidae	Lipogramma evides
Perciformes	Serranidae	Choranthias sp. new species
Perciformes	Serranidae	Serranus notospilus or S. phoebe
Scorpaeniformes	Scorpaenidae	Scorpaenidae 1
Scorpaeniformes	Scorpaenidae	Scorpaenidae 2
Scorpaeniformes	Scorpaenidae	Pterois volitans

5.5 Octocorals

Identification: L. van Ofwegen (Naturalis Biodiversity Center) and N.K. Santodomingo (Naturalis/British Natural History Museum)

Octocorals represent the gorgonians, soft corals, black coral, and seapens. At least 13 species were observed of which 7 species were collected (Table 6).

Order	Family	Genus	
Alcyonacea	Ellisellidae	Nicella guadalupensis	
Alcyonacea	Ellisellidae	<i>Ellisella</i> sp.	
Alcyonacea	Gorgoniidae	Leptogorgia virgulata	
Alcyonacea	Nephtheidae	Stereonephthya sp.	
Alcyonacea	Nidaliidae	<i>Nidalia</i> sp.	
Alcyonacea	Nidaliidae	Chironephthya sp.	
Alcyonacea	Plexauridae	Bebryce Cinerea	
Alcyonacea	Plexauridae	Hypnogorgia pendula	
Alcyonacea	Plexauridae	Thesea guadalupensis	
Alcyonacea	Primnoidae	Callogorgia gracilis	
Antipatharia	Antipathidae	Stichopathes sp.	
Antipatharia	Myriopathidae	Cupressopathes gracilis	
Pennatulacea	Kophobelemnidae	Sclerobelemnon sp.	

Table 6 List of observed octocoral species

5.6 Hard corals

Identification: N.K. Santodomingo (Naturalis/British Natural History Museum) and B.W. Hoeksema (Naturalis)

No hard corals were collected, but at least 7 species were identified (Table 7).

Order Family Species Scleractinia Caryophylliidae Caryophyllia sp. Scleractinia Caryophylliidae Thalamophyllia riisei Scleractinia Caryophylliidae Desmophyllum dianthus Scleractinia Dendrophylliidae Balanophyllia sp. Scleractinia Flabellidae Javania sp. Scleractinia Stylasteridae Stylastra sp.

Table 7 List of observed hard coral species

5.7 Microbial diversity

The microbial diversity in the collected sponges and octocorals is being analysed by Dr. Detmer Sipkema of the Laboratory of Microbiology of Wageningen University. Universal eubacterial primers will be used to amplify 16S rRNA gene fragments, which will subsequently be sequenced to identify the microbial species residing in sponges and octocorals (Sipkema & Blanch 2010). Marine sponges are reservoirs of many unknown uncultured microbial species. It has been suggested that sponges harbor many specific bacterial species and clades that are not found in other environments (Taylor et al. 2007, Simister et al. 2012). The bacterial assemblages of sponges and coral host are of substantial ecological, biotechnological and pharmaceutical importance (Hentschel et al. 2003 & 2006, Taylor et al. 2007, Webster and Taylor 2012). In many cases the bacterial symbionts are either the source or contribute significantly to the production of bio-active secondary metabolites found in sponges (e.g. Taylor et al. 2007, Erpenbeck and van Soest, 2007). As a result there is heightened interest in these bacteria.

6 Lionfish

Special attention was paid to the invasive Indo-Pacific lionfish (*Pterois volitans* and *Pterois miles*). The lionfish is now a significant threat to coral reef ecosystems throughout the Caribbean. During the dives between the depths of 80-115 m, schools of 10-15 individual lionfish clustered together within an area of 25 m² could be observed. Because lionfish can be cryptic and secretive, the actual numbers present may be much greater than those observed. Individual or pairs of lionfish were observed as deep as 165 m (at DIVE 3), often swimming exposed above open stretches of sand. The maximum observed size was estimated at 40cm.

In three decades, since the first documented Atlantic occurrence in the mid-1980s, the invasive lionfish species has spread from the North Western North Atlantic to the Caribbean and Campeche Bank (e.g. Aguilar-Perera and Tuz-Sulub, 2010, Schofield, 2010). Lionfish are generalist predators of fishes and invertebrates with the potential to disrupt the ecology of the invaded range. The extremely rapid expansion of lionfish represents a potentially major threat to coral reef food webs in the Caribbean region by decreasing the survival of a wide range of native animals (Albins and Hixon, 2008).

