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Decontamination protocol of the macroalga *Bostrychia binderi* Harvey (Rhodophyta) for unialgal cultures and laboratory studies[1]

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ABSTRACT - (Decontamination protocol of the macroalga *Bostrychia binderi* Harvey (Rhodophyta) for unialgal cultures and laboratory studies). This study aimed to test the effectiveness of physical and chemical procedures for surface decontamination of *Bostrychia binderi* Harvey. For this, thalli were washed and immersed in chemical solutions (5% liquid detergent, 1% povidone iodine and 0.2% sodium hypochlorite) at different times and combinations. After treatments the thalli were cultivated in laboratory to verify possible negative effects caused by the procedures to alga. Contaminating organisms in the algal culture medium were quantified at the end of cultivation. Washing, spraying, removal of macrofauna individuals with tweezers, thalli immersion in 5% liquid detergent for 60 seconds, after in 0.2% sodium hypochlorite for 2 minutes were effective procedures to eliminate all contaminants analyzed. Thalli immersion in 1% povidone iodine did not affect negatively the thalli growth, whereas immersion in 0.2% sodium hypochlorite for 5 minutes affected. For establishment of *Bostrychia* unialgal cultures we recommend the protocol proposed in this study.
Keywords: Bostrychietum, contaminants, macroalgae, mangrove, 0.2% sodium hypochlorite

RESUMO - (Protocolo de descontaminação da macroalga *Bostrychia binderi* Harvey (Rhodophyta) para culturas unialgais e estudos laboratoriais). Este estudo objetivou testar a eficácia de procedimentos físicos e químicos para descontaminação da superfície de *Bostrychia binderi* Harvey. Para isto, talos foram lavados e imersos em soluções químicas (detergente líquido 5%, iodopovidona 1% e hipoclorito de sódio 0,2%) em diferentes tempos e combinações. Após os tratamentos, os talos foram cultivados para verificar possíveis efeitos negativos causados pelos procedimentos à alga. Contaminantes no meio de cultura dos talos foram quantificados no final do cultivo. Procedimentos de lavagem, remoção física de indivíduos da macrofauna, imersão de talos em detergente líquido 5% por 60 segundos, depois em hipoclorito de sódio 0,2% por 2 minutos, foram eficazes para eliminar os contaminantes analisados. Imersão de talos em iodopovidona 1% não afetou o crescimento dos talos, enquanto que imersão em hipoclorito de sódio 0,2% por 5 minutos afetou. Para estabelecimento de culturas unialgais de *Bostrychia* recomendamos o protocolo proposto neste estudo.
Palavras-chave: Bostrychietum, contaminantes, macroalgas, manguezais, hipoclorito de sódio 0,2%

Introduction

Culture techniques for macroalgae in laboratory are valuable tools in ecophysiological studies for basic research or to obtain relevant products to humans (Fernandes *et al.* 2011). According to Berland *et al.* (1972) contaminants in macroalgal culture medium grow and proliferate faster than the algae do, competing for nutrients and releasing substances capable of inhibiting algal growth. Fernandes *et al.* (2011) say that the establishment and maintenance of macroalgal cultures free of contaminating organisms have been one of the main challenges found by researchers. Contaminating organisms as cyanobacteria and microalgae, fungi and heterotrophic bacteria, protozoa and invertebrates can also grow in these cultures. Commonly, the main contamination source for macroalgal cultures is the biota on the algal surface.

Procedures have been developed and are used to prevent the contamination of cultures. Methods for surface decontamination of macroalgae have used chemical compounds as antibiotics, disinfectant solutions (detergent, povidone iodine, sodium hypochlorite, sodium hydroxide) and also physical methods as brushing and washing (*e.g.* Oliveira *et al.* 1995, Kawai *et al.* 2005, Bravin *et al.* 2006, Shea & Chopin 2007, Fernandes *et al.* 2011, Holdt *et al.* 2014, Saminathan *et al.* 2014, Yong *et al.* 2014, Kerrison *et al.* 2016, Ali *et al.* 2018). However, the methods for surface decontamination of macroalgae must be adjusted for each species (Fernandes *et al.* 2011), because the effects

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of procedures, mainly the use of chemical compounds, can vary among macroalgal species.

