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Experimental effects of sediment burial and light attenuation on two coralline algae of a deep water rhodolith bed in Rio de Janeiro, Brazil

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Abstract – A dense rhodolith bed on deep-water soft bottoms in the Peregrino oil field in Campos Basin, Brazil was recently described. This critical habitat is increasingly subjected to disturbances that promote massive sediment dislodgment. This study aimed to test the combined effects of sediment burial and light attenuation on two main rhodolith-forming coralline algae. Experiments were conducted using the dominant algae *Mesophyllum engelhartii* and *Lithothamnion* sp. Color changes were measured as a response to burial with a thin layer of fine and coarse sediments compared to uncovered samples at two natural light levels. A mesocosm system exposed species to combined treatments of light and burial by sediments that mimic drill cuttings. *M. engelhartii* bleached after 75 days and *Lithothamnion* sp. earlier than that, at 41 days, when buried by fine sediments. Sediments had a strong negative effect on the photosynthesis of coralline algae species within two weeks. Low light levels are not a problem for these deep-water coralline species, but fine sediments have a negative effect after a relatively short time. *Lithothamnion* was more sensitive than *M. engelhartii* in terms of color changes but less sensitive in terms of their fluorescence responses to burial.

Deep-water rhodoliths / sediment disturbance / southeastern Brazil

Résumé – Un lit de rhodolite dense a récemment été découvert dans les eaux profondes du champ de pétrole Peregrino, dans le Bassin de Campos, Brésil. Cet habitat est de plus en plus sujet aux perturbations qui provoquent des déplacements massifs de sédiments. Cette étude a pour objectif de tester les effets combinés de l'enterrement des sédiments et de l'atténuation de la lumière sur les deux principales algues corallines formant les rhodolites. Des expériences ont été réalisées utilisant les algues dominantes : *Mesophyllum engelhartii* et *Lithothamnion* sp. Les changements de couleur ont été mesurés en réponse à l'application d'une couche mince de sédiments fins et épais, en comparaison à des échantillons non recouverts à deux niveaux de lumière naturelle. Un système de mésocosmes a exposé les espèces à des traitements combinés de lumière et d'enterrement

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par imitation de forage. Recouverts de sédiments fins *M. engelhartii* blanchit après 75 jours et *Lithothamnion* sp. au bout de 41 jours. En deux semaines, les sédiments ont un fort effet négatif sur la photosynthèse des espèces de coralline. Des niveaux faibles de lumière ne sont pas un problème pour ces espèces de coralline en eau profonde alors que des sédiments fins ont un effet négatif assez rapide. *Lithothamnion* s'est montrée plus sensible en changements de couleur mais moins sensible que *M. engelhartii* en réponses fluorescentes à l'ensevelissement.

Rhodolithes en eau profonde / pertubation des sediments / Brésil Sud-Est

INTRODUCTION

Terrestrial runoff increases turbidity and therefore sedimentation in coastal environments, threatening coral reefs and shallow-water rhodolith beds in the tropical northeastern region of Brazil. These are the most likely limiting factors for shallow water rhodolith beds under various environmental conditions (Leão *et al.*, 2003; Dutra *et al.*, 2006). In rhodoliths collected from a temperate shallow rhodolith bed, it was demonstrated that burial by fine anoxic sediments can be especially lethal compared to smothering stress caused by partially covering the rhodoliths with gravel or coarse sand (Wilson *et al.*, 2004). Whereas in rhodoliths collected from a tropical shallow rhodolith bed the decrease in both net and gross production rates resulted in a significant decrease in the oxygen production rates of the tested species, clearly demonstrating that burial by a thin sediment layer has a negative impact on the tested rhodolith species (Riul *et al.*, 2008).

Considered a priority area for marine conservation and a major oil production area of Brazil, Campos Basin has the potential for high biological diversity, but it is poorly studied. This critical habitat is increasingly exposed to a range of natural and anthropogenic disturbances that promote massive sediment dislodgment, including calcareous mining, fish dredging and drill-cutting discharges from oil exploration (Radziejewska & Stoyanova, 2000; Jones *et al.*, 2006). This study aimed to test the combined effects of sediment burial and light level attenuation on two main rhodolith-forming coralline algae under simulated short-term disturbances. It was conducted with rhodoliths collected from the Peregrino oil field in Rio de Janeiro State, Brazil, where a dense aggregation of rhodoliths and associated fauna was recently described on offshore soft bottoms at depths of approx. 100 m (Figueiredo *et al.*, 2012; Tâmega *et al.*, 2013).