Their voracious appetite together with their broad habitat distribution has made lionfish successful colonisers of the Caribbean and a grave threat. In the Indo-Pacific, lionfish are found not only on reefs but also on soft bottoms and in nearshore habitats such as seagrass beds and mangroves, and near estuaries (Barbour et al. 2010, Kulbicki et al. 2012).

In the native locations in the Indo-Pacific lionfish have been reported at maximum depth of 75 m. (Kulbicki et al. 2012) indicating the potential capacity of lionfish to disperse via deep waters. In the Atlantic the invasive lionfish have been caught down to 99 m depth off the Carolinas (Meister et al. 2005) and observed from a submersible at 300 m in the Bahamas (Kulbicki et al. 2012). Detailed data on their maximum depth is, however, lacking and they may occur deeper.

Indo-Pacific lionfish are larger and more abundant on invaded reefs in the Caribbean than in native Indo-pacific waters (Darling et al. 2011). Average density estimates on Bahamian coral reefs of 390 lionfish ha⁻¹ (Green and Cote 2009). This is several times higher than in its native ranges, from 2.2 lionfish ha⁻¹ in Palau (Grubich et al., 2009) to 80 lionfish ha⁻¹ in the Red Sea (Fishelson, 1997). Biological and physical factors that control lionfish densities across its native Indo Pacific range are not yet fully understood (Morris and Whitfield 2009). Lionfish are reported to have few natural predators, though Caribbean groupers could function as a biocontrol of invasive lionfish (Mumby et al. 2011). However, the grouper populations are being overexploited, and thus are unlikely to substantially counter the invasive lionfish threat posed towards Atlantic coral-reef ecosystems (Maljković et al. 2008, Mumby et al. 2011).

A common form of control of lionfish is spearfishing of shallow water populations by (recreational) divers. Recent publications by Morris et al. (2011) and Barbour et al. (2011) indicate that large numbers of lionfish would need to be removed regularly to cause a decrease in the overall population. In the model of Morris et al. (2011) 27% of the adult population per month would need to be collected, in the model of Barbour et al. (2011) between 15–65% each year.

Within the context of the "Natuurbeleidsplan Caribisch Nederland 2013-2017" that stresses the importance of controlling invasive species, further study on the lionfish populations in the Dutch Caribbean should be conducted. There are at present key gaps in knowledge on the size of the populations as well as the migration routes of the lionfish within the Dutch Caribbean.

7 Conclusions & Recommendations

Three exploratory dives were made off the southern coast of Bonaire.

Results indicate that hard structures exist below the light dependent coral reefs. These structures appear to consist of fossil reefs and rhodolits beds. By providing hard substrate in a desert landscape of sand, these fossil reefs displayed heightened biodiversity, with species unknown to science. The results warrants further investigation and inventory of species in the deep reef. Acoustic explorations of the deep waters around Bonaire, also in the northern part, followed by submarine dives should lead to the discovery of many more new species and knowledge about the deep parts of the sea around Bonaire.

Large fields of cyanobacteria were discovered at all sites at depths between 45-90 m. These are cause for concern and further study, as they could be indicative of eutrophication and human disturbance in the reefs.

Invasive lionfish were observed aggregating at artificial structures below 100 m. and were found as deep as 165 m. In order to manage the invasion, further research on the estimates of lionfish population density and distribution is required, particularly in the deep reef.

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10 Quality Assurance

IMARES utilizes an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organization has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

11 Justification

Rapport Project Number: C006/14 BO-11-011.05-023

MA

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved:

Dr. A.O. Debrot

Signature:

Date:

4th of June 2014

Approved:

Drs. F. Groenendijk Head Department Maritime

Signature:

Date:

 4^{th} of June 2014

12 Appendix A. Photo gallery of species

12.1 Sponges

12.1.1 AGELASIDA





Agelas flabelliformis (DIVE1/14)

Agelas flabelliformis (DIVE1/14)





Caminus sp. new (DIVE2/35)



Caminus sp. new (DIVE2/35)



Caminus sp. new (DIVE2/35)



