EASTERN ATLANTIC EXPEDITION:  
SUBMERSIBLE AND SCUBA COLLECTIONS 
FOR BIOACTIVE ORGANISMS FROM 
THE AZORES TO WESTERN AFRICA

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Research expeditions conducted by Harbor Branch Oceanographic Institution on the R/V Sea Diver in 1990 and on the R/V Seward Johnson in 1991 explored the waters of the Azores, Madeira Islands, Salvage Islands, Canary Islands, Republic of Cape Verde, and the west African countries Senegal and Sierra Leone. The objectives were to collect marine macro- and microorganisms for the purpose of isolating novel, biologically active compounds with potential as therapeutic agents for human diseases. A total of 1,756 samples of macroinvertebrates and algae were collected at 254 sites, utilizing submersible, scuba, trawl, and dredge. During 69 operational days, 28 diver-scientists using DCIEM dive tables made 547 dives, totaling 403 hr bottom time, to depths of 40 m. Deep-water (40 - 914 m) collections with the Johnson-Sea-Link I submersible discovered rugged, steep volcanic slopes off Madeira, Salvage and Canary Islands, and also dense populations of black coral, gorgonians, and sponges. The dominant taxa consisted of Porifera (49.6%), Cnidaria (19.3%), Rhodophyta (8.4%), Echinodermata (8.0%), Phaeophyta (6.2%), Ascidiae (5.9%), Chlorophyta (2.7%) and Mollusca (2.0%). Biological affinities of many of the algae, cnidarians, sponges, and ascidians from Azores, Madeira, Salvage and Canary Islands are Mediterranean and west African. In contrast, the benthic communities in the warmer water of Cape Verde, Senegal, and Sierra Leone had more tropical and Caribbean similarities. Biological activities of sample extracts were tested with cytotoxic (P388), antitumor (A549), cell adhesion (EL4), and antifungal (Candida albicans) assays. Of the 759 samples tested, approximately 10% showed bioactivity.

INTRODUCTION

Research expeditions were conducted by Harbor Branch Oceanographic Institution (HBOI), Division of Biomedical Marine Research (DBMR), in the eastern North Atlantic Ocean during 1990 and 1991 to collect benthic marine organisms for biomedical research. During 1990, from 15 July to 27 October, HBOI's R/V Sea Diver visited the Azores (Portugal), Madeira and Salvage Islands (Portugal), Canary Islands (Spain), Republic of Cape Verde, and the west African countries Senegal and Sierra Leone (Figure 1). This expedition, which lasted 104 days and traveled over 10,000 nmi, used scuba, trawl, and dredge for collections from intertidal to depths of 380 m. During 1991, from May 24 to June 17, HBOI's R/V Seward Johnson revisited Madeira, Salvage and Canary Islands for collections using the Johnson-Sea-Link I submersible to depths of 914 m. These expeditions were coordinated through the U.S. State Department and through the foreign ministries, navy, and visiting scientists for each country.
Previous expeditions to this region of the Atlantic include the HMS Challenger (Wright and Studer 1889), Mercator (Thiel 1941), Albert (Thomson 1927), and Calypso (Forest 1964). The most thorough survey was the Cancap Project by the Rijksmuseum van Natuurlijke Historie from 1976 to 1986 using the Tydeman (Hartog 1984; van der Land 1987). Samples were collected at depths of intertidal to 4,000 m with benthic grab, dredge, trawl, and scuba at 1,574 stations in the Azores, Madeira, Canaries, Cape Verde, and Senegal.

Deep-water submersible collections have used the French bathyscaphe Archimede to study echinoderms, sponges, and anthozoans in the Azores and Madeira (Peres 1972a, b; Sibuet 1972; Laubier 1985). In addition, an expedition by the University of Azores in 1990 used an unmanned submersible, the ROV Minirover, and scuba to survey from the littoral zone to a depth of 100 m in the Azores.