MATERIAL AND METHODS

Rhodoliths and sediments were collected by dredging at 90-100 m depths offshore from the Cabo Frio region (23°17'776"S-41°14'218"W; 23°21.2´S-41°17'05"W). In the laboratory, samples were sorted into two dominant species of coralline algae, *Mesophyllum engelhartii* (Foslie) Adey and *Lithothamnion* sp. Two experiments were carried out using samples of these rhodolith-forming algae.

The two coralline algae had a thin thallus; that of *M. engelhartii* measured 48-125 μm and that of *Lithothamnion* sp. 50-110 μm in thickness.

Measurement of stress in coralline algae

Changes in coralline algae thallus color (e.g., Figueiredo *et al.*, 2000) and chlorophyll fluorescence analysis (Pulse Amplitude Modulation, PAM), the latter defined by maximum photochemical efficiency or quantum yield on dark-acclimated samples (Kuhl *et al.*, 2001; Wilson *et al.*, 2004; Burdett *et al.,* 2012) or maximum effective quantum yield on light-acclimated samples (Harrington *et al*, 2005), are two non-invasive methods recently used to measure the photosynthetic responses of red coralline algae to stress conditions.

Color change experiment

This experiment was conducted in four aquariums (each 400 L) with the temperature maintained at 15°C using a cooling system. In each aquarium, a pump was used to recirculate the seawater, and the water was partially replaced (20%) each week.

One fragment of a single coralline algae species (approx. 20 mm in diameter) was glued into a plastic Petri dish with underwater epoxy putty (TUBOLIT MEN), and in each dish was fitted a cage made of a plastic frame wrapped with a net (20 μm mesh size) to hold sediments.

Peregrino sediments are of carbonate origin (87%) and consist of gravel (24%), coarse sand (54%), fine sand (16%) and silt (6%). Drill-cutting sediments are made up of coarse sand (28%), fine sand (42%) and silt (30%). The Peregrino sediment was oven-dried at 100°C, sterilized in an autoclave and sieved into two particle size fractions: fine sediments (< 250 μm), which represent the dominant fraction of drill-cuttings, and coarse sediments (250-500 μm), which are dominant at the Peregrino oil field. According to Kjeilen-Eilertsen *et al.* (2004), the most conservative Predicted No Effect Threshold (PNET) for burial by drilling particulate matter is 5 mm. When the thickness of the layer of settled particles exceeds the PNET of burial depth, there is a risk of a negative effect at that location. Therefore, we tested the PNET, which represents the sensitivity of the rhodolith ecosystem, the threshold value for adverse effects caused by burial of the calcareous algae.

Replicate Petri dishes ($n = 4$ for each coralline algae species) were either covered by a thin layer (5 mm thickness) of fine or coarse sediments or not covered (controls) at each light level (3 and 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$), corresponding to the irradiance levels of the natural site. Twelve dishes, one for each treatment and each species, were placed into each of the four aquariums. A blue-wavelength light source was used to provide appropriate light spectra and irradiance levels close to those measured in the field.

The responses of coralline algae to sediment burial and shade were measured by the color changes on the thallus surface. These changes were recorded in the form of photographs taken after 34, 41 and 75 days in culture. The color images were analyzed by matching their color to a standard color chart that represents the color tone and intensity at a scale from 0 to 10 (method in Figueiredo *et al.*, 2000).

Fluorometry Fv/Fm experiment

This experiment was conducted in a mesocosm flow-through system exposed to combined levels of environment variables kept in a refrigerated room. For this experiment, the mesocosm system consisted of two units (racks) with six loops (chambers) in each unit, all made of Plexiglass. The inner diameter of the exposure chambers is 100 mm, and the length is 700 mm. Pumping by propellers provided a water circulation capacity of approx. 5600 L per hour. On the top of each chamber at a distance of 0.16 to 0.20 m, there were three LED bulbs adjusted to produce the desired levels of irradiance.