Characella sp. new (DIVE2/31)



Characella sp. new (DIVE2/31)



Characella aspera (DIVE2/36)







Calthropella lithistina (DIVE2/33)



Pachastrella cf. abyssi (DIVE2/27)



Pachastrella cf. abyssi (DIVE2/28)



Pachastrella cf. abyssi (DIVE3/64)



Pachastrella cf. abyssi (DIVE3/64)

12.1.3 LITHISTIDA







Aciculites cribrosa (DIVE3/52)





Aciculites higginsi (DIVE2/29)

Aciculites higginsi (DIVE2/29)



Corallistes typus (DIVE2/34)



Corallistes typus (DIVE2/34)



Corallistes typus (DIVE2/30)



Corallistes typus (DIVE2/30)



Discodermia dissoluta (DIVE2/26)



Discodermia dissoluta (DIVE2/26)



Discodermia sp. new (DIVE1/12)



Discodermia sp. new (DIVE1/12)



Gastrophanella implex (DIVE1/7)



Gastrophanella implex (DIVE1/7)



Gastrophanella implexa (DIVE1/15)





Leiodermatium lynceus (DIVE3/56) Leiodermatium lynceus (DIVE3/63) Leiodermatium lynceus (DIVE3/63)

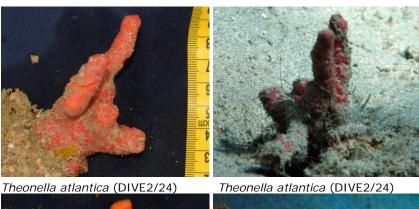




Neopelta perfecta (DIVE2/43)



Neopelta perfecta (DIVE2/43)



Theonella atlantica (DIVE2/25)

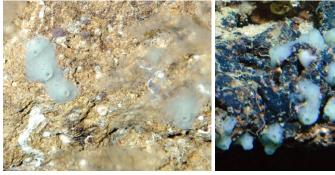


Theonella atlantica (DIVE2/25)



Unidentified Lithistida- not collected - Unidentified Lithistida

12.1.4 HALICHONDRIDA



Parahigginsia sp. new (DIVE3/48)

Parahigginsia sp. new (DIVE3/48)







Spongosorites reutzleri (DIVE2/38) Spongosorites reutzleri (DIVE2/38)

Spongosorites reutzleri (DIVE2/38)



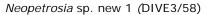
Topsentia pseudoporrecta(DIVE3/50) Topsentia pseudoporrecta(DIVE3/50)

12.1.5 HAPLOSCLERIDA



Calyx sp.new (DIVE3/60)

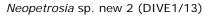






Siphonodictyon viridescens (DIVE3/61)







Neopetrosia sp. new 2 (DIVE1/13)

12.1.6 HOMOSCLEROPHORIDA



Plakinastrella sp. new (DIVE3/49)

12.1.7 POECILOSCLERIDA



Antho sp. new (DIVE1/9)

12.1.8 HEXACTINELLIDA

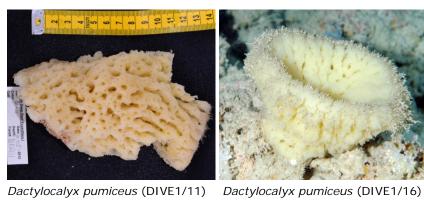


Daedalopelta nodosa (DIVE1/17)





Dactylocalyx pumiceus (DIVE1/10) Dactylocalyx pumiceus (DIVE1/10)





Verrucocoeloidea sp. new DIVE3/46 Verrucocoeloidea sp. new (DIVE3/46)

12.1.9 UNIDENTIFIED – NOT COLLECTED





12.2 Cnidarians (anemones, octocorals, hard corals)







Boloceroides sp.

Boloceroides sp.

Telmactis sp.



Chironephtya sp. (DIVE3/53)



Chironephtya sp. (DIVE3/53)



unidentified



Leptogorgia virgulata (DIVE3/65)



Leptogorgia virgulata (DIVE3/65)



Thesea guadalupensis (DIVE3/66)



Hypnogorgia pendula (DIVE2/39)



Hypnogorgia pendula (DIVE2/39)



Nicella guadalupensis (DIVE2/40)



Nicella guadalupensis (DIVE2/40)



Callogorgia gracilis (DIVE2/41)



Callogorgia gracilis (DIVE2/41)



Not identified



Caryophyllia sp.