The primary objectives of our expeditions were: 1) to collect benthic marine organisms for research on biologically active novel compounds with potential as therapeutic agents; 2) to isolate and culture microorganisms from sediment and marine invertebrates; and 3) to survey marine flora and fauna for studies of systematics, taxonomy, chemotaxonomy, and ecology.

**METHODS**

In 1991 the 54-m R/V Seward Johnson was used for support of operations with the Johnson-Sea-Link I (JSL) submersible. This 4-person research sub is capable of collections to 914 m, using a manipulator, suction hose, collection baskets, color video and 35-mm cameras, and environmental data logger (Tietze and Clark 1987). In 1990 the 30-m R/V Sea Diver was used for support of scuba dives, dredging, and trawling.

Samples were collected in shallow water (0 - 40 m) by wading, snorkeling, or scuba; in mid-depths (40 - 300 m) with a 0.6-m triangle dredge having 2.2 cm x 0.8 cm (7/8 x 5/16-in) mesh, a 0.25-m meiofaunal dredge, and a 4.5-m otter trawl having 4.7 cm (1 7/8-in) stretch mesh); and in deep water (40 - 914 m) with the JSL submersible. The dredge and trawls were deployed with a hydrowinch and
1200 m of 0.9 cm (3/8 in), 6x19 wire rope.

Each sample was photographed in situ if possible, photographed on deck, and labeled (Pomponi 1988). Museum voucher specimens were stored in 5-10% formalin or 70% ethanol, and samples for subsequent biological and chemical analyses were stored frozen at -10 to -18 C. Complete site and sample descriptions were entered into a computerized database (dBase III Plus).

RESULTS AND DISCUSSION

Site Descriptions

Collections were made in all available coastal marine habitats and included lagoons, estuaries, mangroves, muddy and rocky intertidal, grassbeds, shelf slopes, boulders, canyon, rock walls, ledges, caves, shipwrecks, and concrete seawalls and jacks. The islands of the Azores, Madeira, Salvage and Canaries are primarily volcanic, and in general provide similar hardbottom substrates (Table 1). Nearshore, 1 - 3-m boulders are common along with lava pavement slopes and vertical walls. At Cape Verde, in addition to the volcanic rock walls and boulder slopes, rocky reefs with hard coral are also present.

The west African countries of Senegal and Sierra Leone have relatively scarce hardbottom substrate in the coastal zone compared to the other sites. However, offshore rock pinnacles, pavement, and boulders do provide some habitat for rich communities of soft corals, sponges, and tunicates. Coastal intertidal is predominantly sand beach with mud flats and mangrove extending into river mouths.

Hydrology

Summer surface water temperatures ranged from a low of 18 C in the Azores to 30 C in west Africa (Table 1). The volcanic islands from Azores to Cape Verde had relatively clear water (10 - 30-m visibility) and full salinity. In contrast, high precipitation and river runoff generally result in lower visibility and salinity in coastal Senegal and Sierra Leone.

Deep-Water Habitats

The island slopes off Madeira, Salvage, and Canary Islands are similar to depths of 900 m. At 900 m, sand or mud slopes of 10 - 20° with some rock outcrops were observed. From 600 m to 100 m the bottom is commonly a 40 - 70° slope of rock or sand/mud. Rock ledges and outcrops occur on the slopes, and some sites have extremely irregular volcanic ridges and vertical walls. No strong thermoclines were apparent, and temperatures generally ranged from 10 - 12 C at 900 - 600 m, 13 - 16 C at 600 - 300 m, and 15 - 19 C at 300 - 100 m. Deep-water currents averaged 5 - 15 cm/sec but occasionally exceeded 50 - 100 cm/sec. Visibility typically averaged 20 m.

Collections

A total of 1,756 samples were collected using scuba, (973), submersible (467), dredge and trawl (201), and snorkel and wading (115) (Figure 2a). Although the majority of samples were collected at scuba depths of 3 - 20 m (10 - 60 feet), a total of 572 samples were collected at depths of >46 m (>150 ft) to 914 m (3,000 ft) (Figure 2b). Use of the JSL submersible in 1991 enabled the collection of 194 deep-water samples in Madeira and Salvage Islands and 273 samples in the Canaries (Figure 3a).

A total of 254 sites were sampled, including 46 submersible dive sites, 125 scuba, and 65 trawl and dredge (Figure 3b). During the 1990 collections, 16 scientific divers made a total of 379 scuba dives, with a cumulative bottom time of 282 hours. In 1991, 12 divers completed 168 dives and accumulated 121 hours of bottom time. An average of 7.8, 10.1, and 9.3 samples/site were collected using scuba, sub, and trawling, respectively. Dredging resulted in an average of 2.1 samples/site due to the extremely rugged underwater terrain. Several sites in Madeira and Canaries that produced only
a few samples from dredging were discovered to have a high diversity and abundance of organisms when viewed by submersible. The sub proved much more efficient when collecting on the rocky island slopes and pinnacles. Direct observation and maneuverability of the submersible, and dexterity of the manipulator were essential for successful deepwater collections. In Sierra Leone, where the offshore bottom was primarily flat sand, mud or smooth rock, the trawl also proved efficient.

Table 1. Collection sites and habitats from the 1990 R/V Sea Diver and 1991 R/V Seward Johnson expeditions.

<table>
<thead>
<tr>
<th>Location</th>
<th>Habitat</th>
<th>Surface Temp. (C)</th>
<th>Visibility (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZORES</td>
<td>Volcanic rock slope and wall, caves, lagoon, jetty, 1-5 m boulders, cobble, sand/shell, mud.</td>
<td>18</td>
<td>10-20</td>
</tr>
<tr>
<td>MADEIRA</td>
<td>Volcanic rock slope and wall, caves, 1-5 m boulders, cobble, sand/shell, mud.</td>
<td>21 (Aug)</td>
<td>10-20</td>
</tr>
<tr>
<td>(14-17 Aug 1990, 26 May-1 Jun 1991)</td>
<td>Madeira, Islas Desertas Porto Santo.</td>
<td>18-19 (May)</td>
<td></td>
</tr>
<tr>
<td>SALVAGE ISLANDS</td>
<td>Volcanic rock pavement, slope, boulders, sand.</td>
<td>23 (Aug)</td>
<td>10-25</td>
</tr>
<tr>
<td>CANARY ISLANDS</td>
<td>Volcanic rock slope, wall, caves, tide pools, algal nodules, sand, rock, grass bed.</td>
<td>23-24 (Aug)</td>
<td>10-25</td>
</tr>
<tr>
<td>CAPE VERDE</td>
<td>Volcanic slope, wall, cave, tide pool, coral, boulders, sand/shell, mud, algal nodules.</td>
<td>25-27</td>
<td>10-20</td>
</tr>
<tr>
<td>(6-15 Sep 1990)</td>
<td>Sao Tiago, Fogo, Razo, Branco, Santa Luzia, Sao Vicente, Boa Vista.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SENEGAL</td>
<td>Rock slope, boulders, mud/sand slope, mud flat, mangroves, shipwrecks.</td>
<td>27-30</td>
<td>0-3 (nearshore), 10-15 (offshore)</td>
</tr>
<tr>
<td>(20-26 Sep 1990)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIERRA LEONE</td>
<td>Rock slope, boulders, tide pools, sand flat, mangroves.</td>
<td>28</td>
<td>0-3 (nearshore), 6-9 (offshore)</td>
</tr>
<tr>
<td>(1-7 Oct 1990)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2. a. Total number of samples collected by each method on 1990 and 1991 expeditions.  
b. Depth distribution (feet) of samples.

Figure 3. a. Number of samples by each method and location.  
b. Number of collection sites by each method and location. AZ = Azores, MAD = Madeira/Salvage Island, CAN = Canaries, C.V. = Cape Verde, SEN = Senegal, S.L. = Sierra Leone

Taxonomy and Distribution

The dominant taxa of the 1,756 samples consisted of Porifera (sponges - 49.6%), Cnidaria (hydroids, hard and soft corals, etc. - 19.3%), Rhodophyta (red algae - 8.4%), Echinodermata (sea urchins, starfish, sea cucumbers - 8.0%), Phaeophyta (brown algae - 6.2%), Ascidiacea (tunicates - 5.9%), Chlorophyta (green algae - 2.7%), and Mollusca (2.0%) (Figure 4). Miscellaneous polychaete worms, bryozoans, and crustaceans made up 2.3% of the collections. Sponges were the dominant
taxa at each of the countries except for Senegal, where more cnidarians were collected (Figure 4). Although collections were targeted on sponges, cnidarians, and ascidians, the relative distribution of taxa from the collections tends to reflect the distribution in nature.

Many factors undoubtedly contribute to the species distributions found in these collections. The latitudinal difference from the Azores to Sierra Leone is 3,300 km (1,800 mi), with a surface water temperature gradient of approximately 10°C. The Gulf Stream, which moderates the temperatures of the Azores, splits into the southern-flowing Canaries Current past Madeira, Canaries and Cape Verde. This, in turn, splits into the eastern-flowing Guinea Current along the coast of west Africa and the western-flowing North Equatorial Current. Thus larval dispersion would be possible for eurythermic species with long planktonic stages. Many of the species present in the Azores had Mediterranean affinities, whereas many genera from Cape Verde, Senegal and Sierra Leone had tropical and subtropical western Atlantic similarities. The latter occurrence has been postulated as due to the compression of the Atlantic gyre currents during the last glacial stage, which would allow for quicker transport of larvae from western to eastern Atlantic (Boekschotan and Best 1988). The following descriptions are based on the 1990 collections, which are more completely analyzed than the recent 1991 collections.

Algae

Diversity of macroalgae appeared greatest in the Azores, where a total of 100 species were identified. Populations of red and brown algae were common to depths of 20 - 25 m. In contrast, in Madeira, Salvage, and Canary Islands, dense macroalgal habitats only extended to 1 - 3 m. Below this depth, dense populations of Diadema urchins scoured the rock, and Diadema were observed at depths >100 m in the Canaries. In Cape Verde, Diadema were less dense and had less impact on the macroalgae.

Eleven species of red algae were new distribution records for the Azores and were primarily from the Mediterranean and west Africa (personal communication, S. Fredericq, Smithsonian Institution, 1990). Biogeographical affinities of Azores and Madeira macroalgae were primarily warm temperate Euroafrican (Prud'homme van Reine 1988). The algae in the warmer waters of Cape Verde had stronger tropical and Caribbean affinities (personal communication, S. Reed, Smithsonian Institution, 1990). A species of Chondriella which was collected in Cape Verde was previously reported only from Argentina.

The algal communities of the tropical west African countries Senegal and Sierra Leone were depauperate, possibly due to high turbidity and silt accumulation from river runoff and relatively sparse hardbottom. All the species, however, had pantropical distributions and most had close affinities with Western Atlantic/Caribbean species (personal communication, R. Sims, Smithsonian Institution, 1990). Additional to algal habitats, mangrove communities were only found in Senegal and Sierra Leone.

Porifera

Cape Verde had the greatest diversity of sponges with 58 species, compared to 39 in the Canaries, 33 in Azores, 29 in Senegal, 24 in Sierra Leone, and 21 in Madeira and Salvage Islands. The Cape Verde Porifera were dominated by the orders Choristida (14 species), Dendroceratida (12 species), Poecilosclerida (8 species), and Axinellida (7 species). It is probable that the dense Diadema populations of Madeira, Salvage and Canary Islands have adversely affected the recruitment or survival of invertebrate larvae (e.g., sponges, gorgonians, and ascidians) as well as of macroalgae.

The tropical water of Cape Verde and west Africa had greater diversity of choristids, axinellids, and lithistids compared to Azores, Madeira, and the Canaries. The Cape Verde and west African sponge fauna also showed strong tropical Caribbean affinities.
Figure 4. Phylogenetic distribution of samples collected on 1990 and 1991 expeditions. POR = Porifera, CNI = Cnidaria, CHO = Asciacea, ECH = Echinodermata, MOL = Mollusca, RHO = Rhodophyta, CHL = Chlorophyta, PHA = Phaeophyta, CYA = Cyanophyta, MISC = Miscellaneous.
Ascidiacea

Only 5 species of ascidians were collected from the Azores, Madeira, Salvage and Canary Islands, whereas 34 species were identified from Cape Verde, Senegal and Sierra Leone. Ascidians were particularly abundant in Senegal, and were dominated by the solitary Pyuridae. By contrast, in other tropical areas of the Atlantic, Pacific and Indian Oceans, colonial aplousobranchs predominate (personal communication, F. Monniot, Paris Museum, 1990). This occurrence may be the result of longer larval transport of the Pyuridae, which may allow for colonization on the sparse and widely disjunct hard-bottom habitats of west Africa.

Cnidaria

In the Azores, Madeira, Salvage and Canary Islands, only 10 species of Cnidaria (primarily Anthozoa) were collected, including 3 black corals and one hard coral Madracis. Cape Verde, Senegal, and Sierra Leone showed strong tropical affinities and were represented by 33, 23, and 31 species, respectively. The Gorgoniidae Lophogorgia and Plexauridae Eunicella dominated the octocorallian community at both Cape Verde and Senegal; however, Senegal also had dense populations of Alcyoniidae soft corals. In Sierra Leone, Muriceopsis replaced Eunicella as the dominant plexaurid octocoral. The zoanthid Polythoa was common on the rocky reefs of these tropical countries but was rare in the Canaries and absent to the north in Madeira and Azores. Although there are no true coral reefs in Cape Verde and tropical west Africa, hard coral communities exist which are dominated by Porites, Siderastrea, and Millepora (IUCN 1988).

Submersible Collections

The deep-water (40 - 914 m) submersible collections in Madeira/Salvage Islands and Canary Islands in 1991 were dominated by Cnidaria (26 and 22 species, respectively), and Porifera (52 and 41 species, respectively). The Anthozoa were dominated by the families Antipathidae, Paramuriceidae, Dendrophylliidae, Elliselliidae, and Primnoidae. The gorgonians Isidella, Paramuricea, Ellisella, and Calliagoria, and also 7 species of Antipathes black coral, were common to abundant at all depths from 100 to 700 m. Two species of the scleractinian Dendrophyllia were also common at 100 - 335 m depths.

Dominant deep-water sponge orders were Choristida, Halichondrida, Axinellida, Haplosclerida, and Lithistida. The axinellids Phakellia and Teichaxinella were common between 100 and 365 m. The choristids Pachastrella, Poecillastra, and Stelletta were also common along with the halichondrid Spongosorites and haplosclerid Strongylophora. The stony lithistid sponges were dominated by Corallistes, Discodermia, and Theonella. Although the hexactinellid glass sponges Euplectella and an unidentified Hexactinellida were relatively uncommon, some sites had dense populations of Hyalonema, which were up to 1 m in height, and were shaped like inverted sombrero hats.

Biochemistry

Crude extracts of 759 samples from the 1990 expedition were screened with the following assays to test for biologically active compounds: P388, mouse leukemia cell, cytotoxicity screen; A549, human lung cancer cell, antitumor screen; EL-4, mouse lymphoma cell, cell adherence assay and screen for protein kinase C enzyme activators; Candida albicans, pathogenic fungus, antifungal screen (Table 2).

Approximately 10% of the samples that were tested proved to be active. Although 13.8% (104) of the samples showed A549 activity, only 7.8% (59) had concurrent low P388 cytotoxicity which could be indicative of a suitable antitumor compound. Sponges, brown algae, and ascidians showed the greatest percent activity. Other phyla, however, which had few specimens collected, e.g. mollusks, echinoderms, bryozoans, and polychaetes, also had active species. Several species which had strong bioactivities at low concentrations have been identified from these collections.
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underway on these lead samples to isolate the active compounds and to elucidate the chemical structures.

Table 2. Bioactivity summary of samples from 1990 R/V Sea Diver expedition. POR = Porifera, CNI = Cnidaria, CHO = Ascidiacea, RHO = Rhodophyta, PHA = Phaeophyta.

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>POR</th>
<th>CNI</th>
<th>CHO</th>
<th>RHO</th>
<th>PHA</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388</td>
<td>10.7</td>
<td>12.4</td>
<td>21.9</td>
<td>5.0</td>
<td>7.7</td>
<td>9.7</td>
</tr>
<tr>
<td>A549</td>
<td>23.7</td>
<td>10.1</td>
<td>17.1</td>
<td>6.0</td>
<td>2.6</td>
<td>13.8</td>
</tr>
<tr>
<td>EL4</td>
<td>12.1</td>
<td>7.0</td>
<td>7.3</td>
<td>3.0</td>
<td>12.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>16.1</td>
<td>7.0</td>
<td>12.2</td>
<td>7.0</td>
<td>23.8</td>
<td>13.2</td>
</tr>
<tr>
<td>No. of samples</td>
<td>298</td>
<td>129</td>
<td>41</td>
<td>100</td>
<td>78</td>
<td>759</td>
</tr>
</tbody>
</table>

SUMMARY

Expeditions by Harbor Branch Oceanographic Institution were conducted in 1990 and 1991 with the R/V Sea Diver and R/V Seward Johnson to the Azores, Madeira, Salvage and Canary Islands, Republic of Cape Verde, and the west African countries Senegal and Sierra Leone. A total of 254 sites were sampled with the Johnson-Sea-Link I submersible, scuba, trawl, and dredge from intertidal to depths of 914 m. The dominant taxa of the 1,756 samples were Porifera (49.6%), Cnidaria (19.3%), and macroalgae (17.3%). Biogeographical affinities for many of the algae, sponges, anthozoans, and ascidians from Azores, Madeira, Salvage and Canary Islands were Mediterranean and west African. In contrast, the benthic communities in the warmer waters of Cape Verde, Senegal, and Sierra Leone had more tropical and Caribbean similarities. Deep-water (40 - 914 m) submersible collections discovered rugged, steep volcanic slopes off Madeira, Salvage and Canary Islands, and also dense populations of black coral, gorgonians, and sponges. Approximately 10% of the samples that were screened for bioactivity showed positive results among the four assays. Species with the best activities will be further analyzed to isolate the active compounds and to determine the chemical structures.

ACKNOWLEDGMENTS

We thank the field biologists, chemists, divers, and crews of R/V Sea Diver, R/V Seward Johnson, and Johnson-Sea-Link I submersible who endured the long hours at sea and made the expeditions possible. The following taxonomists assisted with field collections and species identifications: Walli deWeerdt (Smithsonian Institution, Porifera), Suzanne Fredericq (Smithsonian Institution, macroalgae), Sherry Reed (Smithsonian Institution, macroalgae), Robert Sims (Smithsonian Institution, macroalgae) and Francoise Monniot (Paris Museum, asciidae). We also thank all the foreign scientists and observers who helped on both expeditions: Helen Martins, Ester Serrao, Manuel Biscoito, Miguel Moreira (Portugal); Fatima Martin, Jose Bernabe, Ricardo Haroun (Spain); Ivone Fernandez, Angelo Cardosa (Cape Verde); Abdoulaya Djiba, Chiekh Cossoko (Senegale); Ernest Ndmahina (Sierra Leone). This is contribution number 934 from Harbor Branch Oceanographic Institution, Inc.

LITERATURE CITED


