Marine Pollution Bulletin 95 (2015) 81–88

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

The effect of sediment mimicking drill cuttings on deep water rhodoliths in a flow-through system: Experimental work and modeling

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A R T I C L E I N F O

Article history:
Received 4 February 2015
Revised 17 April 2015
Accepted 18 April 2015
Available online 29 April 2015

Keywords:
Environmental impact
Sediment load
Photosynthesis
Correlation analysis
Calcareous algae
Multivariate data analysis

A B S T R A C T

The impact of sediment coverage on two rhodolith-forming calcareous algae species collected at 100 m water depth off the coast of Brazil was studied in an experimental flow-through system. Natural sediment mimicking drill cuttings with respect to size distribution was used. Sediment coverage and photosynthetic efficiency (maximum quantum yield of charge separation in photosystem II, $\phi_{PSIImax}$) were measured as functions of light intensity, flow rate and added amount of sediment once a week for nine weeks. Statistical experimental design and multivariate data analysis provided statistically significant regression models which subsequently were used to establish exposure–response relationship for photosynthetic efficiency as function of sediment coverage. For example, at 70% sediment coverage the photosynthetic efficiency was reduced 50% after 1–2 weeks of exposure, most likely due to reduced gas exchange. The exposure–response relationship can be used to establish threshold levels and impact categories for environmental monitoring.

1. Introduction

Rhodolith beds are benthic communities dominated by calcareous red algae (Rhodophyta: Corallinales and Sporolithales) which build calcified nodules (rhodoliths) (Bosence, 1983; Foster, 2001). Rhodoliths with multi spherical structures are classified within the morphological group "boxwork" (Basso, 1998). Due to the structure, the rhodoliths are inhabited by other organisms increasing the biodiversity of soft-bottom communities (Bordehore et al., 2003; Steller et al., 2003; Figueiredo et al., 2007; Harvey and Bird, 2008; Sciberras et al., 2009) and therefore they play an important ecological role in coastal areas (Hall-Spencer, 1998; Ávila and Riosmena-Rodriguez, 2009; Steller et al., 2009; Riosmena-Rodriguez et al., 2010).

The largest occurrence of rhodolith beds in the world are found in the southwest Atlantic along most of the Brazilian continental shelf (Kempf, 1970; Foster, 2001; Lavrado, 2006; Amado-Filho et al., 2012). Calcareous algae are found down to approximately 250 m depth (Littler et al., 1986, 1991). These communities may be disturbed and have a potential to get buried due to natural sedimentation and anthropogenic activities such as fish-trawling and mining (Nelson, 2009). Rhodolith beds are increasingly exposed to discharges of drill cuttings from oil and gas activities, for instance in the Gulf of Mexico and on the Brazilian shelf (Davies et al., 2007). It has been demonstrated that fine sediments (<250 μm grain size) may reduce the photosynthetic activity of coralline algae to a larger extent than coarse calcareous sediments from shallow nutrient-rich estuarine and coastal environment (Wilson et al., 2004; Harrington et al., 2005; Riul et al., 2008) as well as deep rhodolith soft-bottoms (Villas-Bôas et al., 2014). The ability to withstand sedimentation varies greatly among calcareous algae species (Harrington et al., 2005; Villas-Bôas et al., 2014). Different species may have different survival strategy towards sedimentation, for example slow growth and low metabolic demand, shooting branches above thallus surface, translocation of photosynthesis through cell-fusions from healthy to damaged area of thallus, or tilting (Steneck, 1986; Dethier and Steneck, 2001).

Abbreviations: PLS, partial least squares; $\phi_{PSIImax}$, photosynthetic efficiency (maximum quantum yield of charge separation in photosystem II).

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http://dx.doi.org/10.1016/j.marpolbul.2015.04.040
0025-326X/

The purpose of the present study was to measure the possible impact of sediment coverage on two rhodolith-forming calcareous algae species collected at approximately 100 m water depth at the Peregrino oil field off the coast of Brazil.

Due to the slow growth of the calcareous algae (Adey and Macintyre, 1973; Foster, 2001), photosynthetic efficiency (maximum quantum yield of charge separation in photosystem II, $\phi_{PSIImax}$) was selected as endpoint. A decrease in photosynthetic efficiency is generally considered to be a response to environmental stress (Genty et al., 1989; Wilson et al., 2004; Evertsen and Johnsen, 2009).

Natural sediment mimicking drill cuttings with respect to size distribution was used in the study. Although water based drilling fluids consist of water soluble and non- or low-toxic components (Bakke et al., 2013) some laboratory studies on single species (Larsson and Purser, 2011; Larsson et al., 2013) and soft bottom seabed communities (Trannum et al., 2010; Bakke et al., 2013) have shown larger effects of drill cuttings (water based) compared to natural sediments. However, initial short- and long-term toxicity testing with the algae species used in the present work verified that there was no significant difference in photosynthetic efficiency when comparing sediment and drill cuttings from Peregrino (Reynier et al., in press). It was therefore assumed that this sediment was considered relevant as a surrogate for the drill cuttings from Peregrino. Besides, discharge of drill cuttings from the laboratory experiments would not be permitted.

Sediment coverage and photosynthetic efficiency were studied as functions of light intensity, flow rate and added amount of sediment for nine weeks in an experimental flow-through system especially built for the experiments. Statistical experimental design and multivariate data analysis and statistics have been useful in previous toxicological and environmental studies (Eide and Johnsen, 1998; Søfteland et al., 2009) and were used to obtain multivariate regression models. These models were subsequently used to establish exposure–response relationship for photosynthetic efficiency as function of sediment coverage. In addition, color changes indicating stress or mortality of the calcareous algae were recorded.

2. Material and methods

2.1. Sampling site

The organisms used in the present study were collected at the Peregrino oil field 80 km off the coast of Brazil, in the Campos Basin area. Drilling started in November 2010 and the field came in production in May 2011. The water depth in the area is approximately 100 m. The seabed sediments consist in general of sand and silt with a hard surface (Salgado et al., 2010). In order to avoid impact of drilling activities to the calcareous algae habitat, the drilling platform and the discharge were located approximately 1.5 km away from the habitat.

2.2. Species and their environment

A field assessment was carried out at the Peregrino oil field to identify rhodolith-forming species (Tâmega et al., 2013). Rhodoliths and the associated fauna were collected by dredging 22 sampling sites at 94–103 m depth in June and November 2010 and April 2011. For specimen identification, a selected number of the calcareous algae samples were preserved in 10% formalin solution, and associated fauna samples were preserved with magnesium chloride (8%) for 30 min and then fixed in ⩾70% alcohol. The main organisms identified were the long lived encrusting calcareous algae and bryozoans.

The choice of species for the experiments was based on their ecological importance and abundance at the Peregrino oil field, their resistance to sampling, and survival under laboratory conditions. For species identification the calcareous algae were sectioned using histological techniques for optical microscopy according to Moura et al. (1997) and identification followed descriptions of Woelkerling (1988). Two of the most abundant species of encrusting calcareous algae covering rhodoliths were chosen. Organisms for the exposure studies were kept alive in a flow-through system under controlled levels of temperature and light intensity (15 °C and 15 μmol m^{-2} s^{-1}, respectively) similar to natural conditions.

2.3. The calcareous algae

The two dominant species of calcareous algae, *Mesophyllum engelhartii* (Foslie) Adey and *Lithothamnion* sp. (Figueiredo et al., 2012), were chosen for the exposure studies. Rhodoliths free of epibionts and with the most common size (40–60 mm in average diameter) and shape (compact-bladed to bladed) were selected and tagged with numbers glued by epoxy putty (TUBOLIT MEN) to their surface. The calcareous algae used in the exposure studies were healthy specimens, purple to pinkish in surface color with bleached spots.

2.4. Sediment

Natural sediment mimicking drill cuttings with respect to size distribution was used in the study. Composition of the sediment grain size was based on sieving analysis of drill cuttings from one of the wells at the Peregrino oil field (well A-18).

The dominant drill cutting particle size is medium to fine (63–250 μm). Natural sediment was sieved and the fine and coarse fractions were mixed at a ratio of 3:1 in order to mimic drilling particles. Initial toxicity testing verified that this natural sediment and the drilling particles from Peregrino had comparable effects on the two calcareous algae species (Reynier et al., in press).

2.5. The flow-through system

A novel large-scale flow-through system was designed to test the effects of particles on selected species as a function of added sediment amount, light intensity, water flow rate and exposure time. The entire flow-through system consisted of four units with eight loops in each unit placed in a rack system. Each loop consisted of an exposure chamber where the organisms were positioned. The water movement was driven by a propeller as illustrated in Fig. 1. Water flow could be varied within each of the four units. Light intensity and added amount of sediment could be varied independently within each loop. Each exposure chamber was optically shielded from its neighbors and was illuminated by three blue diodes (peak wavelength 465 nm) of the Lambertian type (Seoul Semiconductor type B42182). The entire experimental system of four units, each with eight loops, was placed inside a temperature regulated chamber with the same temperature as the inlet water to avoid temperature fluctuations. A cooling system with the capacity to cool 2 L min^{-1} of sea water to 15 °C was established outside the cooled room. In addition to a biological filter the water was mechanically filtered using 25 μm and 5 μm filters in series. A 20 L seawater reservoir for the chilled water was placed inside the cooled room.

2.6. Statistical experimental design

Photosynthetic efficiency and sediment coverage were studied as function of the three design variables light intensity, flow rate