Caryophyllia sp.

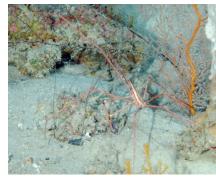


Desmophyllum dianthus



Thalamophyllia riisei

12.3 Crustaceans



Not collected





Plesionika longicauda (DIVE2/42)

12.4 Echinoderms (sand dollars, brittlestars, seastars, basket seastars, crinoids, sea cucumbers)





12.5 Fish

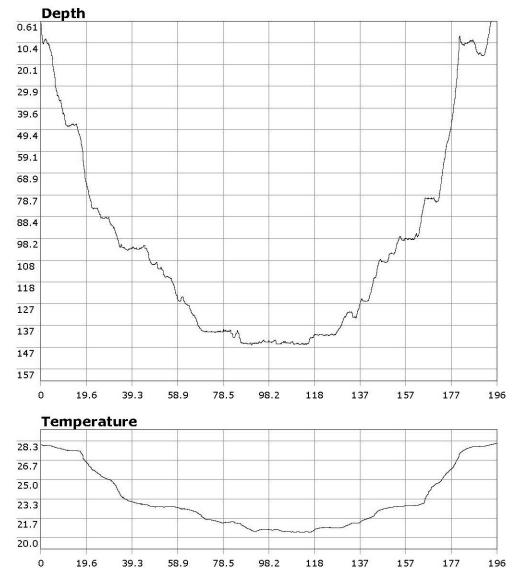




12.6 Mollusks

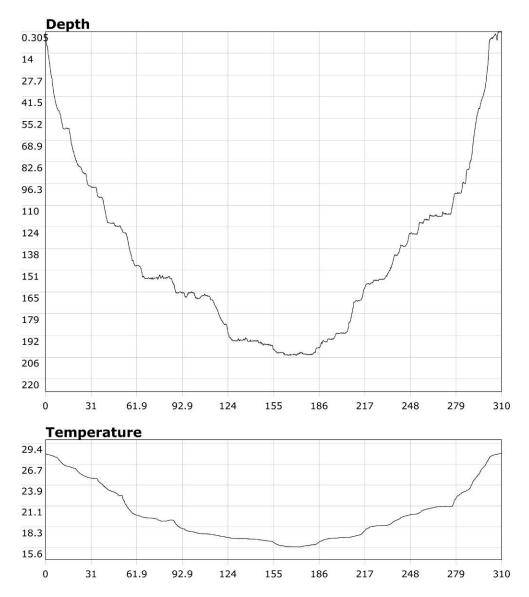


13 Appendix B. Dive profiles



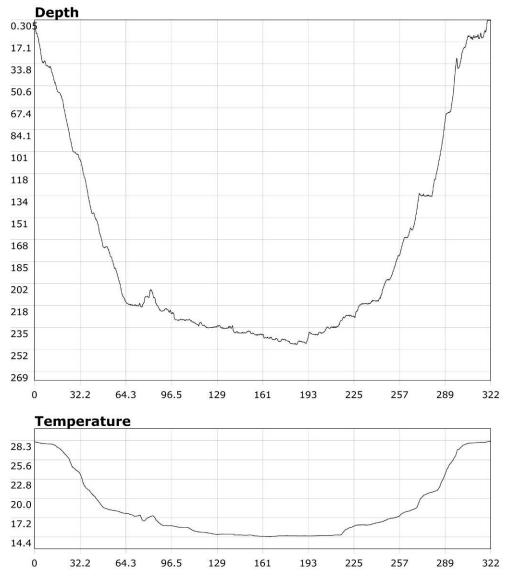
13.1 Dive 1: off Kralendijk

Kralendijk Pier (DIVE 1) depth (m) and temperature (°C) profile (x-axis in minutes of submersible dive time)



13.2 Dive 2: Airport pier

Airport pier (DIVE 2): depth (m.) & temperature (°C) profile (x-axis in minutes)



13.3 Dive 3: Cargill pier

Cargill pier (DIVE 3): depth (m.) & temperature (°C) profile (x-axis in minutes)

14 Appendix C. Media exposure

Newspapers & Magazines

Associated Press Bionews: May 2013 5: p. 2-3 "Bonaire Deep Reef Exploration Underway" Bionieuws: 22 June 2013 Front page Antilliaans Dagblad: 24 May 2013 Antilliaans Dagblad: 16 June 2013 Resource: 10 June 2013 *Volkskrant:* 26 July 2013 Front page and 'Binnenlands nieuws': "Nieuwe garnalen, sponzen en bacteriën in Neerlands rif" (interview met Erik Meesters en Lisa Becking) WageningenWorld 2013 (3). - p. 16 - 17. "<u>Afzakken naar onbekende wateren [mariene ecologie]</u>" Wageningen International

Radio

Labyrinth Radio interview with Erik Meesters: 1 September 2013

Television

Local news Bonaire NOS

Websites:

WUR

http://www.wageningenur.nl/nl/show/Bijzondere-vondsten-in-het-diepe-rif-van-Bonaire.htm http://www.wageningenur.nl/nl/show/Resource-Op-zoek-naar-de-bodemschatten-van-Bonaire.htm http://www.wageningenur.nl/nl/show/Met-duikboot-het-diepe-rif-van-Bonaire-verkennen.htm https://twitter.com/imares_wur

http://resource.wur.nl/wetenschap/detail/op_zoek_naar_de_bodemschatten_van_bonaire/

Other

http://www.naturalis.nl/nl/over-ons/nieuws/expedities/de-diepzee-van-bonaire/ https://twitter.com/NWOALW http://www.natuurbericht.nl/?id=10925 http://www.dcnanature.org/exploring-bonaires-deep-reefs/ http://www.dutchwatersector.com/news-events/news/6159-research-institute-imares-maps-bonaire-s-deepreef-biodiversity-hotspot-with-submarine.html http://www.versqeperst.com/nieuws/215026/curasub-onderzoekt-diepe-rif-bonaire.html http://www.actueelnieuwsnederland.nl/artikel/100007742/bijzondere-vondsten-diepe-rif-bonaire.html http://www.bonaireexclusief.nl/algemeen/nieuws.shtml http://maritiemnieuws.nl/47191/met-duikboot-het-diepe-rif-van-bonaire-verkennen/ http://maritiemnieuws.nl/47381/bonaire-deep-reef-expedition-1/ http://www.offshoremanagement.nl/nieuwsr/106/Met-duikboot-het-diepe-rif-van-Bonaire-verkennen http://globedivers.org/2013/07/06/bonaire-uw-archeology-artifacts-imares-la-jarre-espagnole-1700-1800/ http://waternieuws.blogspot.nl/2013_05_01_archive.html http://www.lachispa.eu/nieuws/uniek-onderzoek-diepe-riffen-bonaire/ http://drimble.nl/nieuws/bonaire/ http://www.rijksdienstcn.com/nieuws/bonaire-deep-reef-expedition-1 http://www.bonaire.nu/2013/05/24/onderzoekers-gaan-per-duikboot-rif-bonaire-verkennen/ http://www.biodiversiteit.nl/nieuws/expeditie-diep-koraalrif-bonaire-van-start/

http://www.nieuws360.com/laatste-nieuws/zand-koraalduivel-en-cyanobacterien-in-diepe-riffen-bonaire/ http://www.easybranches.nl/natuur-nieuws/zand-koraalduivel-en-cyanobacterien-in-diepe-riffen-van-bonaire/ http://www.dolfijnfm.com/nieuws/nieuws-van-de-eilanden/14240-curasub-brengt-rif-bonaire-in-kaart http://www.anp360.nl/plaatsen/Bonaire?date-to=2013-06-30

http://www.bootjesgek.nl/2013/05/duikboot-imares-verkent-rif-bonaire/

http://www.nieuws.be/nieuws/Met_duikboot_het_diepe_rif_van_Bonaire_verkennen_49b88c95.aspx http://ataghans18.wadukuri.com/browser.php?indx=9919403&item=441

http://www.dolfijnfm.nl/nieuws/nieuws-van-de-eilanden/14240-curasub-brengt-rif-bonaire-in-kaart http://bonaireprikbord.com/prive/?p=582

http://phys.org/news/2013-05-exploring-bonaire-deep-reef-submarine.html