Seawater was cooled to a temperature of 15°C, simulating the most frequent temperature of the sea bottom, and the blue-wavelength light source was adjusted to provide 50 and 100% light levels (6 and 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The latter refers to the most frequently measured irradiances at the sea floor. A flow of 45 Hz, referring to the pump frequency, simulated the sea bottom current (corresponding to 0.07 $cm\ s^{-1}$).

The Peregrino sediments were treated in the same way as in the color change experiment; however, for this experiment, the mix used was proportioned to mimic drill-cuttings from the Peregrino Oil Field (3:1, coarse to fine sediments) and Peregrino natural sediments (500 µm). Whole standardized rhodoliths (5 cm larger diameter) were buried covered by a thin layer (5 mm thickness) of sediments or remained uncovered. The two species of rhodolith-forming coralline algae, *Mesophyllum engelhartii* and *Lithothamnion* sp., were exposed to combined treatments of light level (50% and 100%) and burial (by sediments that mimic drill cuttings or Peregrino natural sediments or no burial). So, each species was submitted to six different treatments, and four replicates (rhodoliths) of each treatment were used.

After 15 days, the samples were removed from the mesocosm system to measure their fluorescence responses to treatments. The rhodoliths were transferred to a tray with sterilized sea water and carefully brushed to remove biofilm and sediments. Then, they were placed into a completely dark box for dark-adaptation for 20 minutes prior to the fluorescence measurements. Photosynthetic efficiency was determined by measuring the variable chlorophyll fluorescence of photosystem II with a pulse amplitude modulated (PAM) fluorometer (Diving PAM, Walz, Germany) attached to a computer that displays the Fv/Fm value. Blue light was used with a length of the saturating pulses of 0.6 s, saturation pulse intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, gain of 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and dumping of 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A decrease in Fv/Fm values was assumed to indicate a negative response of the coralline algae's photosynthetic apparatus to the tested environmental variables (Wilson *et al*., 2004, Burdett *et al*., 2012).

Three fluorescence measurements were made on the surface of each rhodolith, keeping a consistent distance of 3 mm between the fiber optic sensor (2 mm in diameter) and the coralline algae surface using a black plastic spacer (Harrington *et al*., 2005). The average of these three measurements for each rhodolith was used to perform the statistical analysis.

Statistical analysis

The responses of the coralline red algae *Mesophyllum engelhartii* and *Lithothamnion* sp. to different sediment compositions and light levels, given by Fv/Fm measurements and color changes, were analyzed using a bi-factorial orthogonal analysis of variances (2-way and 3-way ANOVA) (Underwood, 1997). Two levels of each parameter were used, so no post-hoc test was needed to assess the effects of sediments and light. Changes in color were plotted over time, and the analysis of variance was performed for a single time (41 days) when significant differences were detected.

RESULTS

Color change experiment

Throughout the experiment, the two coralline algae species in the non-covered treatments remained healthy in color, with the same scale of tone and intensity for the shaded (3 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and unshaded (9 $\mu mol \cdot m^{-2} \cdot s^{-1}$) individuals. Both species remained mostly healthy when buried by coarse sediments, changing to a paler color (tone after 41 days and intensity after 75 days). In contrast, fine sediments negatively affected both coralline algae, causing bleaching. A deleterious response was found after 75 days for *Mesophyllum engelhartii* and after a shorter period of 41 days for *Lithothamnion* sp. There was no significant interaction detected between the effects of light level and sediment burial on color changes for either coralline algae species (Figs 1 and 2, Tables 1 and 2).

Fluorometry Fv/Fm experiment

Overall, there was a marked pattern of higher Fv/Fm values of *Lithothamnion* sp. compared to *Mesophyllum engelhartii* but no significant interaction between coralline algae species, sediment composition and light level. Both sediment compositions had a strong negative effect on the two coralline algae species. The maximum photochemical quantum yield of the controls was considered a suitable representation of healthy coralline algae ($Fv/Fm \geq 0.5$) and was significantly higher compared to the values of stressed coralline algae ($Fv/Fm \geq 0.1$) (Fig. 3, Table 3).

DISCUSSION

The thallus thickness of *Mesophyllum engelhartii* in this study was far thinner than has been recorded in shallow waters elsewhere (Figueiredo *et al.*, 2012). *Lithothamnion* sp. also has a thin crust. This pattern of crusts with thin thalli replacing thick crusts in deep water is expected given the low light environment (Figueiredo & Steneck, 2002).

The two coralline algae appear to have adapted to the low light environment of deep water, as the photosynthetic peak reached 0.5–1.5% of the maximum surface irradiance. The P-I curve of net primary production (photosynthetic rates versus irradiance) indicates that *Mesophyllum engelhartii* is more efficient at absorbing low irradiance than *Lithothamnion* sp. Their

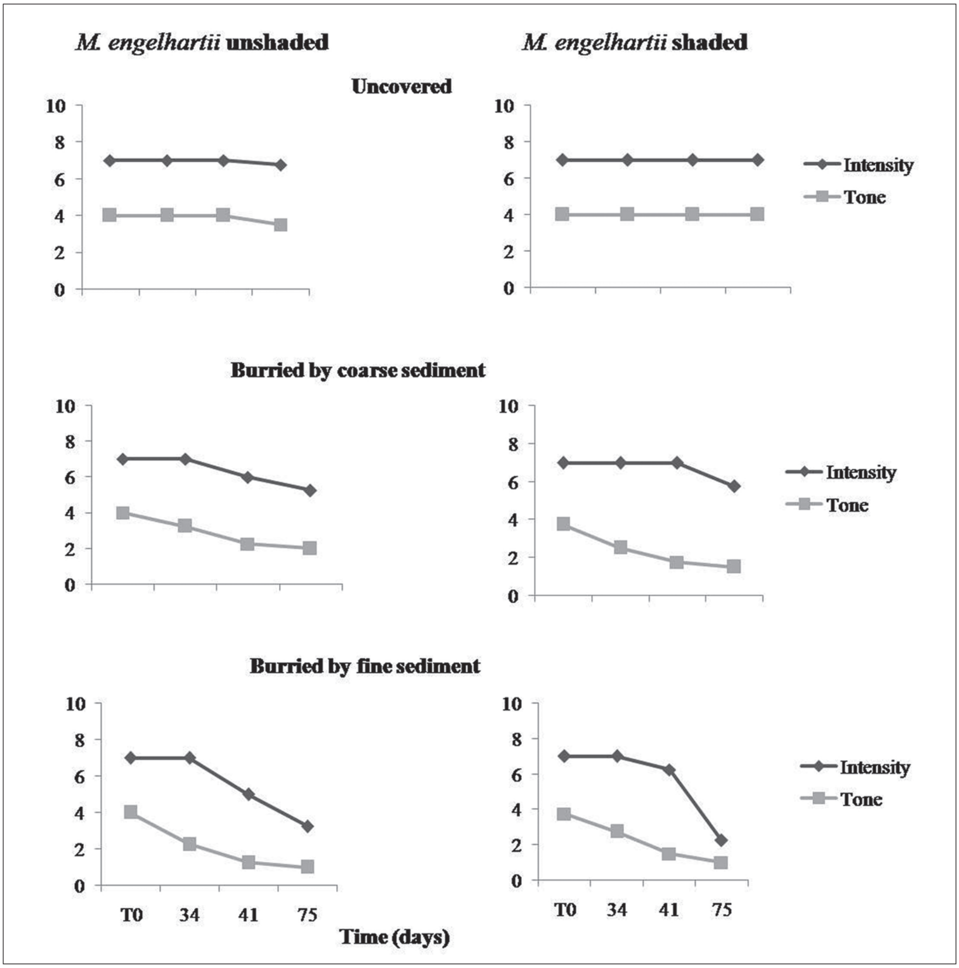

Fig. 1. Changes in the color of the thallus surface of *Mesophyllum engelhartii* after 34, 41 and 75 days.

Table 1. Analysis of variance for the color change responses (A=Intensity; B=Tone) to different sediment compositions and light intensities in *Mesophyllum engelhartii* after 41 days (n = 4)

A - Intensity	*D.F.*	*M.S.*	*F*	*P- value*
Sediment (S)	2	5.042	1.995	0.165
Light (L)	1	0.167	0.066	0.800
S × L	2	2.042	0.808	0.461
Error	18	2.528		
B - Tone				
Sediment (S)	2	15.042	29.270	< 0.001
Light (L)	1	0.042	0.081	0.779
S × L	2	0.292	0.568	0.577
Error	18	0.51389		

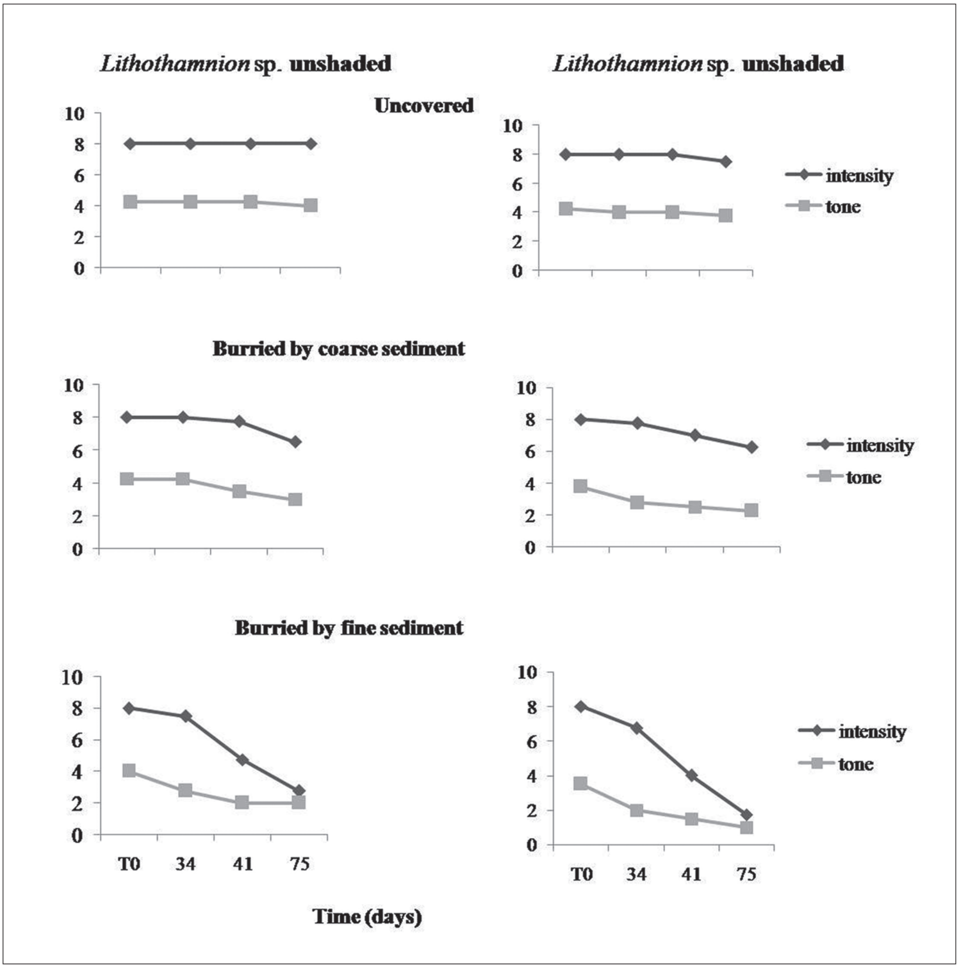

Fig. 2. Changes in the color of the thallus surface of *Lithothamnion* sp. after 34, 41 and 75 days.

Table 2. Analysis of variance for the color change responses (A=Intensity; B=Tone) to different sediment compositions and light intensities in *Lithothamnion* sp. after 41 days (n = 4)

A - Intensity	*D.F.*	*M.S.*	*F*	*P- value*
Sediment (S)	2	30.042	14.420	< 0.001
Light (L)	1	1.500	0.720	0.407
S × L	2	0.375	0.180	0.837
Error	18	2.083		
B - Tone				
Sediment (S)	2	11.292	17.298	< 0.001
Light (L)	1	2.042	3.128	0.094
S × L	2	0.292	0.447	0.647
Error	18	0.653		

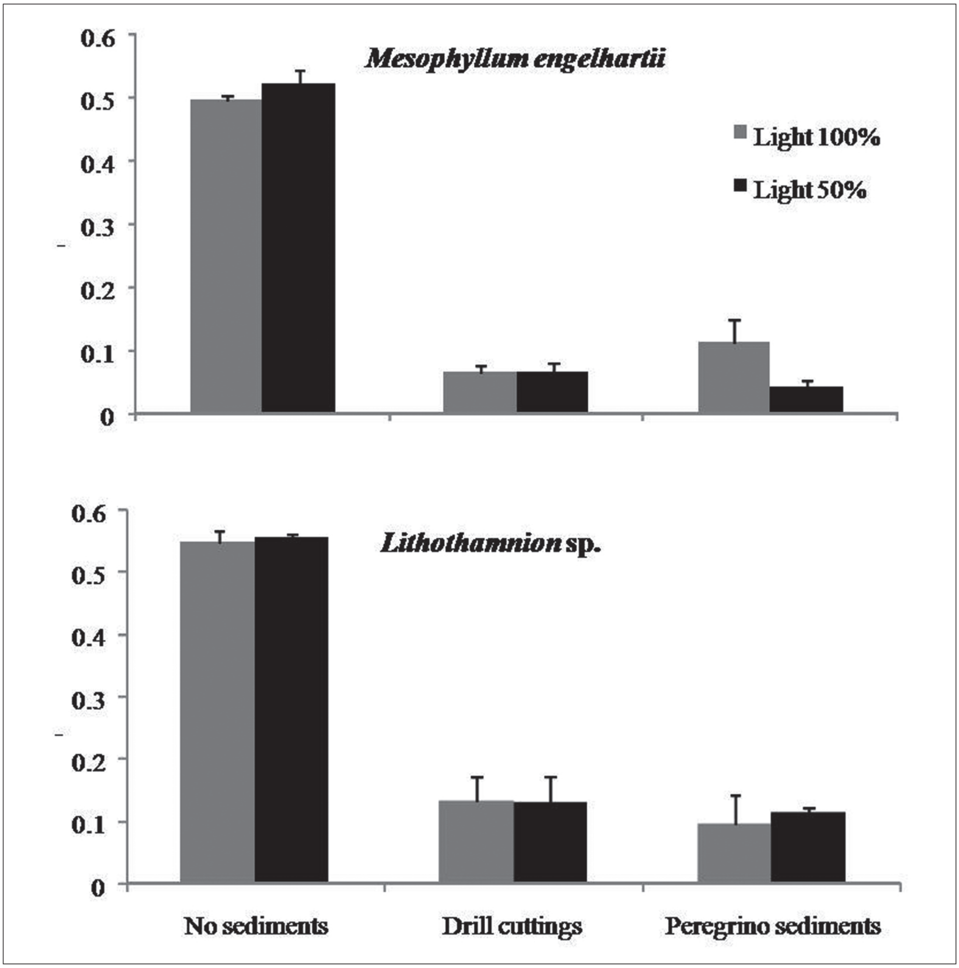

Fig. 3. Maximum photochemical efficiency (Fv/Fm) of dark-acclimated samples of *Mesophyllum engelhartii* and *Lithothamnion* sp. exposed to different sediment compositions and light intensities (T=15 days).

Table 3. Analysis of variance for the fluorescence (Fv/Fm) responses of *Mesophyllum engelhartii* and *Lithothamnion* sp. to different sediment compositions and light intensities (n = 4)

Effect	*SS*	*df*	*MS*	*F*	*P-value*
Species (Sp)	0.02	1	0.02	9.53	$p \leq 0.05$
Sediment (Sd)	2.022	2	1.011	371.736	$p \leq 0.0001$
Light (L)	0.0001	1	0.0001	0.063	$p \geq 0.05$
Sp × Sd	0.003	2	0.001	0.556	$p \geq 0.05$
Sp × L	0.001	1	0.001	0.598	$p \geq 0.05$
Sd × L	0.003	2	0.001	0.715	$p \geq 0.05$
Sp × Sd × L	0.006	2	0.003	1.234	$p \geq 0.05$
Error	0.056	36	0.001		$p \geq 0.05$

saturation points, measured by change in dissolved oxygen, are 9 μmol photons $m^{-2} \cdot s^{-1}$ for *Lithothamnion* sp. and 27 μmol photons $m^{-2} \cdot s^{-1}$ for *M. engelhartii* (Figueiredo *et al.*, 2012).

Nonetheless, based on the maximum photochemical efficiency results, *Lithothamnion* sp. was more efficient compared to *Mesophyllum engelhartii* in this study.

Overall, the sediments had a major impact on the rhodoliths independent of particle size fraction, being either the fine sediments that mimic drill-cuttings or the coarse natural sediments from the Peregrino oil field.

A high proportion of dead relative to live rhodoliths has been attributed to an increase in the fine sediment load that reduces bed vitality (Harvey & Bird, 2008). Under controlled conditions, the addition of a thin sediment layer resulted in a 30% reduction of the irradiance, decreasing the *Lithothamnion* sp. net production in 70% (Riul *et al.*, 2008). Burial by fine sediments may be lethal or cause significant stress to rhodolith-forming coralline algae by blocking light and reducing oxygen and nutrient uptake (Wilson *et al.*, 2004), which may result in major changes to the associated community (Figueiredo *et al.*, 2007).

The highest level of irradiance (9 $\mu mol \cdot m^{-2} \cdot s^{-1}$) did not interfere with the "healthy" conditions expected on the outermost pigmented layer of the thalli of these two deep water coralline species. *Lithothamnion* sp. was more sensitive and quickly responded to stress, changing color once buried by fine sediments, whereas *Mesophyllum engelhartii* gradually turned pale in color and eventually bleached. Under a layer of coarse sediments, both coralline algae became paler in color over time, but neither bleached.

Experiments using fragments of the coralline algae species *Hydrolithon reinboldii, Neogoniolithon fosliei* and *Porolithon onkodes* from the Great Barrier Reef, Australia showed that sediment deposition can have a negative effect on the photosynthetic activity of coralline algae. When coralline algae were exposed to fine estuarine silt and offshore sediments, their photosynthetic activity decreased more than when exposed to the same amount of fine calcareous and coarse estuarine sediments (Harrington *et al.*, 2005).

The presence of fine sediment with a high organic load and hydrogen sulphide content was rapidly lethal to the rhodolith-forming species *Phymatolithon calcareum.* The main anthropogenic hazard for maerl algae and the rich communities that depend on them is smothering by fine sediment, whether produced by trawling or maerl extraction or resulting from barriers to natural tidal flow (Wilson, *et al.,* 2004).

In summary, *Lithothamnion* was more sensitive than *Mesophyllum engelhartii* in terms of color changes over the long term but less sensitive in terms of short-term fluorescence responses to burial with different sediment compositions. This pattern may help to explain the dominance of the latter species and may be used to assess the impact of natural sediment disturbances compared to drill-cutting discharges from oil activities.

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REFERENCES

AMADO-FILHO G.M., MANEVELDT G., MANSO R.C.C., MARINS-ROSA B.V., PACHECO M.R. & GUIMARÃES S.M.P.B., 2007 — Structure of rhodolith beds from 4 to 55 meters deep along the southern coast of Espírito Santo State, Brazil. *Ciencias marinas* 32(4) : 399-410.

BAHIA R.G., ABRANTES D.P., BRASILEIRO P.S., PEREIRA FILHO G.H. & AMADO FILHO G.M., 2010 — Rhodolith Bed structure along a depth gradient on the Northern Coast of Bahia State, Brazil. *Brazilian journal of oceanography* 58(4) : 323-337.

BURDETT H.L., HENNIGE S.J., FRANCIS F.T.-Y. &. KAMENOS N.A., 2012 — The photosynthetic characteristics of red coralline algae, determined using pulse amplitude modulation (PAM) fluorometry. *Botanica marina,* DOI: 10.1515/bot-2012-0135.

DUTRA L.X.C., KIKUCHI R.K.P., & LEÃO Z.M.A.N., 2006 – Effects of sediment accumulation on reef corals from Abrolhos, Bahia, Brazil. *Journal of coastal research, Proceedings of the international coastal symposium*, *Special Issue* 39 : 633-638.

FIGUEIREDO M.A.O., KAIN (JONES) J.M. & NORTON T.A., 2000 – Responses of crustose corallines to epiphyte and canopy cover. *Journal of phycology* 36 : 17-24.

FIGUEIREDO M.A.O. & STENECK R.S., 2002 — Floristic and ecological studies of crustose coralline algae on Brazil's Abrolhos reefs. In: *Proceedings of the 9th International Coral Reef Symposium*, Bali, 1 : 493-498.

FIGUEIREDO M.A.O., MENEZES K.S., COSTA-PAIVA E.M., PAIVA P.C. & VENTURA C.R.R., 2007 — Experimental evaluation of rhodoliths as living substrata for infauna at the Abrolhos Bank Brazil. *Ciencias marinas* 33 : 427-440.

FIGUEIREDO M.A.O., COUTINHO R. VILLAS-BOAS A.B., TÂMEGA F.T.S. & MARIATH R., 2012 — Deep-water rhodolith productivity and growthin the southwestern Atlantic. *Journal of applyed phycology*, DOI: 10.1007/s10811-012-9802-8.

HARRINGTON L., FABRICIUS K., EAGLESHAM G.C. & NEGRI A., 2005 — Synergistic effects of diuron and sedimentation on photosynthesis and survival of crustose coralline algae. *Marine pollution bulletin* 51 : 415-427.

HARVEY A.S. & BIRD F.L., 2008 — Community structure of a rhodolith bed from cold-temperate waters (southern Australia). *Australian journal of botany* 56 : 437-450.

JONES D.O.B., HUDSON I.R. & BETT B.J., 2006 — Effects of physical disturbance on the cold-water megafaunal communities of the Faroe- Shetland Channel. *Marine ecology progress series* 319 : 43-54.

KJEILEN-EILERTSEN G., TRANNUM H., JAC R., SMIT M., NEFF J., DURELL G., 2004 — Literature report on burial: derivation of PNEC as componente in the MEMW model tool. Environmental Risk Management System Report. www.rf.no /rf-akvamiljo. 25p.

KUHL M., GLUD R.N., BORUM J., ROBERTS R., RYSGAARD S., 2001 — Photosynthetic performance of surface-associated algae below sea ice as measured with a pulse-amplitude-modulated (PAM) fluorometer and O2 microsensors. *Marine ecology progress series* 223 : 1-14.

LEÃO Z.M.A.N., KIKUCHI R.K.P. & TESTA V., 2003 — Corals and coral reefs of Brazil. *In:* Cortés J. (ed.). *Latin America Coral Reefs*. Amsterdam, Elsevier Science, pp. 9-52.

RADZIEJEWSKA T. & STOYANOVA V., 2000 — Abyssal epibenthic megafauna of the Clarion-Clipperton area (NE Pacific): changes in time and space versus anthropogenic environmental disturbance. *Oceanological studies Gdansk* 29 : 83-101.

RIUL P., TARGINO CH., FARIAS J.N., VISSCHER P.T., HORTA P.A., 2008 — Decrease in Lithothamnion sp. (Rhodophyta) primary production due to the deposition of a thin sediment layer. *Journal of the marine biological association of the United Kingdom* 88(1): 17-19. doi:10.1017/S0025315408000258

SCIBERRAS M., RIZZO M., MIFSUD J.R., CAMILLERI K., BORG J.A., LANFRANCO E. & SCHEMBRI P.J., 2009 — Habitat structure and biological characteristics of a maerl bed off the northeastern coast of the Maltese Islands (central Mediterranean). *Marine biodiversity* 39 : 251-264.

TÂMEGA F.T.S., SPOTORNO-OLIVEIRA P. & FIGUEIREDO M.A.O., 2013 — *Catalogue of the benthic marine life from Peregrino oil field, Campos Basin, Brazil.* Rio de Janeiro, Instituto Biodiversidade Marinha, 140 p.

UNDERWOOD A.J., 1997 — *Experiments in ecology. Logical design and interpretation using analysis of variance*. Cambridge, Cambridge University Press.

WILSON S., BLAKE C., BERGES J.A. & MAGGS C.A., 2004 — Environmental tolerances of free-living coralline algae (maerl): implications for European marine conservation. *Biological conservation* 120 : 283-293.