The genus *Bostrychia* Montagne belongs to Rhodomelaceae (Rhodophyta) and includes macroalgae with distribution in temperate and tropical regions, occurring in continental aquatic and marine environments, mainly in salt marshes and mangroves (King & Puttock 1989). Species of *Bostrychia* form the structure of the mangrove community known as Bostrychietum. This term was proposed by Post (1936) and includes mainly rhodophytes as *Bostrychia*, *Caloglossa* (Harvey) G. Martens and *Catenella* Greville, but also cyanobacteria and chlorophytes which associate themselves to pneumatophores of *Avicennia* L., to rhizophores and stems of *Rhizophora* L. and *Laguncularia* C.F.Gaertn (West 1991, West *et al.* 1993, Pedroche *et al.* 1995, Yokoya *et al.* 1999, Fontes *et al.* 2007, García *et al.* 2016).

Bostrychia spp. have growth by apical cells present in the branches and *Polysiphonia*-type life history. Tetrasporophyte life stages (diploid) release tetraspores (haploid) which develop male and female gametophytes (haploid). Male gametophytes produce and release spermatia (haploid) which fertilize the carpogonium (haploid) present at the end of carpogonial branches produced by the female gametophytes. After fertilizing, the formation of carposporophytes (diploid) occurs, which produce and release carpospores (diploid) which develop tetrasporophytes (West *et al.* 1993, West *et al.* 2001).

Sediment and several mangrove organisms (*e.g.* microalgae, protozoa, fungi, nematodes, microcrustaceans, annelids, small molluscs, chironomid larvae, trombiculid mites) are associated to the Bostrychietum (García *et al.* 2016, Vieira *et al.* 2018). Epiphyte macroalgae and endophyte fungi may also be associated to *Bostrychia* spp. (De Felício *et al*. 2015). So, laboratory cultures of *Bostrychia* spp. free of contaminating organisms (unialgal cultures) are difficult, and protocols for surface decontamination adjusted for species of *Bostrychia* are needed.

In this context, the present work aimed to test the effectiveness of physical and chemical procedures for surface decontamination of *Bostrychia binderi* Harvey. This species was used as model in order to develop a decontamination protocol for obtaining unialgal cultures for laboratory studies with this genus. Our hypothesis is that washing with distilled water, afterward washing and spraying with sterilized seawater, physical removal of macrofauna individuals and epiphyte macroalgae, and thalli immersion treatments in 5% liquid detergent and afterward in 0.2% sodium hypochlorite are effective for surface decontamination of *B. binderi*.

Material and methods

Collection and maintenance of cultures - Specimens of *Bostrychia binderi* Harvey (BRASIL. Paraíba: Rio Tinto, Área de Proteção Ambiental da Barra do Rio Mamanguape, *s. d.*, Henrique Douglas dos Santos Borburema, JPBN° 63215) were collected in the mangrove of the "Área de Proteção Ambiental da Barra do Rio Mamanguape" (6°46'15.00"S and 34°56'15.00"O) in February 2017. During field collection, most of the estuarine sediment was removed with *in situ* water.

In laboratory remaining sediment adhered on the thalli was physically removed by several washing and spraying with sterilized seawater and macrofauna individuals associated (*e.g.* molluscs, crustaceans and annelids) were removed with tweezers. Thalli parts with filamentous and crustose macroalgae were removed by cutting with scalpels. Removal of macrofauna individuals and cutting of thalli parts with epiphytes were performed under a stereoscopic microscope. All instruments used in the handling of macroalgae were sterilized in ethanol (70°) and tweezers were flamed. The seawater used in all procedures was sterilized through filtering using sterilized cellulose membrane filters (0.45 µm pore), followed by heating in a laboratory drying oven at 90 ºC for two hours (after cooling, it was heated up again) (Borburema *et al.* 2020). The seawater was not autoclaved to preserve its vitamins.

Specimens of *B. binderi* (ca. 900 mg) were kept in 1000 mL Erlenmeyers flasks containing 700 mL of culture medium in constant aeration. The water temperature was 25 ºC, photonic flux density of 60 - 80 µmol photons m^{-2} s^{-1} and a photoperiod of 12:12 h (light:dark cycle) for maintenance of macroalgae in laboratory for one week before of the chemical procedures to test surface decontamination. The culture medium was sterilized seawater enriched with von Stosch's solution (8 mL L^{-1}) prepared as described by Edwards (1970) and modified with reduction of 50 % in the vitamin concentrations (Yokoya 2000).

Tests for surface decontamination with chemical procedures - We tested the effectiveness of thalli immersion treatments in chemical solutions after several washing and spraying with sterilized seawater and physical removal of macrofauna individuals and epiphyte macroalgae (as described above).

Tetrasporophyte plant thalli (3 cm in primary axis length with lateral branches, weighing 200 mg ± 20, n = 5 per treatment) of *B. binderi* were washed with distilled water and immersed in liquid detergent based on 5% sulfonic acid (w/w) (Fernandes *et al.* 2011) during 60 seconds (Saminathan *et al.* 2014). Posteriorly, the detergent was completely removed from the thalli by several washing with sterilized seawater. Then, thalli were immersed in 1% povidone iodine (POVIDINE ®) (Saminathan *et al.* 2014, Ali *et al.* 2018) for: 15 seconds (treatment 1), 30 seconds (treatment 2), 1 min (treatment 3) and 2 min (treatment 4). Other thalli were immersed in sodium hypochlorite (0.2% active chlorine L^{-1} of deionized water) (Holdt *et al.* 2014) for: 1 min (treatment 5), 2 min (treatment 6) and 5 min (treatment 7). The chemical solutions were completely removed from all thalli by several washing with sterilized seawater. A control (n = 5) was performed with thalli that were not washed with distilled water and immersed in any of the tested substances.

This study did not aim to compare the effectiveness between 1% povidone iodine and 0.2% sodium hypochlorite, but to compare the effectiveness of the treatments (1-7) in relation to the control. The laboratory glassware used in all procedures was sterilized: immersed in 0.2% sodium hypochlorite overnight and afterward washing and drying in laboratory drying oven at 120 ºC at least four hours.

Thalli were cultivated for three weeks in glass transparent containers (100 mL) containing 50 mL of culture medium which was changed weekly. The culture medium and other cultivation conditions (temperature, photonic flux density and photoperiod) were the same as described above for maintenance of macroalgae in laboratory before of treatments for surface decontamination.

Biomass values of the thalli were measured weekly on analytical balance during the replacement of the culture medium. Before weighing, thalli were gently blotted dry with paper towels to remove excess water. Relative growth rates (RGR) were calculated at the end of the cultivation and were estimated using the formula recommended by Yong *et al.* (2013): $[(W_t/W_i)^{1/t} - 1] \times 100$, being W_t the wet weight after t days, W_i the initial wet weight, and t is the cultivation period. The wet weights and RGRs were measured as proxies of thalli health.

At the end of the cultivation, 30 mL of culture medium from each replicate were discarded, and 20 mL of formaldehyde were added, the final formaldehyde concentration was of 4%. Rose Bengal dye was also added. 4% Formaldehyde was utilized to fix and preserve the contaminants and Rose Bengal dye to color them facilitating afterward the visual counting. A volume of culture medium (1 mL) from all replicates was taken away near the thalli surface by pipetting and deposited into SedgEwick-Rafter counting chamber so that the contaminants could be counted under stereoscopic and optical microscopy.

Statistical analyses - The number of contaminants (individuals mL^{-1}) in thalli culture medium immersed in 5% liquid detergent and 1% povidone iodine at different times was compared to the control by a one-way analysis of variance (ANOVA), whereas the number of contaminants in thalli culture medium immersed in 5% liquid detergent and 0.2% sodium hypochlorite (at different times) was compared to the control by the following tests: Mann-Whitney (for mean number of ciliates, microcrustaceans and nematodes between control and 1 minute), ANOVA and Tukey's post hoc test for annelids among control and immersion treatments for 1, 2 and 5 minutes.

ANOVA and Tukey's test were performed to compare the thalli weights observed at the three cultivation weeks for the same treatment. Final weights measured from immersion treatments in 5% liquid detergent and 1% povidone iodine were compared to the control by ANOVA, whereas a Kruskal-Wallis test was used to compare the final weights from immersion treatments in 5% liquid detergent and 0.2% sodium hypochlorite to the control. RGRs of the thalli immersed in 5% liquid detergent and 0.2% sodium hypochlorite were compared to the control by ANOVA and Tukey's test. Kruskal-Wallis test was applied to compare RGRs of the thalli immersed in 5% liquid detergent and 1% povidone iodine to the control.

Data were checked for normality (Shapiro-Wilk test) and variance homogeneity (Levene's test). The statistical analyses were performed using the R program (4.0.0 version) and the significance value adopted was of 5%.

Results and Discussion

Ciliates, microcrustaceans, nematodes, annelids and small gastropods in samples of thalli culture medium from all treatments and control were quantified by stereoscopic and optical microscopy. Immersion treatments in 5% liquid detergent (60 seconds) and after in 1% povidone iodine did not have effectiveness to eliminate contaminants on the thalli surface, and the mean number of contaminants (individuals mL^{-1}) was not significantly different among these treatments and control (table 1).

Immersion treatments in 5% liquid detergent for 60 seconds and after in 0.2% sodium hypochlorite for 2 and 5 minutes were effectiveness to eliminate ciliates, microcrustaceans, nematodes and small gastropods, reducing significantly the number of annelids (table 1). The treatment 5 (thalli immersion in 5% liquid detergent and after in 0.2% sodium hypochlorite for 1 minute) had the lowest effectiveness in the elimination of contaminants. Small gastropods were eliminated in all treatments with 0.2% sodium hypochlorite (table 1).

We observed a biomass loss in *B. binderi* at the second weighing (after one week of cultivation) in all treatments with chemical procedures (figure 1). This biomass loss occurred possibly due to negative effects of the chemical compounds on the macroalga. However, at the third weighing (after three weeks of cultivation) we observed recovery of the thalli with biomass gain (figure 1). Final weights of the thalli did not differ among treatments.

RGRs of the thalli were calculated with the weights obtained between the second and third weighing (after recovery of the thalli). Significant differences among RGRs of the thalli immersed in 1% povidone iodine for 30 seconds (1.69% $day^{-1} \pm 0.26$) from those immersed for 15 seconds (0.76% $day^{-1} \pm 0.25$) and 2 minutes (0.65% $day^{-1} \pm 0.63$) were observed, but when compared to the control (1.15% $day^{-1} \pm 0.42$) and 1 minute (1.13% $day^{-1} \pm 0.63$) there were no significant differences. Thalli immersed in 0.2% sodium hypochlorite for 5 minutes had the lowest growth (0.46% $day^{-1} \pm 0.14$), being significantly different from the thalli growth immersed for 2 minutes (1.19% $day^{-1} \pm 0.30$), 1 minute (1.02% $day^{-1} \pm 0.22$) and control (figure 2). These data indicate that immersion in 1% povidone iodine did not affect negatively the thalli growth, whereas immersion in 0.2% sodium hypochlorite for 5 minutes affected.

Results observed in this study indicate that only 5% liquid detergent for 60 seconds is not effective for surface decontamination of species of *Bostrychia*, because in immersion treatments in this compound and 1% povidone iodine there was no elimination of contaminating organisms. Although immersion treatments in this compound and 0.2% sodium hypochlorite have been effective, suggesting that 0.2% sodium hypochlorite is effective for surface decontamination of the macroalga. However, 5% liquid detergent is relevant for removing fat tiers on contaminating organisms, contributing to the action of sodium hypochlorite (Holdt *et al.* 2014).

Table 1. Mean values (individuals mL^{-1}) with standard deviations of contaminants counted in culture medium samples of the treatments for surface decontamination of *Bostrychia binderi* Harvey and control. Statistical analyses were performed among: control and treatments with 5% liquid detergent and 1% povidone iodine; and among control and treatments with 5% liquid detergent and 0.2% sodium hypochlorite. Asterisk (*) indicates significant differences. The values correspond to five replicates (n = 5).

	Contaminating Organisms (Individuals mL^{-1})				
	Ciliates	Microcrustaceans	Nematodes	Gastropods	Annelids
Control	759.6; ± 160	17.6; ± 7.0*	33.6; ± 9.9*	0.4; ± 0.5	1.6; ± 0.5*
Treatments 5% liquid detergent and 1% povidone iodine					
15 sec	720; ± 113.6	18.8; ± 7.0	28.8; ± 5.5	0.6; ± 0.8	1.4; ± 1.1
30 sec	613.4; ± 159.1	15; ± 9.2	38; ± 9.2	0.6; ± 0.5	1; ± 0.7
1 min	594.6; ± 204.4	18; ± 4.9	34; ± 8.8	0.8; ± 0.83	2; ± 0.7
2 min	603.2; ± 194.1	22.6; ± 9.6	30.6; ± 4.2	1; ± 0.7	1; ± 0.7
p values (ANOVA)	0.49	0.69	0.48	0.83	0.26
Treatments 5% liquid detergent and 0.2% sodium hypochlorite					
1 min	538.8; ± 626.7	0.2; ± 0.4*	0.6; ± 0.5*	0	0.6; ± 0.8
2 min	0	0	0	0	0.2; ± 0.4*
5 min	0	0	0	0	0.2; ± 0.8*
p values	0.30[a]	> 0.01[a]	0.01[a]	-	0.03[b]

a: p value Mann-Witney test and b: p value Tukey's test.

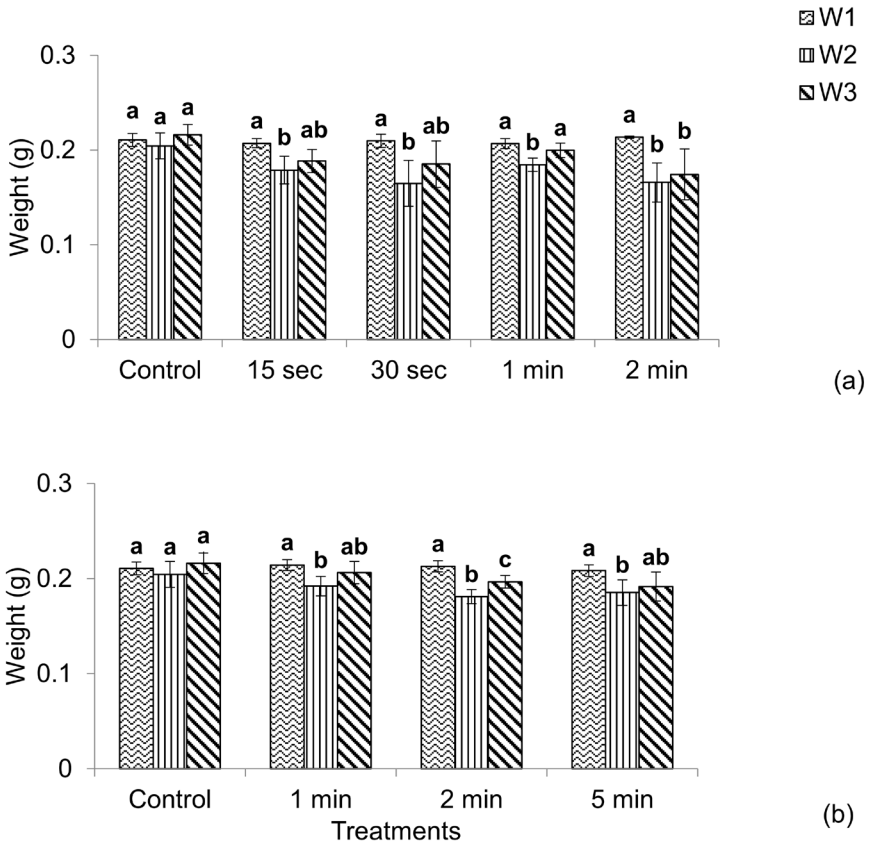

Figure 1. Mean weights (g) with standard deviations of the cultivated thalli. Initial weight (W1), weight in the second week (W2) and in the third week (W3). a. Control and immersion treatments in 5% liquid detergent and after in 1% povidone iodine. b. Control and immersion treatments in 5% liquid detergent and after in 0.2% sodium hypochlorite. Columns are means and bars standard deviations. Different letters above bars indicate significant differences among weights over three weeks for the same treatment. The values correspond to five replicates (n = 5).

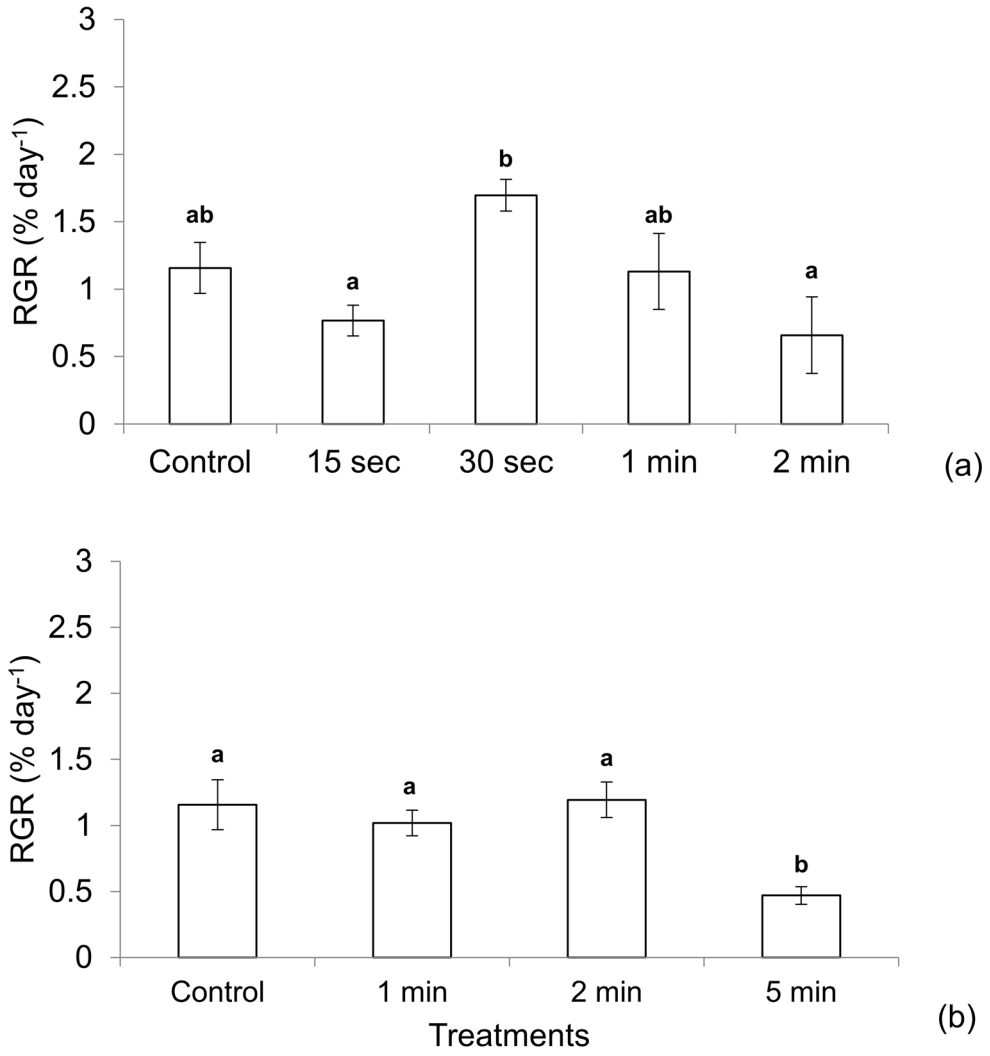

Figure 2. Relative growth rates (RGRs) of the cultivated thalli. RGRs were calculated with weights obtained at the second and third weighing (after recovery of the thalli). a. Control and immersion treatments in 5% liquid detergent and after in 1% povidone iodine. b. Control and immersion treatments in 5% liquid detergent and after in 0.2% sodium hypochlorite. Columns are means and bars standard errors. Different letters above bars indicate significant differences among treatments. The values correspond to five replicates (n = 5).

Diatoms were observed in all treatment and control samples. Nevertheless, they were not quantified because procedures to inhibit growth of these microalgae in culture medium are well-known, using GeO_2 (Germanium Dioxide) which does not cause negative effects on the macroalgae (Lewin 1966, Markham & Hagmeier 1982, Shea & Chopin 2007, Miranda *et al.* 2012, Saminathan *et al.* 2014). Diatoms in macroalgal culture medium may grow and proliferate faster than the algae do, competing for nutrients and releasing substances capable of inhibiting algal growth (Berland *et al.* 1972). In our study, these negative effects probably did not occur because the thalli biomass did not vary significantly among weeks in the control (which has not been subjected to chemical procedures for decontamination), and RGRs of control thalli differed only from the immersion treatment for 5 minutes, likely due to the effect of sodium hypochlorite (discussed below).

Saminathan *et al.* (2014) suggest that thalli immersion in liquid detergent (0.5% for 10 minutes) followed by 1% povidone iodine for 30 seconds reduces bacteria and eliminates zooplankton and ciliates on *Gracilaria dura* (C.Agardh) J.Agardh. In our study, 1% povidone iodine was not efficient to eliminate ciliates and zooplankton (*e.g.* microcrustaceans) on the algal surface, maybe the effective decontamination found by Saminathan *et al.* (2014) was caused by a longer thalli immersion time in detergent (10 minutes).

Fernandes *et al.* (2011) verified the potential of chemical agents for surface decontamination of macroalgae (using *Hypnea musciformis* (Wulfen) J.V.Lamouroux as a model) and found that detergent (5%) and sodium hypochlorite (1.01%) were effective solutions. In our study, we tested sodium hypochlorite in a lower concentration (0.2%) because *Bostrychia* spp. have thallus more delicate than *Hypnea* J.V.Lamouroux. Gigartinales and Gracilariales (*e.g. Hypnea* sp., *Kappaphycus* Doty, *Chondrus* Stackhouse, *Gracilaria* Greville) have phycocolloids in the thallus, which provide more rigidity and consistency to the thallus. Procedures for surface decontamination of these macroalgae (*e.g.* Holdt *et al.* 2014, Yong *et al.* 2014, Saminathan *et al.* 2014, Ali *et al.* 2018) are well-known due to their economic importance.

We observed during the cultivation that the immersion treatment in sodium hypochlorite for 5 minutes caused a slight loss of pigmentation in the thalli (thallus part bleaching).

Probably for this reason, the lowest growth was observed in this treatment, since pigment loss results in photosynthetic impairment. Fernandes *et al.* (2011) recorded also this effect on thalli treated with detergent and sodium hypochlorite. However, as there was thalli recovery and immersion for 2 minutes in sodium hypochlorite was sufficient for eliminating contaminants without great negative effects on the macroalga, we recommend the use of this chemical compound for 2 minutes, corroborating with Holdt *et al.* (2014).

For studies with species of *Bostrychia*, as well as other estuarine macroalgae, it is recommended to remove sediment in the field due to high amount of sediments frequently found on macroalgae from these environments. Afterward in laboratory, remaining adhered sediments and biota must be eliminated following protocols.

After applying the decontamination procedures described in this study, it is recommended to repeat the procedures if necessary (if contaminants were found in the culture medium yet). For other species belonging to the genus *Bostrychia*, preliminary analyses of thalli immersion times in 5% liquid detergent and 0.2% sodium hypochlorite are recommended, so that the macroalgae do not lose pigments nor damages are caused by overexposure to the chemical compound.

Data obtained in this study allowed us to determine procedures for the removal of contaminating organisms on *B. binderi*. We concluded that thalli immersion procedures in 5% liquid detergent and after in 1% povidone iodine were not effective, whereas immersion procedures in 5% liquid detergent and after in 0.2% sodium hypochlorite were effective. Since the RGRs of the thalli immersed in sodium hypochlorite for 5 minutes significantly differed from the immersed for 2 minutes and control, whereas the RGRs of the thalli immersed for 2 minutes did not differ from the control, we recommend a 2-minute immersion to avoid a longer exposure of the macroalgae to the compound. We recommend the physical removal of remaining annelids with sterilized tweezers under stereoscopic and optical microscopes. The protocol for surface decontamination of *B. binderi* is summarized in table 2.

Table 2. Decontamination protocol of the macroalga surface.

1: During field collection, remove the estuarine sediment through washing with *in situ* water.
In laboratory:
2: Remove remaining sediments adhered on the thalli by washing and spraying with sterilized seawater.
3: Remove macroinvertebrates which are still associated to the thalli using sterilized tweezers.
4: Remove epiphytes by cutting with scalpels.
5: Wash the thalli with distilled water.
6: Immerse the thalli in 5% liquid detergent (DETERBIO Neutral + deionized water) for 60 seconds.
7: Remove completely the liquid detergent on the thalli with sterilized seawater by several washing.
8: Immerse the thalli in 0.2% sodium hypochlorite for 2 minutes.
9: Remove completely the sodium hypochlorite on the thalli with sterilized seawater by several washing.
10: Maintain the macroalgae in culture medium (sterilized seawater and nutrient solution).

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Author Contributions

Henrique Douglas dos Santos Borburema: Substantial contribution to the study concept and design; contribution to data collection; contribution to data analyses and interpretation; contribution to preparation of the manuscript, figures and tables.

Êmille Natane de Araújo Barbosa: Contribution to the study design; contribution to manuscript preparation.

George Emmanuel Cavalcanti de Miranda: Substantial contribution to the study concept; contribution to critical revision, adding intellectual content.

Conflicts of interest

There is no conflict of interest.

Literature cited

Ali, M.M., Sani, M.Z.B., Hi, K.K., Yasir, S.M., Critchley, A.T. & Hortado, A.Q. 2018. The comparative efficiency of a brown algal-derived biostimulant extract (AMPEP), with and without supplemented PGRs: the induction of direct, axis shoots as applied to the propagation of vegetative seedlings for the successful mass cultivation of three commercial strains of *Kappaphycus* in Sabah, Malaysia. Journal of Applied Phycology 30: 1913-1919.

Berland, B.R., Bonin, D.J. & Maestini, S.T. 1972. Are some bactéria toxic for marine algae? Marine Biology 12: 189-193.

Bravin, I.C., Yoneshigue-Valentin, Y. & Yokoya, N.S. 2006. Formação de calos e regeneração de segmentos apicais de *Hypnea musciformis* (Wulfen) Lamouroux (Gigartinales, Rhodophyta): obtenção de culturas axênicas e efeitos de diferentes concentrações de ágar. Revista Brasileira de Botânica 29: 175-182.

Borburema, H.D.S., Lima, R.P. & Miranda, G.E.C. 2020. Effects of ocean warming, eutrophication and salinity

variations on the growth of habitat-forming macroalgae in estuarine environments. Acta Botanica Brasilica 34: 662-672.

De Felício, R., Pavão G.B., De Oliveira, A.L.L. et al. 2015. Antibacterial, antifungal and cytotoxic activities exhibited by endophytic fungi from the Brazilian marine red alga *Bostrychia tenella* (Ceramiales). Brazilian Journal of Pharmacognosy 25: 641-650.

Edwards, P. 1970. Illustrated guide to the seaweeds and seagrass in the vicinity of Porto Aransas, Texas. Contributions to Marine Science, University of Texas, Austin.

Fernandes, D.R.P., Yokoya, N.S. & Yoneshigue-Valentim, Y. 2011. Protocol for seaweed decontamination to isolate unialgal cultures. Brazilian Journal of Pharmacognosy 21: 313-316.

Fontes, K.A.A., Pereira, S.M.B. & Zickel, C.S. 2007. Macroalgas do "Bostrychietum" aderido em pneumatóforos de duas áreas de manguezal do Estado de Pernambuco, Brasil. IHERINGIA, