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ESTRESSORES LOCAIS E SUAS AMEAÇAS À BIOLOGIA DE ALGAS CALCÁRIAS

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Samara Dumont Fadigas

Estressores locais e suas ameaças à biologia de algas calcárias

Esta Dissertação foi julgada adequada para obtenção do Título de "Mestre em Oceanografia", e aprovada em sua forma final pelo Programa de Pós-graduação em Oceanografia.

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“Enquanto você dorme o tempo anda
Enquanto você anda o tempo corre
Enquanto você corre o tempo voa
A gente voa alto e o tempo não volta”
(Don L - Roteiro pra Aïnouz, Vol. 3 – 2017)

“Vai e vai, ganha esse mundo sem olhar
pra trás
E vai, só não esquece de voltar”
(Djonga – Ladrão – 2019)

RESUMO

Algas calcárias vermelhas são organismos fotossintetizantes que apresentam depósitos de carbonato de cálcio ($CaCO_3$) em suas paredes, apresentando assim uma estrutura rígida que forma depósitos rochosos no substrato marinho. Essas algas também são responsáveis pela formação de rodolitos e consequentemente de bancos de rodolitos que cobrem o leito marinho criando microhabitats que abrigam inúmeras espécies. Entretanto, as espécies de rodolitos se veem ameaçadas devido ao aumento das atividades antrópicas e a eventuais catástrofes. Algumas mudanças no ambiente como aumento na frequência de chuvas ou aumento da descarga continental em ambiente marinho pode trazer graves consequências aos bancos de rodolitos, como por exemplo o aumento da turbidez no meio e o aumento do soterramento dos leitos. Tais eventos podem causar inúmeros efeitos na fisiologia dessas algas. Por serem organismos fotossintetizantes, a redução da disponibilidade de luz devido ao aumento da turbidez ou ao soterramento pode alterar a produtividade dos rodolitos, causando alteração fisiológicas como redução da atividade fotossintética. Por isso, nesse trabalho, foram realizados dois experimentos. O primeiro avaliou as alterações na produtividade de algas calcárias um cenário com aumento da concentração de nutrientes e redução da disponibilidade de luz num curto intervalo de tempo. Desse experimento obtivemos dados preliminares sobre o comportamento fisiológico da alga em aspectos de produtividade. No segundo experimento, algas foram expostas a um cenário de redução de disponibilidade de luz devido ao rompimento de uma barragem de rejeito de minério de ferro, simulando assim o soterramento desses organismos. Com o segundo experimento obtivemos dados que demonstravam a redução da produtividade em algas soterradas pela lama e utilizamos técnicas de manejo para reestabelecer a condição inicial de saúde da alga com a limpeza do organismo. Demonstrando assim, que é possível recuperar bancos de rodolitos que sofreram soterramento.

Palavras-chave: Alga calcária 1. Produtividade 2. Disponibilidade de luz 3.

ABSTRACT

Crustose coralline red algae are photosynthetic organisms that exhibit deposits of calcium carbonate ($CaCO_3$) on their cellular walls, thus presenting a rigid structure that forms rocky deposits on the marine substrate. These algae are also responsible for the formation of rhodoliths and consequently of rhodolith beds that cover the seabed creating microhabitats that house innumerable species. However, species of rhodoliths are threatened due to anthropic activities and catastrophes. Some changes in the environment such as increased rainfall frequency or increased continental discharge (rich in nutrients) in the marine environment can have serious consequences for rhodolith beds, such as increased turbidity in the middle and increased bed burial. Such events can have numerous effects on the physiology of these algae. Because they are photosynthetic organisms, the reduction of the light availability due to the increase of the turbidity or the burial can change the productivity of the rhodoliths, causing physiological changes as a reduction of the photosynthetic activity. Therefore, in this work, two experiments were carried out. The first evaluated the changes in calcareous algae productivity in a scenario with increased nutrient concentration and reduced light availability in a short period of time. From this experiment we obtained preliminary data on the physiological behavior of algae in aspects of productivity. In the second experiment, algae were exposed to a scenario of reduced light availability due to the rupture of an iron ore tailings dam, thus simulating the burial of these organisms. With the second experiment we obtained data that showed the reduction of the productivity in algae buried by the mud and we used management techniques to reestablish the initial condition of algae health with the cleaning of the organism. Demonstrating thus, that it is possible to recover banks of rhodoliths that have undergone burial.

Keywords: Calcareous algae 1. Productivity 2. Availability of light 3.

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

LISTA DE ABREVIATURAS E SIGLAS

APACA – Environmental Protection Area of Costa das Algas
CCA – crustose coralline red algae
HNHI – High Nutrient High Irradiance
HNLI – High Nutrient Low Irradiance
LNHI – Low Nutrient High Irradiance
LNLI – Low Nutrient Low Irradiance
RVSSC – Wildlife Refuge of Santa Cruz

SUMÁRIO

1 INTRODUÇÃO GERAL

Rodolitos são estruturas calcárias de vida livre formadas por algas vermelhas coralíneas (Corallinales, Rhodophyta) (Foster, 2001). Bancos de rodolitos são compostos por rodolitos e apresentam distribuição global, dos trópicos aos polos, de zonas e marés até mais de 200m de profundidade (Littler et al., 1991; Foster, 2001). A biodiversidade desses bancos de rodolitos é ameaçada por variações nos fatores abióticos, como aumento da frequência e intensidade de tempestades, com consequentes mudanças na turbidez da água, que podem afetar a saúde e a produtividade dos rodolitos no longo prazo (Riul et al., 2009). No entanto, essas variações raramente são testadas empiricamente nessas comunidades (Horta et al., 2016). Condições ambientais que normalmente variam com a profundidade, como a luz, a sedimentação e o movimento da água, podem afetar as características dos bancos rodolitos, como vitalidade, fauna e flora associadas e abundância bentônica.

Condições associadas a um evento extremo como os acidentes de Mariana ou Brumadinho devem receber atenção especial (Horta et al., 2016). No entanto, os impactos da mineração de sedimentos e impactos de sombreamento em algas calcárias nos bancos de rodolitos ainda são pouco compreendidos (Fabricius, 2005; Villas-Boas et al., 2014).

O efeito prejudicial físico e químico desses rejeitos de minério tem sido associado a perdas de biodiversidade (Gomes et al., 2017), mortalidade de produtores primários relacionados à toxicidade de metais (Costa et al., 2017) e insuficiente irradiância e absorção de nutrientes para sustentar a fotossíntese e metabolismo basal (Riul et al., 2008). Entre esses produtores, há uma falta especial de informações sobre esses impactos nas algas calcárias (Isaac e Ferrari, 2017). Somando-se à falta de informação, até onde sabemos, não houve tentativa de aplicar técnicas físicas para mitigar o impacto ou até mesmo a recuperação de qualquer taxa bentônica como algas calcárias após a exposição aos sedimentos.

A distribuição e a sobrevivência de algas calcárias dependem de condições nos quais luz, temperatura e sedimentação são consideradas como principais limitadores do crescimento e da estrutura (Hinojosa-Arango, 2013). A intensificação de atividades antropogênicas aumenta os impactos sobre as algas, especialmente na disponibilidade de luz através da redução desta causada especialmente pela deposição de finas camadas de sedimento na superfície de rodolitos, por exemplo (Riul, 2008; Wilson et al., 2004; Figueiredo et al, 2015). O sombreamento pode ser causado por enterramento (Steller e Foster, 1995; Dutra et al., 2006; Riul et al., 2008), bem como pelo aumento da turbidez do ambiente (Foster, 2001; 2013). Ambos os eventos causam redução da disponibilidade de luz, alterando a fisiologia do organismo, como a desova, reprodução e crescimento de larvas (Littler, 1991; Wilson, 2004; Riul, 2008).

Portanto, avaliamos nesse trabalho impactos de alguns estressores locais de fonte antropogênica em algas calcárias. Tais como o aumento na concentração de nutrientes na coluna d'água e redução da disponibilidade de luz causada pelo aumento de descargas continentais na região costeira. Conhecendo os efeitos desses impactos que estão ocorrendo com uma frequência cada vez maior, avaliamos então os efeitos de eventos extremos como o soterramento de rodolitos causado pelo lama do rompimento de uma barragem de rejeito de minério de ferro. Com isso podemos sugerir estratégias de manejo para evitar impactos em futuros crimes ambientais como os de Mariana e Brumadinho que possam ocorrer.

1.1 OBJETIVOS

Avaliar os impactos de estressores locais (aporte de nutrientes e redução da disponibilidade de luz por sombreamento ou soterramento) em algas calcárias através de experimentação e sugerir práticas de manejo para os impactos causados pelo soterramento.

1.2 OBJETIVOS ESPECÍFICOS

Avaliar, *in vitro*, os efeitos da redução da disponibilidade de luz na produtividade de algas calcárias e o efeito do aumento de nutrientes em ambientes mais profundos na produtividade dessas algas. Trabalhamos com a hipótese de que a contribuição de corpos de água ricos em nutrientes e plumas de sedimentos pode causar mudanças fisiológicas em algas calcárias de ambientes profundos.

Avaliar a eficácia da limpeza da mineração de Mariana na fotossíntese de macroalgas, com destaque para as algas calcárias incrustantes formadoras de rodolitos, da espécie *Lithophyllum margaritae* (Hariot) Heydrich. Testamos a hipótese de que os procedimentos de limpeza após algumas horas de exposição podem mitigar os efeitos negativos do rejeito.

CAPÍTULO 1

O capítulo 1 da presente dissertação não é uma publicação efetiva. A intenção da autora na elaboração dos capítulos da dissertação é para a defesa e obtenção de título.

Impacts of local stressors on the physiology of crustose coralline red algae from Eastern Brazil

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Abstract
The Brazilian North-Northeast continental shelf shelters the largest known rhodolith beds in the world which cover large areas of the country. The richest marine flora of the coastal zone is found in the state of Espírito Santo (Eastern Brazil) and this diversity has been partially associated to the presence of extensive areas of rhodolith that extend from the intertidal zone. The biodiversity of rhodolith beds are threatened by variations in abiotic factors such as increases in water turbidity which may affect the health and productivity of these beds in the long. A 5-day microcosm experiment with calcareous red algae collected in the coast of Espirito Santo (45 m depth) was carried out. After 5-day of experiment with different concentrations of nutrients and light availability, we obtained data of gross photosynthesis, concentration of pigments and photosynthetic yield, and the effects were evaluated after analysis of data. In the short exposure experiment, productivity did not show any significative changes, but we could see impacts on pigments concentration and photosynthetic yield. The
present work has presented introductory results on the increasingly common impacts that may occur on rhodolith beds on Brazilian eastern coast, providing the basis for studies that bring other types of stressors, not so common, but which cause even greater impacts than those cited in this study.

Keywords: Calcareous Red Algae; Eastern Brazil; Physiologic stress

1. Introduction

Rhodoliths are made up of superimposed calcified thalli of coralline red algae (Rhodophyta), which form a multi-layered arrangement around a nucleating particle, such as coral rubble, bivalves, foraminifera or bryozoans, constituting a complex network with bioclastic sediment infilling matrix (e.g., Bosence 1979; 1983; 1985). It grows by fragments or reproduction by spores and present slow growth (0.5−1.5 mm per year) and can be long lasting (> 100 years), being resilient to different environmental disturbances and therefore considered as non-renewable resources (Gagnon et al., 2012; Riosmena-Rodríguez et al., 2017). Rhodoliths occur in a variety of sizes, shapes and aggregated species by extensive areas (Foster, 2001) and after death, form deposits of fossil material (Bassi et al., 2009; 2013; Checconi et al., 2010). The structure of the individual rhodoliths can vary from being composed of a single coralline species (Riosmena-Rodriguez et al., 1999; Steller et al., 2003) to a structure with a composition of several species denominated boxwork (Bosence, 1983; Basso, 1998; Baarli et al., 2012).

Rhodoliths exhibit global distribution, occurring from tropics to poles (Bosence, 1983; Foster 2001), besides dimensions and morphology that may vary with depth (Littler et al., 1991; Amado-Filho et al., 2007, Riul et al., 2009). The largest known rhodolith beds in the world are found on the Brazilian coast (Foster, 2001; 2013), covering large areas of the North-Northeast continental shelf of the country (Bahia, 2010). In Brazil, the richest marine flora of the coastal zone is found in the state of Espírito Santo (Eastern Brazil) and this diversity has been partially associated to the presence of extensive areas of rhodolith that extend from the intertidal zone up to 120 m along the continental shelf (Guimarães, 2006; Amado-Filho, 2007; 2010).

Ecologically, crustose coralline algae forming rhodoliths present a morphological complexity and distribution of individuals in beds that provides a set of microhabitats with gaps and shelters that sustain a rich biodiversity, including commercially important species and often endemic species, besides being of great ecological importance (Foster, 2013; et al., 2009). One of the ecologic importance is the ability to deposit $CaCO_3$ in its cell wall, forming rigid structures and sequestering atmospheric carbon through carbonate accumulation over a long-term scale, being also responsible for the global CO2 balance (Testa & Bosence, 1999; Bosence & Wilson, 2003; Amado-Filho et al., 2012).

The biodiversity of rhodolith beds are threatened by variations in abiotic factors such as increases in temperature (Nelson et al., 2012; Amado-Filho et al., 2012), frequency and storm intensity with consequent changes in water turbidity which may affect the health and productivity of these beds in the long-term (Riul et al., 2009). However, these variations are rarely empirically tested in these communities (Horta et al., 2016). Environmental conditions that normally vary with depth, such as light, sedimentation and water movement, may affect the characteristics of rhodolith beds, such as vitality, associated fauna and flora, and benthic abundance. The distribution and survival of these organisms are regulated by many factors, in which light, temperature and sedimentation are considered as main limiters of growth and structure (Hinojosa-Arango, 2013).

Intensification of climate change and anthropogenic activities have been increasing impacts on rhodoliths, especially in the availability of light through reduction of light caused especially by the deposition of thin layers of sediment that are deposited on the surface (Wilson et al., 2004; Riul et al., 2008; Figueiredo et al., 2012). Shading can be caused either by burial (Steller & Foster, 1995; Dutra et al., 2006; Riul et al., 2008) as well as increased turbidity of the environment (Foster, 2001; 2013). Both events cause reduced light availability in the environment, altering the physiology of the organism, such as larval nesting, reproduction and growth (Littler, 1991; Wilson et al., 2004; Riul et al., 2008).

Physiologic aspects such as gross photosynthesis, calcification rates and pigments concentration can be affected by increase of temperature and acidification (Noisette et al., 2013; McCoy and Kamenos, 2015; Vásquez-Elizondo and Susana Enríquez, 2016;

In this work, we evaluated in vitro the effects of light reduction on rhodoliths and the effect of increasing of nutrient in deeper environments in physiology of these organisms. We work with the hypothesis that the contribution of nutrient-rich water bodies and sediment plumes can cause physiological changes on rhodoliths from deeper environments.

In this work, we evaluated in vitro the effects of light reduction on red calcareous algae and the effect of increasing of nutrient in deeper environments in these algae physiology. We work with the hypothesis that the contribution of nutrient-rich water bodies and sediment plumes can cause physiological changes on these algae from deep environments.

2. Material and Methods

2.1. Study area, sampling procedures and experimental design

The Espirito Santo coast is considered a transition zone between the tropical and subtropical Brazilian coast, creating oceanographic and climatic conditions that provide a high number of species and diversity of marine habitats that occur in the southern part of the state (Guimarães, 2006). Such richness and abundance have also been attributed to the presence of a consolidated substrate, represented by rocky shores and sandstone reefs encrusted with calcareous algae (Guimarães, 2003). Therefore, the coastal region of the Espírito Santo State has important conservation areas with biodiversity richness included in the Environmental Protection Area of Costa das Algas (APACA) and the Wildlife Refuge of Santa Cruz (RVSSC). APACA comprises one of the world's largest rhodolith beds that is being threatened by climate change and local stressors.

Algae were sampled on Jan 26th and 27th 2018, between depths of 45 and 49 m in the continental shelf of Espírito Santo State using a trawler net (50 cm in diameter x 30 cm in height) (Table 1). The experiment was carried out at the Oceanographic Base of the Federal University of Espírito Santo in Aracruz (Espírito Santo State) in a temperature-controlled environment at 22°C using a chiller (GELAQUA) and variable irradiance with a photoperiod of approximately 13h.

Twenty aquariums filled with 0.5 L of seawater from the study area and containing 3 organisms each were randomly placed in two tanks and maintained submersed. All aquariums were covered with two sheets of cellophane paper (blue and green) to simulate red light reduction, and 10 of them were also covered with a 95% shading screen allowing only a 5% irradiance. All samples were aerated with aeration pumps.

Samples were labelled as A1 to A5, B1 to B5, C1 to C5, and D1 to D5 according to the treatment submitted: samples from A1 to A5 (HNLI - High Nutrients/Low Irradiance) were covered with shading screen and during the experiment were given a high concentration of nutrients (80 µM NH_4, 5 µM PO_4, 40 µM NO_3) (Gouvea et al., 2017). Samples from B1 to B5 (LNLI - Low Nutrients/Low Irradiance) were covered with shading screen and did not receive nutrients. Samples from C1 to C5 (HNHI - High Nutrients/High Irradiance) were not covered with shading screen and received addition of high concentration of nutrient. Samples from D1 to D5 (LNHI - Low Nutrient/High Irradiance) were not covered with shading screen or received nutrient addition, being the control samples.

During the five days of experiment aquarium water was changed and nutrient was added to the HNLI and HNHI samples every day.

2.2. Variables of interest

Every day, fluorescence (Fo) baseline, maximum quantum fluorescence (Fm), variable fluorescence and the maximum quantum yield (Fv/Fm) of the PSII were obtained using a portable fluorimeter (Water PAM, Walz, Effeltrich, Germany) after a 30 minutes adaptation of the algae into the dark.

To calculate the gross primary productivity, dissolved oxygen were obtained using an oxygen sensor Fibox 4 (PreSens, Regensburg, Germany) before and after a 30 minutes incubation under irradiation of 210 µmol photons m^2s^{-1} (to obtain photosynthesis rate) and also in the dark (to obtain respiration rate).

The values of gross primary productivity ratio (mg O_2 L^{-1} g^{-1} h^{-1}) were calculated using the oxygen rate using the following equation:

$$\text{Photosynthesis rate} = \frac{\Delta O_2}{\Delta t * m}$$

Where, ΔO_2 (mg L^{-1}) is how much oxygen was produced during the incubation period; Δt is the incubation period (h), and m is the fresh biomass of the sample (g).

Irradiance value was obtained by adjusting data to exponential function proposed by Jassby and Platt (1976):

$$\text{P} = P_{max}\left[1 - e^{(\alpha E/Pmax)}\right]$$

Where, P is the photosynthetic rate at a given light intensity, P_{max} is the maximum value of photosynthetic activity, α is the photosynthetic efficiency, and E is light intensity (µmol $quantam^{-2}$ s^{-1}).

2.3. Pigments analysis

Small amount (0.5 - 1.5 g) of fresh biomass were sampled for analysis of accessory pigments of phycobiliproteins and chlorophyll. Fragments were washed with distilled water and macerated in liquid nitrogen and then transferred to a 15 mL falcon tube in which was added 4 mL of a phosphate buffer solution (0.1 M, pH 6.8) and homogenized. Samples were stored in darkness at 4°C for 24 h and then centrifuged for 2 min at 3000 rpm. The supernatant was recovered, and spectrophotometric measurements were carried at 498 nm, 614 nm, 651 nm, and 750 nm wavelengths (Bel Spectro LGS53, BEL Analytical Equipment Ltd., Brazil). The concentrations of phycobiliproteins fractions (allophycocyanin, phycocyanin and phycoerythrin) were calculated according to equations proposed for Kursar et al., (1983). In the remainder of the falcon sample, 4 ml of acetone (80%) was added and again the falcon was agitated in order to homogenize the mixture and allow the maximum extraction of chlorophyll. Samples were stored in the refrigerator for 24 hours and after falcons were again centrifuged for 2 minutes at 3000 rpm. The supernatant was recovered, and spectrophotometric measurements were carried at 646 nm, 663 nm and 750 nm wavelengths by spectrophotometer (Bel Spectro LGS53, BEL Analytical Equipment Ltd., Brazil). Concentrations of chlorophyll were calculated according to equations proposed for Ritchie (2008).

Phycoerythrin is located on the end of the rods and is the complex with the shortest wavelength absorption maximum, phycocyanin is located between the end of the rods and the core and absorbs at intermediate wavelength and allophycocyanin forms the core of the phycobilisome and absorbs at the longest wavelength (Blankenship, 2014). Phycoerythrin is an accessory pigment to Chlorophyll and it role seems to be especially important under low-light conditions (Beer et al., 2014).

2.4. Statistical analyses

Two-way ANOVA were used to test all variables. Previous tests were performed to verify the homoscedasticity (Levene’s test) and presence or absence of normal distribution (Kolmogorov-Smirnov test). LSD post-hoc was used in the evaluation of differences among treatments.

When no significant interaction among descriptors was verified, individual tests were carried out to verify the differences among groups. To test effects of different concentrations of nutrients and availability of light on response of different parameters tested, statistical analyses were performed using Statistica 7.1 (Statsoft Inc.) software.

3. Results

3.1. Physiological impacts

Data from incubations are non-parametric and to compare different treatments, a two-way ANOVA of log transformed data were performed and LSD post-hoc was used in the evaluation of differences among treatments, which showed that none of the treatments were significant (Table 2). After 5-days experiment, species presented no significant reduction or increase of net photosynthesis between day 1 and 5 ($p = 0.9483$); Figure 1a, Table 2).

There was a significant increase in photosynthetic yield from day 1 to day 5 ($p < .005$; Figure 1b, Table 3) in the experiment. Data from pigments presented a difference between concentrations of treatments, in which the concentration of phycoerythrin presented a significant difference between other pigments ($p < .005$; Figure 1c, Table 4).

4. Discussion

The sensitivity of organisms can be measured indirectly by their ability to occupy large regions or be restricted in their distribution to an environmental condition (Ehrlén and Morris, 2015; Dean and Pandolfi, 2015) Some species of calcareous algae are shown to be more suitable than many other photosynthetic organisms when they occupy less productive environments, where light is limited, whether at great depths, availability of nutrients, suspension and/or deposition of sediments (Riul, 2008; Bessell-Browne et al., 2017). Although gross photosynthesis data between day 1 and day 5 and between the treatments show some fluctuations, these differences are not significant, therefore, little can be inferred about how the treatments could have changed the productivity data. It was expected since rhodoliths were all known by their resilience (Amado-Filho, 2012; Amado-Filho, 2017).

Phycobiliproteins, besides being photosynthetic pigments that are accessory to photosynthesis and function as an energy capture antenna for chlorophyll a, perform other functions such as protecting algae against photo oxidation and serving as a nitrogen reserve (Beer, 2014; Blankenship, 2014).

Our results demonstrated that increase of nutrients and decrease of light availability can cause physiological effects on calcareous algae, such as photosynthetic yield and pigments concentration. However, our data did not show significative effects on their productivity.

5. Final considerations

Knowing the impacts of local stressors on rhodoliths beds becomes more and more urgent as there is an increase of stressors in this environment. Knowing that the increase in nutrients concentration and the reduction of light availability in the marine environment caused by the increase in freshwater intake can have an impact on their physiology, the present work has presented introductory results on the increasingly common impacts that may occur on rhodoliths beds on Brazilian eastern coast, providing the basis for studies that bring other types of stressors, not so common, but which cause even greater impacts than those cited in this study.

6. Ethical declarations

6.1. Conflict of interest

The authors declare no actual or potential conflict of interest.

6.2 Contribution

SDF and LEOG participated in sampling and experiment procedures. All authors analysed the data, wrote the manuscript and approved the final article.

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Table captions

Table 1. Time, Coordinates, depth and number of crustose coralline red algae (CCA) sampled at each trawler time in the Eastern Brazil.

Date	Start time	Lat. S	Lat. W	deep (m)	Number of CCA
26/01/2018	13:50	20º 07.255’	39º 58.010’	45	16
27/01/2018	17:00	20º 03.307’	39º 54.039’	49	566

Table 2.Two-way ANOVA of log transformed gross O_2 production of CCA considering different nutrient concentration and light availability(factors) (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Intercept	108.0094	1	108.0094	**640.7119**	*
Treatment	3.6877	3	1.2292	**7.2917**	*
Factor	0.1786	1	0.1786	1.0597	0.311011
Treatment*Factor	0.0601	3	0.0200	0.1189	0.948352

Table 3.Two-way ANOVA of log transformed photosynthetic yield considering different treatments and days (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Intercept	5.017208	1	5.017208	**233.6170**	*
Treatment	0.348396	3	0.116132	**5.4075**	*
Days	1.217762	4	0.304441	**14.1757**	*
Treatment*Days	0.398186	12	0.033182	1.5451	0.125585

Table 4. Two-way ANOVA of log transformed pigments concentration after experiment, considering different treatments (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Intercept	0.035590	1	0.035590	43.13508	*
Treatment	0.000767	3	0.000256	0.30969	0.818291
Pigments	0.054642	3	0.018214	**22.07536**	*
Treatment*Pigments	0.001415	9	0.000157	0.19057	0.994446

Figure caption

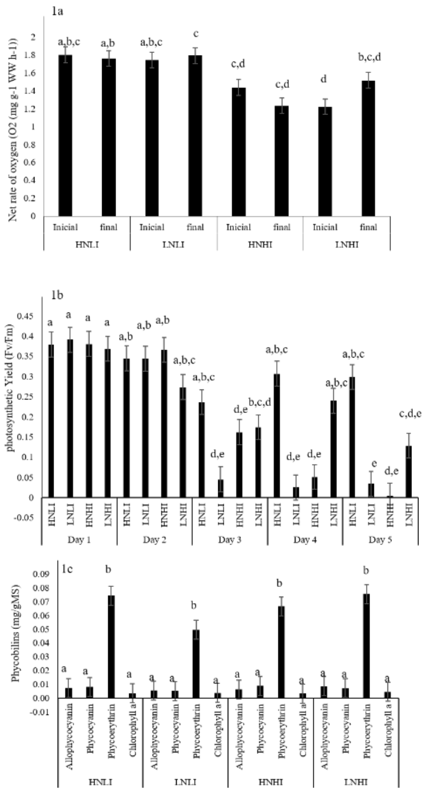

Figure 1: Graphical results for: a) Mean of gross photosynthesis data, obtained through incubation, between different treatments (HNHI, LNLI, HNLI, LNHI) among day 1 and day 5. Graph shows no significant difference between sample means with different treatments (p =0.9483; Table 2). b) mean of photosynthetic yield from day 1 to day 5c) phycobilin and chlorophyll a concentrations in the end of the experiment. Different letters represent significant differences among species behaviour considering LSD post-hoc test.

CAPÍTULO 2

O capítulo 2 da presente dissertação é referente ao artigo submetido.

Management can reduce the impact of mining tailing sediment on *Lithophyllium margaritae* (Hariot) Heydrich

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Abstract
The last Brazilian mining tailing accidents, failure of Mariana's and Brumadinho's ore tailing dams, are considered as being among the worst environmental disasters worldwide. Despite such consideration, information from short to long-term impacts are scarce, especially the impacts of the sediment (e.g. metals concentration) on fleshy macroalgae and crustose coralline red algae (CCA), that are widely distributed in the impacted zone. CCA are important carbon sinkers and support huge biodiversity/fishery biomass, and no cleaning management has been applied after the last Brazilian cases. We evaluated, *in vitro*, the effects of mining tailing in different fleshy macroalgae and CCA species, and the effect of occasional cleaning on recover of

Lithophyllium margaritae, the most vulnerable among evaluated CCA species. A short exposure of 24 h was utilized to evaluate the relative vulnerability of fleshy macroalgae and CCAs. A 14-days microcosm experiment with *L.margaritae* was carried out. After 7-day of mud exposure, the surface was cleaned, and the recover was evaluated after 7 additional days. In the short exposure experiment, mud compromised the primary production of all fleshy macroalgae and two of the evaluated CCAs. In the longer trial, all tested concentrations of mud disrupted primary production after one week. However, after cleaning, all treatments recovered their productivity significantly. A simple cleaning process, simpler than those done in accidents with oil spill, can reduce or eradicate the impact of mud, considering an action conducted for one week. Therefore, legislation regarding tailing contamination should consider cleaning actions immediately after accidents to avoid environmental disasters in marine coastal areas with the consequent losses of environmental goods and services. The increase frequency of accidents reinforces the need of further discussions regarding the application of urgent management tools considering different key groups and environments.

Keywords: Mining spill, Crustose coralline red algae, Photosynthesis related parameter, Eastern Brazil.

1. Introduction

The management of coastal areas has always faced multiple challenges related to anthropic activities, such as overfishing, environmental crimes, habitat destruction and losses of environmental goods and services, most of them with social and economic consequences for coastal communities (Moberg and Folke, 1999; Chee, 2004; FAO, 2016). Tragedies of anthropic activities reported around the world foster adoption of management protocols after events considering different kinds of impacts (Bartolome and Veiga, 2002; Rico et al., 2008). Most of protocols focus on mitigation or restoration of the ecosystem functioning, besides preserving wild life, contributing to the recognition and cultural knowledge of natural systems (Fernandes et al., 2016). For exemple, after decades of spill accidents, relatively efficient technologies and protocols that reduced the environment impact have been produced (Chapman et al., 2007; Si-Zhong et al., 2009; Fingas, 2012). On the other hand, mining activity did not receive the same attention and accidents around the world are contributing for the crisis of biodiversity loss announced in the last years (Miranda et al., 2003; Fernández-Caliani et al., 2009; Murguía et al., 2016; Sonter et al., 2018).

Fundão and Córrego do Feijão dams (joint-venture of BHP Billinton and Vale S.A) disruptions are considered among the worst environmental disasters worldwide and the most severe catastrophes for Brazilian history. Fundão dam (SAMARCO mining company) disruption, on November 5th, 2015, spilled approximately 50 million cubic meters of iron ore tailings (Escobar, 2015). The tailing sediment was mainly composed of Fe (57.2 %), SiO_2 (14.1 %), Al (1.3 %) and other trace metals (Cr, Cd and Pb; Pires et al., 2003). On November 21st, the mining tailing from the Fundão dam reached the marine region. It caused numerous social, economic and environmental impacts in one of the most important water and food provision to local communities (Cohen et al., 2014; Gomes et al., 2017). Upon reaching coast, the tailing plumes spread sediment along Doce river's adjacent coastal environments (Miranda and Marques, 2016). Among them, the environmental protection area Costa das Algas should be highlighted, a marine protected area (MPA) with bottom-dominated by rhodolith beds (Magris et al., 2018). Rhodoliths are built by crustose coralline red algae (CCA) (Bourguignon et al., 2018), which are important biofactory of carbonate and carbon sink (Hill et al., 2015, Horta et al., 2016), being substrate and niche for an abundant and socioeconomically important flora and fauna, with some endemic and/or threatened representatives (e.g. *Lutjanus spp*. and *Caranx* spp.; Mazzei et al., 2017). On January 25th, 2019, Córrego do Feijão ore reject dam collapsed in Brumadinho, Minas Gerais, 125 km from Fundão dam that collapsed on November 5th, 2015 (Sá, 2019). The tailing from Brumandinho dam may contain traces of Ni, Ma, Cd, Fe_2O_3, NH_3, SiO_2, Hg, As, silt and clay, but the level of toxicity of mud is still uncertain (Franco and Wentzel, 2019). Despite having still insufficient information about Brumadinho, this new tragedy calls attention for global discussion regarding urgency of management tools.

In a scenario of growing threats represented by global and local stressors, an extreme event like Mariana or Brumadinho accidents should receive special attention. However, the impacts of mining sediment burial and shading impacts on CCA are still poorly understood (Fabricius, 2005; Villas-Boas et al., 2014; Reynier et al., 2015; Osterloff et al., 2016). For example, flood periods increase the river runoff, which can release certain amount of sediment from the mining spill still in the Doce river basin (Gomes et al., 2017; Magris et al., 2018), that may hit the rhodolith beds in the area. The physical and chemical harmful effect of these ore tailings have been associated with biodiversity losses (Gomes et al., 2017), mortality of primary producers related with metal toxicity (Costa et al., 2017) and insufficient irradiance and nutrient uptake to sustain photosynthesis and basal metabolism (Riul et al., 2008). Among these producers, there is especial lack of information of these impacts on rhodolith (Horta et al., 2016). Adding to the lack of information, as far as we know, there was no attempt to apply physical techniques to mitigate de impact or even to recovery any benthic taxa of rhodolith after tailing sediment exposure. Since rhodoliths have a key-role as niche for socioeconomically important flora and fauna and ecosystem's carbon balance (Hill et al., 2015, Horta et al., 2016, Mazzei et al., 2017), impacts over rhodolith bed builders could result on cascade loss of niche, biodiversity and disruption of ecosystemic services provided by these environments (Martin and Hall-Spencer, 2017).

Incidents such as Brumadinho and Mariana tragedy and their impacts on environment and their biota have been reported numerous times (Olsgard and Hasle, 1993; Lancellotti and Stotz, 2004; VandenBerge et al., 2011; Thygesen et al., 2017). Recent studies have demonstrated the impacts of dam rupture on socioeconomic and environmental aspects along the Doce river and estuary (Aires et al., 2018; Cordeiro et al., 2019). Hatje et al. (2018) estimated transport of dissolved metals, such as Fe, Ba, Al, Hg, Co, Fe, Ni, As, Ba, Cr, Cu, Mn, Pb and Zn, along more than 650km, between the dam and the plume of the Doce River in the Atlantic. Impacts in the region of the mouth of Doce river and adjacent marine area were also investigated (Marta-Almeida et al., 2016; Magris et al., 2018). Recently, Magris et al. (2018) demonstrated that the most impacted marine areas were the Santa Cruz wildlife refuge and the environmental protection area Costa das Algas (Coast of macroalgae) that embrace an ecosystem rich in coral reefs, seagrass meadows, rocky reefs and rhodolith beds, which was the one that suffered the highest impact.

Facing the ecological importance of CCA and the current and future impacts of ore dams' ruptures on rhodolith beds, this work aims on evaluate the effectiveness of cleaning the sediments from rupture of mining tailing dam on macroalgal photosynthesis, with emphasis on the crustose coralline red algae *Lithophyllum margaritae* (Hariot) Heydrich. We tested the hypothesis that cleaning procedures after few hours of exposure can mitigate the tailings burial effects on *L. margaritae* productivity.

2. Material and Methods

2.1. *Sampling procedures and experimental design*

Rhodolith samples were collected by scuba diving at a depth between 5 and 10 m at Rancho Norte (27°16'29.6" S; 48°22'26.2" W), located on the marine biological reserve of Arvoredo, (Florianópolis, Santa Catarina, Brazil). This area is out of Doce river plume influence, a necessary aspect to evaluate specimens never exposed to this kind of sediment. To evaluate effects and impacts of tailing burial on macroalgae, seven species were selected for short-term exposure experiments regarding to photosynthesis parameter. Three macroalgae (*Ulva lactuca* (Linnaeus 1753), *Sargassum vulgare* (C.Agardh 1820) and *Jania rubens* (Linnaeus) J.V.Lamouroux, 1816 and four CCA representatives, with wide tropical distribution (*Lithophyllum margaritae (Hariot) Heydrich*, *Lithophyllum atlanticum, Mesophyllum erubescens* (Foslie) Me.Lemoine, and *Lithothamnion crispatum,* Hauck, 1878), were exposed to sediment of the mining tailing for 24 hours according to the methods described by Riul et al. (2008). The algae were incubated in 200 mL sealed glass jars containing filtered natural seawater under 150 μmol photons m^{-2} s^{-1} irradiance at room temperature on a shaker. Photosynthesis and respiration rates of four individuals were measured during a 1-hour incubation period on a magnetic stir-plate using oxygen spot optodes (Pyroscience GmbH, Germany). Following the incubation, the algae were exposed to a 1:60 mud:seawater dilution treatment for 24 hours, which simulated turbidity values observed at the edges of the documented Doce River plume. Photosynthesis and respiration rates were measured again after the 24-hour exposure.

To evaluate the influence of management practices, *Lithophyllum margaritae* (Figure 1a) was the CCA specie used since it is the taxon most vulnerable to mud exposure as demonstrated in our first experiment as in the Doce river influence area.

A 14-day microcosm experiment was carried out, including a 7-day exposure to the sediment of the mining tailing and a 7-days physiological recovery after cleaning procedure (Figure 1b). Specimens with a diameter range from 5 to 7 cm (Figure 1a) were kept in acclimation for one week, with cultivation on sterile seawater (salinity = 36), controlled conditions of temperature (20°C ± 2), and continuous aeration. During the exposure days fleshy macroalgae were excluded, once their thallus lost consistency after one-week exposure and could not support management.

To evaluate effects and impacts of tailing burial on macroalgae, seven species were selected for short-term exposure experiments. Three macroalgae (*U. lactuca, S. vulgare* and *J. rubens*) and four CCA representatives, with wide tropical distribution (*L. margaritae, L. atlanticum, M. erubescens* and *L. crispatum)*, were exposed for 24 hours according to the methods described by Riul et al. (2008) with 4 replicates. These species were exposed to the lower concentration of sediment, with turbidity values observed in the edges of the documented Doce River plume. To evaluate the influence of management, a 14-day microcosm experiment was carried out, including a 7-days exposure to the sediment of the mining tailing and a 7-days physiological recovery after cleaning procedure (Figure 1b). For primary productivity measurements, algae were incubated in 200 mL sealed glass jars containing filtered natural seawater under 150 µmol photons.m-2 s^{-1} irradiance at room temperature on a shaker. Photosynthesis and respiration rates of four individuals were measured during a 1-hour incubation period on a magnetic stir-plate using oxygen spot optodes (Pyroscience GmbH, Germany). Following the incubation, the algae were exposed to a 1:60 mud:seawater dilution treatment for 24 hours, which simulated turbidity values observed at the edges of the documented Doce River plume. Photosynthesis and respiration rates were measured again after the 24-hour exposure.

To simulate the effect of the mining tailing on CCA, 30 kg of the sediment of the mining tailing (for metals concentration see Gomes et al., 2017) were sampled near the Doce river mouth (~30 days after the mining spill). The sediment was obtained in river sand banks by scraping with a shovel (5-10 cm over solid bottom), stored in polyethylene vessels and transported to the Phycology Laboratory (samples were protected from sun and heat; LAFIC, CCB – UFSC). The experiment was accomplished on July 2017.

All treatments were accomplished in 260 mL erlenmeyers, in water bath at 20 ºC, under continuous and individual aeration, in a culture chamber with controlled temperature (20 ±2 °C) and photoperiod of 12 h (starting at 6 a.m.), and PAR obtained with fluorescent lamps at 100 µmol photons.m^{-2} s^{-1}.

For exposure procedures, the experimental design consisted of 4 treatments in an increasing gradient of contamination: without addition of the sediment of the mining tailing (control samples therefore C1), and with 12.5 g L^{-1}, 25 g L^{-1} and 50 g L^{-1} sediment of the mining tailing addition (called C2, C3 and C4, respectively). The nomenclature treatment will be used for all manuscript for all samples. During the first 7 days of the experiment the organisms were maintained at same conditions of acclimation period, and sediment addition of different treatments was done only at first day in each experimental unity. After the addition of the sediment, each vial was sealed with plastic film to reduce seawater losses through evaporation. On the 7th day, after physiological measurements described in next sections, *L. margaritae* samples were gently washed with seawater, to remove the superficial sediment of the mining tailing. After those procedures, the 7-days recovery experimental period started. To evaluate the oxidoreductive conditions of metal-rich muddy average, incubations were performed with the same mud concentrations, but without addition of the *L. margaritae* for all treatments. The nomenclature time-treatment will be used in all manuscript will be used for *L. margaritae* treatments (without or with mining tailing addition, in all C2, C3 and C4 concentrations treatments) and the conditions of time (exposition or recovery).

2.2. Variables of interest

To observe differences among treatments and the sediment of the mining tailing deposition over *L. margaritae*, at the end of the 14-day experiment, samples were photographed with Leica S8APO magnifying glass (Wetzlar, Germany). Details of surface and area of cover by the sediment was observed as a qualitative descriptor of the interaction between the sediment with CCA.

In vivo chlorophyll-a fluorescence was quantified using a portable fluorimeter (Water PAM, Walz, Effeltrich, Germany), at the beginning of experiment (previously to sediment addiction), after 7-days exposure and after 7-days experimental recovery. In each measurement the fluorescence (Fo) baseline, maximum quantum fluorescence (Fm), variable fluorescence and the maximum quantum yield (Fv/Fm) of the PSII were obtained. All measurements were carried after adaptation of the *L. margaritae* to the dark for 30 minutes.

Gross primary productivity of *L. margaritae* was obtained using a portable oximeter (YSI 5100, Yellow Springs, USA). A total of four incubation periods were performed: initial incubation on the first day of experiment; final incubation on the 7th day of the experiment; initial incubation of recovery period on the 8th day of the experiment and final incubation on the 14th day of the experiment. The measurements of gross primary productivity ratio of the oxygen rate (mg O2.L-1.g-1. h-1) were applied using the following equation:

$$\text{Photosynthesis rate} = \frac{\Delta O_2}{\Delta t * m}$$

The differences of concentration (ΔO2; mg L^{-1}) was assessed comparing the measurements of oxygen concentration (mg L^{-1}) at beginning and at the end of incubation; Δt = incubation period (h); and m= fresh biomass of the sample (g). Samples were incubated under irradiation of 300 µmol photons.m-2 s^{-1} for 30 minutes. Irradiance value was obtained by adjusting data to the exponential function proposed by Jassby and Platt (1976). The irradiance value set for the experiment was equal to the exponential relation between maximum value of photosynthetic activity (Pmax), and photosynthetic efficiency (α):

$$\mathrm{P} = P_{max}\left[1 - e^{(\alpha E/Pmax)}\right]$$

Variation on dissolved oxygen average rates was obtained for difference among measurements at beginning and after thirty minutes of incubation. Incubations under light conditions (gross primary productivity) were performed before the incubation in darkness (respiration rates). To obtain *L. margaritae* respiration rate, dissolved oxygen measurements were also obtained before and after incubation in darkness for 30 minutes. Incubations under light and darkness conditions were also performed to compare data of gross primary production and oxidative effect of metal-rich muddy medium.

2.3. Pigment analysis

Samples of 0.5 to 1.5 g fresh biomass were collected for analysis of accessory pigments of phycobiliproteins. Fragments were washed with distilled water and macerated in liquid nitrogen and then transferred to a 15 mL falcon tube in which was added 4 mL of a phosphate buffer solution (0.1 M, pH 6.8) and homogenized. Samples were stored in darkness at 4°C for 24h and then centrifuged for 2 min at 3000 rpm. The supernatant was recovered, and spectrophotometric measurements were carried at 498 nm, 614 nm, 651 nm, and 750 nm wavelengths (Bel Spectro LGS53, BEL Analytical Equipment Ltd., Brazil). The concentrations of phycobiliproteins fractions (allophycocyanin, phycocyanin and phycoerythrin) were calculated according to equations proposed by Kursar et al. (1983). Phycoerythrin is located on the end of the rods and is the complex with the shortest wavelength absorption maximum, phycocyanin is located between the end of the rods and the core and absorbs at intermediate wavelength, and allophycocyanin forms the core of the phycobilisome and absorbs at the longest wavelength (Blankenship, 2014). Phycoerythrin is an accessory pigment to Chlorophyll and its role seems to be especially important under low-light conditions (Beer et al., 2014).

2.4. Statistical analysis

One-way ANOVA were used to test physiological differences on responses according to treatments (24 hs exposition to 1:60 mud:seawater dilution). Two-way ANOVA were used to test the other variables. Previous tests were performed to verify the homoscedasticity (Levene's test) and presence or absence of normal distribution (Kolmogorov-Smirnov test). LSD post-hoc was used in the evaluation of differences among species.

When no significant interaction among descriptors was verified, individual tests were carried out to verify the differences among groups for both time or sediment levels. To test effects of different concentrations of the sediment of the mining tailing on response of different parameters tested, statistical analyses were performed using Statistica 7.1 (Statsoft Inc.) software. Tests were performed to verify whether data were parametric or non-parametric, and homoscedasticity of the data was also tested.

3. Results

3.1. Description of the different concentration of sediment in CCA surface

The deposition of the sediment from the mining tailing follow the differences in concentration. No sediment was observed in the C1 (control) samples. The lowest concentration of mud (C2) presented a thin layer of sediment deposited for all surface (Figure 1c). The height of the layer increased with the highest concentration of sediment (C4; Figure 1d).

3.2. Physiological impacts

After 24 hours of exposure, among the evaluated species, fleshy macroalgae and *Lithophyllum* representatives presented significant reduction in the photosynthesis, with *L. margaritae* being the most impacted CCA species according to productivity parameter, despite being more resistant than Ulva (Figure 2b, Table 1).

Considering the recover trial, gross photosynthesis after one week reduced to negative values. However, after algal washing, gross photosynthesis recovered to control values. Treatments with mud presented significant decrease ($p \geq 0.05$) of gross photosynthesis among all samples (Figure 3a, Table 2). There was no significant difference of gross photosynthesis between samples treated with mud and samples after recovery ($p = 0.201933$) (Figure 3a, Table 2).

The decrease of oxygen concentration in samples without *L. margaritae* was observed on data of metal-rich muddy medium incubations following the increase of muddy concentration with significant difference on incubations performed in darkness ($p = 0.005885$; Figure 3b, Table 3).

The difference in concentration of phycobilin by wet weight between different treatments with mud was only significant for allophycocyanin, which increased compared to other pigments ($p < 0.001$; Figure 3c, Table 4).

4. Discussion

4.1. Tailing impacts in fleshy macroalgae and CCAs

The significant reduction in the photosynthesis of fleshy macroalgae and CCAs, and the negative O_2 balance caused by the tailing burial demonstrates that there are important limitations to photosynthesis under the impact of mud burial. Higher concentrations of mud increase oxygen consumption, reducing the oxygen availability for benthic organisms. This negative balance helps to understand the general scenario described in Doce river, as in others similar disasters, with losses of species and ecosystem goods and services (Gomes et al., 2017).

In our experiment, gross productivity showed a reduction on oxygen that was observed in all samples that were submitted to sediment dilutions. Regardless of sediment concentration, significant differences among treatments were observed, with a major reduction of productivity for samples exposed to highest mud concentration. Loss of CCA organisms due to burial or sedimentation is a consequence mainly from reduction of light due to burial (Hall-Spencer and Moore, 2000; Wilson et al., 2004; Reyniear et al., 2015). Riul et al. (2008) experimentally demonstrate reduction of net and gross photosynthesis rates in rhodoliths buried by fine sediment, thus reiterating the negative impact of this type of stress to rhodoliths. So, rhodoliths are highly susceptible to anthropogenic disturbance such as trawling harvesting and reduced water quality (Israel et al., 2010; Reyniear et al., 2015). Burial by a sediment layer, even thin, may cause long-term stress in these organisms and may even be lethal due to anoxia in environment caused by sediment accumulation (Wilson et al., 2004; Figueiredo et al., 2012) and by reduction of light penetration by sediment (Basso, 2012; Reyniear et al., 2015; Aguirre et al., 2017).

CCA of *Phymatolithon calcareum* buried by sediment presented a significant reduction of photosynthetic yield in relation to C1 samples, independently of sediment type (Wilson et al., 2004). In the same experiment, it was also verified that greatest losses in photosynthetic performance occurred in samples buried with mud, compared to gravel and sand burial, due to the low oxygenation of muddy sediment, its compacted structure. This can be noticed analysing concentration of oxygen on water samples data that decreased with increasing concentration of mud (Wilson et al., 2004).

The initial disaster remediation programs proposed by Samarco were summarized in revegetation of the banks and the mouth of Doce river (Samarco, 2017), as well as quality monitoring of water and the plume advance through over flights in the river and at the mouth were summarized in the river banks and mouth waters of the Doce river (Samarco, 2017), and an additional monitoring water quality and plume advancement through river and river mouth (Samarco, 2016). Current remediation programs aim to work on impact assessment of tailings, recovery of areas and treatment of sediments, also on the construction and operation of sediment containment structures for the storage of the materials removed from the river channels and their surroundings, on diagnosis of impacts on water quality and aquatic biodiversity, followed by actions for recovery and conservation of aquatic fauna and supporting financially studies and implementing repairing actions in the Conservation Units that were affected by the collapse (Renova Foundation, 2019). Therefore, we also discuss short- and long-term approaches and actions for Samarco's disaster remediation, specific to CCA.

4.2. Approaches to disaster remediation

In situ, the main prevention of rhodolith beds burial in both deep and shallow water environments is through the action of currents and waves (Marrack 1999; Steller et al., 2003; Wilson et al., 2004). However, in cases when the mining tailing sediment reach the ocean, wave and currents movement may not be able to remove contaminated sediment deposited on CCA that colonize depths around 30 m. Due to dominance of northeasterly winds during fair weather in the coast of Espírito Santo, fine sediments tend to be deposited to the south of the Doce river mouth, to depths of up to 30 m (Quaresma et al., 2015). In this depth, extensive beds of rhodoliths of great ecological and economic importance are found (Guimarães and Amado-Filho, 2008; Riul et al., 2009; Amado-Filho et al., 2010; Amado-Filho and Pereira-Filho, 2012). So, management actions for sediment disturbance (e.g. burial impacts) include procedures such as water and sediment cleanliness to restore natural condition of the environment, as it was imposed in accidents such as the rupture of the Placer Dome dam in the Philippines (Miranda et al., 2003) and Aznalcóllar dam in Spain (Bartolome and Veiga, 2002).

Brazilian legislation punishes with fine and/or seclusion in cases of environmental crime in a comprehensive manner, without specifying a punitive action for accidents involving dam rupture (Brasil, 1998; Garcia and Fonseca, 2018). However, there are no specific protocols for environmental remediation for mining tailings impacts (Kossoff et al., 2014; Carvalho, 2017).

According Kossoff et al. (2014), once removed the sediment that cover target communities, monitoring programs are needing to certify environment quality and health, in short and long term. Thus, regulation and monitoring provide the necessary structure and controls for management.

We demonstrated through our experiment that it is possible to manage short term burial impacts on L. margaritae by cleaning the algae surface, thus recovering the productivity parameter to inicial values, e.g before treatment with mud. This recovery is only related to producitivty parameter, however, in this work, we did not evaluate the effetcs related to mud toxicicty which will be testes on futher experiments. Our results also point out that a simple management tool can mitigate the impact of tailing burial on producitivy parameter, which reinforce the necessity of efforts for cleaning the sea bottom as well the Doce river environment as recommended in cases related with oil spill.

É importante considerar também efeitos da presença de metais no sedimento na fisiologia de rodolitos, que podem se acumular na estrutura da alga (Darrenougue et al., 2018) ou até mesmo alterar aspectos fisiológicos como crescimento (Bohm et al., 2003).

In accidents such as the DWH oil spill, measures taken to restore benthic ecosystems (e.g. coral and seaweed) consisted of booming to divert the oil from the reef, water and key organism cleaning and transplantation of species (Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016; Hale, et al., 2017). Also, cooperative management can help protect against multiple threats and provide a framework for monitoring, education, and outreach.

Together with rhodolith bed cleanness actions, understanding sediment and water conditions, and effects of burial on community structure, and hence biodiversity, are crucial for determining the magnitude of short- and long-term effects (Macleod et al., 2016).

Regarding Samarco dam rupture and effects on rhodolith bed, for a long-term approach, resilient mitigation (Cohen-Shacham et al., 2016) should be adopted, which involves proposing solutions capable of facing current and future threats and adopting solutions ecosystem-based and of relevance to the resilience of socioecological systems. Actions aimed at protecting, sustainably managing and restoring ecosystems. Habitat and risk maps should be cross checked in the process of MPAs proposition and management. Monitoring and evaluation of not only the direct and indirect effects of the Fundão Dam disruption on rhodolith beds in the short and long term, but also of other pressures that affect the environmental quality of the basin and the well-being of its populations addressing the challenges of society in an effective and adaptive way while providing the human well-being and benefits of CCA.

5. Final considerations

Following the mainstream efforts, after almost three years, the impacts on Doce river influence zone still unknow and understood, especially impacts on phytobenthic communities, which should be suffering influences of the mining tailing sediment.

Mitigating the impacts of dam failures in the marine environment is an important goal, as reduction of primary production can promote cascade impacts with socioeconomic consequences. CCA cleaning and/or transplantation should be considered as a necessary management action. In addition to these remediation measures, monitoring is necessary to determine the success of the eventual management. Concomitantly to the CCA cleanness actions, long-term approaches should be adopted (e.g. protection and restoration of the ecosystem). The application of short- to long-term approaches on the influence area of the Doce river, as any other area influenced by contaminated plumes, are essential to better manage mining tailing disaster, being an important step towards reducing overall impacts.

6. Ethical declarations

6.1. Conflict of interest

The authors declare no current or potential conflict of interest.

6.2. Contribution

SDF and GBC participated in sampling and experiment procedures. All authors analyzed the data, wrote the manuscript and approved the final article.

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Figure captions

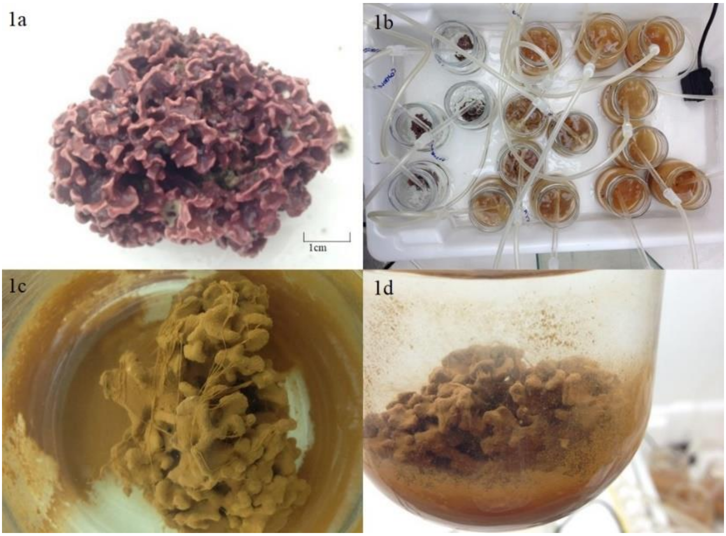

Figure 1. Crustose coralline red algae a) *Lithophyllum margaritae* b) used in the 14-day microcosm experiment of exposure to the sediment of the mining tailing, and the visible changes in sediment deposition c) from the thin layer of sediment deposition (C2) d) to the highest concentration of sediment (C4).

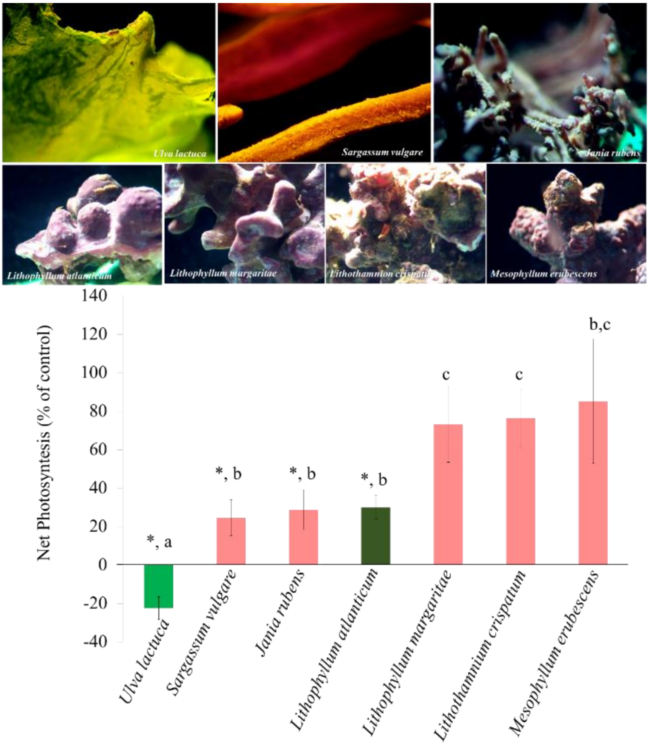

Figure 2: a) Relative impact of the mining waste from the mud on net photosynthesis of macroalgae (green, brown and dark pink bars) and rhodolith-forming species (light pink bars) after a 24h-exposure (data are expressed in percentage to values measured before exposure). Data represent mean ± SE (n=4) and * indicates significant differences to control (C1) treatment (One-way ANOVA, p≤0.05; Table 1). Different letters represent significant differences among species behaviour considering LSD post-hoc test.

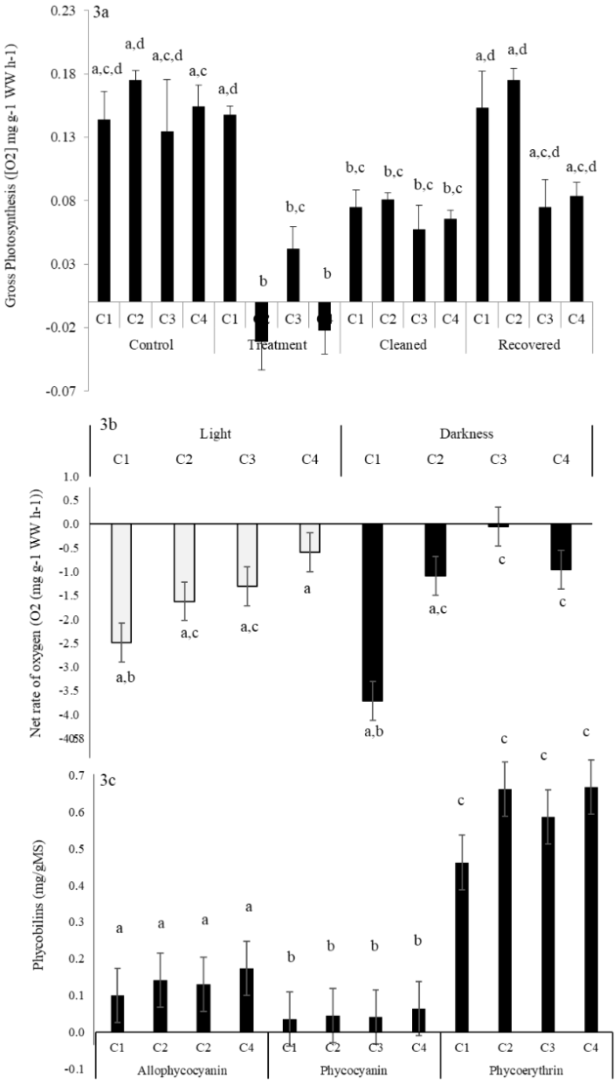

Figure 3. Graphical results of *Lithophyllum margaritae* for: a) Mean of gross photosynthesis data, obtained through incubation, between concentrations (C1, C2, C3 and C4) among different treatments. Graph shows significant decrease between sample means with different concentrations ($p < .000$; Table 2). Graph also shows that there was no significant gross photosynthesis differences

between control and recovered samples ($p = 0.201933$; Table 2) b) Mean of net rate of oxygen concentration in the medium without CCA (mud oxygen balance on seawater) and c) phycobilin concentrations after the recover treatment. Different letters represent significant differences among species behaviour considering LSD post-hoc test.

Table caption

Table 1. One-way ANOVA results of comparing evaluated species and the significant differences of the treatment in relation to the control for each species (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Species	26398.15	6	4399.69	**5.34072**	**0.0047**
Ulva sp.	42079.00	1	42079.00	**49.26**	**0.000**
Sargassum sp.	245.00	1	242.00	**5.91**	**0.050**
Jania sp.	1425.78	1	1425.78	**6.78**	**0.041**
Lithophyllum sp.	14.05	1	14.05	**0.68**	**0.044**
L. margaritae	3.65	1	3.65	**7.07**	**0.038**
L. crispatum	4.65	1	4.65	3.79	0.099
M. erubescens	0.20	1	0.20	0.25	0.645

Table 2. Two-way ANOVA of log transformed gross O2 production of L. margaritae considering different mud concentration and management moments (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Intercept	37.72908	1	37.72908	**1541.557**	**0.000**
Management	2.34334	3	0.78111	**31.915**	**0.000**
Mud concentration	2.09081	3	0.69694	**28.476**	**0.000**
Management*Mud	2.83919	9	0.31547	**12.889**	**0.000**

concentration

Table 3. Two-way ANOVA of log transformed net O2 production medium without CCA (mud oxygen balance on seawater), considering different mud concentration (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Treatment	70.01530	1	70.01530	**37.07411**	**0.000**
Light	0.02194	3	0.02194	0.01162	0.915068
Darkness	30.17948	3	10.05983	5.32682	0.005885

Table 4. Two-way ANOVA of log transformed of phycobilin concentrations after the recover treatment considering different mud concentration (Bold F-values indicate significant p-values. *p<0.001).

Source	Sun of squares	Degree of freedom	Mean squares	F -value	p-value
Pigments	3.224822	1	3.224822	**824.2178**	**0.000**
Allophycocyanin	2.766426	2	1.383213	**353.5292**	**0.000**
Phycocyanin	0.072504	3	0.024168	6.1770	0.001695
Phycoerythrin	0.050252	6	0.008375	2.1406	0.072335

CONSIDERAÇÕES FINAIS

A biologia das algas calcárias é influenciada diretamente por variações ambientais (por exemplo variação na exportação de nutrientes e de turbidez da água influenciadas pela vazão de rios) e impacto humano nos ecossistemas (por exemplo o rompimento barragem de rejeito de minério). Baseado nisso, experimentos sobre **i)** a influência do aporte de nutrientes e turbidez da água, e **ii)** o efeito da cobertura por rejeito de minério na biologia de algas calcárias (por exemplo, *Lithophyllum margaritae*) foram realizados. Os impactos na fisiológica das algas demonstraram a rápida resposta no rendimento quântico e nas concentrações de pigmentos quando se encontram sob alterações no aporte de nutrientes e turbidez da água. Enquanto a redução da produtividade ocorreu durante impacto de cobertura de rejeito de minério de ferro. A limpeza das algas como estratégia de manejo se mostrou eficaz, dando suporte para novas estratégias de manejo destas algas em locais sob impacto de minério de rejeito de ferro.

Variações na produtividade não foram significativos sob o aumento nas concentrações de nutrientes e turbidez da água durante os experimentos. No entanto, o aumento de nutrientes e a diminuição da disponibilidade de luz afetam o rendimento fotossintético e a concentração de pigmentos. Por exemplo, ficobiliproteinas funcionam como uma antena de captura de energia para a clorofila a, e realizam outras funções, como proteger as algas contra a oxidação foto e servem também como uma reserva de nitrogênio. Com o aumento de estressores locais, existe uma tendência de aumento de impactos visíveis nas concentrações de pigmentos.

Os impactos na produtividade no experimento no qual a lama do rompimento da barragem de Mariana foi utilizada apresentou resultados significativas na redução nos dados de fotossíntese bruta. Logo após a limpeza dos organismos, a produtividade voltou a crescer, mostrando a eficácia do manejo. Assim técnicas de manejo, como a limpeza das algas em ambientes impactados por rejeito de minério de ferro, podem ser aplicadas na sua recuperação, por exemplo o rompimento da barragem de Mariana que atingiu o oceano Atlântico no Leste do Brasil e demais acidentes que vierem a ocorrer.

Ambos experimentos abordaram aspectos da biologia de CCA importantes para ampliarmos os conhecimentos sobre esse grupo de algas. Nesse trabalho verificamos a eficácia de técnicas de manejo e buscamos, em projetos futuros, implementar novas estratégias de manejo de CCA que possam ser impactados com diferentes estressores locais.