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Experimental settlement study in the Eastern Mediterranean deep sea (Ionian Sea)

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ABSTRACT

In this study, we examined the potential growth of organisms on different artificial substrates on a bathymetrical transect in the deepest part of the Mediterranean Sea in the Ionian Sea (Nestor site) after long-term deployment. As this area is characterized by small temperature and salinity fluctuations between the deep-water layers, it is an ideal study site to determine variations in the community structure of settled organisms with depth and material type. Four new experimental platforms (GKSS prototype), each in one depth (4500, 3500, 2500 and 1500 m) and with five artificial substrates (titanium, aluminum, glass, limestone and shale) in two orientations (horizontal and vertical) were deployed for 155 days at the study site. After retrieval all substrates in every depth were visually inspected. The absence of macrofouling was evident and only a loosely adhered biofilm could be observed. Scanning Electron Microscopy revealed the presence of substrate attached bacteria although a direct counting could not be achieved. With the use of molecular-biological approaches substrate attached bacteria in biofilms could be detected at all depths, substrates and both orientations.

1. Introduction

Surfaces exposed to the marine environment typically develop a layer of attached organisms, a condition that is generally called biofouling [1]. Research on fouling is vast and has a wide variety as the environmental and socioeconomic damages have pointed out the need to understand the process of settling and to create antifouling compounds [2–6]. Furthermore, recent concern about fouling problems of very expensive oceanographic instruments that are deployed in long-term studies, have pointed out the need to better understand biofouling. Several studies showed that biofouling in general induces scattering and absorption of optical signals which comprises the integrity of data, that it reduces significantly equipment lifetime [7–9] or can cause biocorrosion [10]. In order to secure correct data transmission and equipment lifetime and to protect them from biofouling, it is important to understand the nature of biofouling and sedimentation in deep sea waters.

Biofouling research in shallow waters is vast compared to deep waters. Generally due to technical difficulties, in situ experimental studies to test hypotheses on biofouling communities in the deep sea are lacking. Some work has been done on biofilm development at extreme depths (3500 m) in the Arctic Ocean [11], mid-ocean hydrothermal vents [12] and recently at a depth of 2400 m in the Western Mediterranean [13]. Epibenthic megafauna and their influence on deep-sea benthic communities was studied in the Arctic using a long-term observatory at 2500 m water depth [14]. Between these extremes research has been done only for biofilms along one transect from euphotic to aphotic depths (0–160 m) in the Clyde Sea [15] and in the Indian Ocean (500, 1200, 3500, and 5100 m depth) focusing on the role of micro- and macro-biological growth on corroded surfaces [16,17].

Despite the technical difficulties of experimental work in the deep-sea, there is a need for experimentation to understand the ecology and the processes regulating the structure of deep-sea communities [18]. With the future perspective to deploy a neutrino telescope in the Mediterranean Sea an experimental study was conducted at the Nestor 4.5 site for observing, to the authors' knowledge for the first time, organisms that settle on artificial substrata in the deep-sea after a long-term deployment of approximately half a year.

2. Study site

The Eastern Mediterranean Sea is one of the most oligotrophic marine environments known. It has a high nitrate to phosphate (N:P) ratio (28:1) in the deep water and a highly unusual P limitation of the primary productivity [19–21]. The phosphorous limited system corresponds with a low phytoplankton biomass and primary

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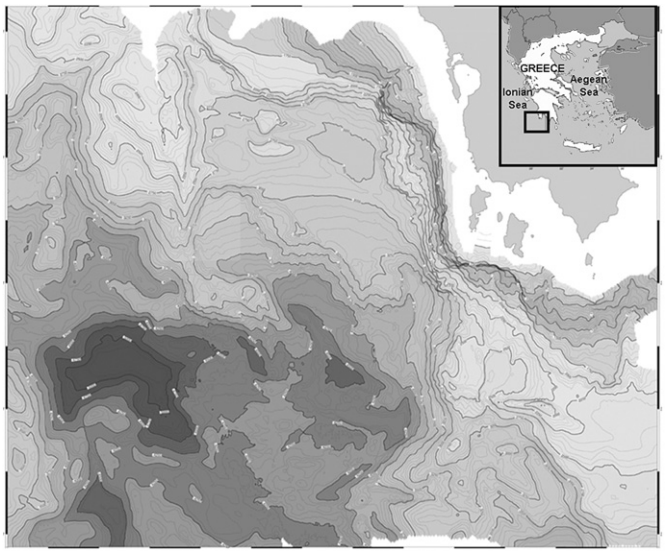

Fig. 1. Multibeam map of area under investigation (source: HCMR).

production [22–25]. The proposed study site lies in the Ionian Sea (Fig. 1), at the deepest part of the Mediterranean with 5121 m depth [26], far from effluents of major rivers thus resulting in extremely clear water [27]. This area is characterized by small temperature and salinity fluctuations between the deep-water layers [28]. The area has been proposed for the deployment of a neutrino telescope.

3. Material and methods

3.1. Experimental setup

A new experimental platform was constructed at the GKSS (Geesthacht, Germany/First-identity number 2006-9876-a) (Fig. 2) that hosts five different materials with a surface of ($5 \times 5 \times 0.5$ cm) each with seven replicates in two orientations (horizontal and vertical). Each device ($700 \times 627 \times 226$ cm) had a closing mechanism using an acoustic deep-sea releaser (CUM Model) for protecting a wash-off effect during retrieval. Four of these devices were deployed in May 2007 with R/V Aegaeo using mooring line technology at four depths (1500, 2500, 3500 and 4500 m). The mooring line was retrieved in October 2007 during a research cruise with R/V Aegaeo. Total deployment duration was 155 days.

Artificial substrates—Materials used in this study were: two metals (titanium, aluminum), two materials that are typical natural hard substrates in the Mediterranean (limestone, shale) and glass (used for the spheres of the photo multipliers of the neutrino telescopes).

3.2. Collection of samples

Immediately after the mooring line was retrieved all samples were visually documented. Two replicates of each sample (according to substrate, depth and orientation) were sampled and conserved in 5% Formaldehyde for later Scanning Electron analyses. For the molecular-biological analyses samples were taken with a sterile swap, put into a sterile Eppendorf tube. All samples were than immediately stored at -20 °C until further processing in the laboratory.

3.3. Scanning electron microscopy

For counting and identifying organisms settled on the artificial substrates Scanning Electron Microscopy (SEM) was used [17,29,30].

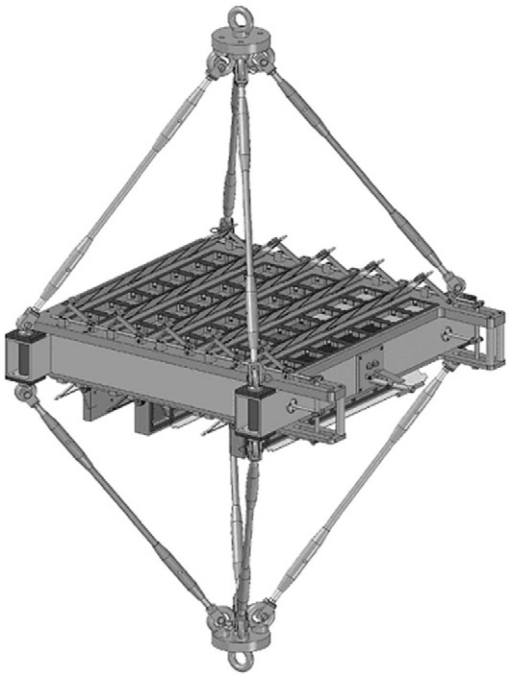

Fig. 2. Experimental prototype sampler (GKSS—Geesthacht, Germany).

Therefore all samples were passed through washing steps with double distilled water and lightly blotted [31]. Afterwards samples were placed in Alpha 1–2 freeze drier (Martin Christ GmbH). All freeze dried samples were sputter-coated with gold in a Cressington 100 auto Cool Sputter Coater. Samples were then photo documented with Nikon D 100 camera that was attached to a CamScan Scanning Electron Microscope (SEM) Series 4. For each sample five random photo-replicates at a magnification of 10 000, 5000, 2000 and 500 were taken. A total of 560 SEM photos were analyzed.

3.4. Molecular biological analyses

For detecting if in all samples substrate attached bacteria are present, the swap samples taken directly after the retrieve were used for extracting 16S DNA. This was done for each sample with the use UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc.) following the manufacturer's recommendations. Integrity of DNA was checked on 1% (wt/vol) agarose gels. DNA concentrations were determined fluorometrically with ND-1000 Spectrophotometer (NanoDrop Technologies, Inc.). Extracted DNA was than used for PCR amplification. The primers used for PCR were 6-carboxyfluorescein (6-FAM)-labeled primer 27F (59-AGA GTT TGA TCC TGG CTC AG-39) and 1492R (59-GGT TAC CTT GTT ACG ACT T-39) [32], which give approximately a 1503-bp product of the 16S rDNA gene. PCR products were than purified with the Qiaquick PCR Purification Kit (Qiagen). Purified DNA concentrations were determined fluorometrically with ND-1000 Spectrophotometer (NanoDrop Technologies, Inc.). Integrity of DNA was checked after the PCR and purification on 1% (wt/vol) agarose gels.

4. Results and discussion

After deployment duration of 155 days the absence of macro-organisms was evident in all four depths, five substrate types and orientations in this extreme environment. In a long term deployment study conducted in the extreme habitat of Arctic at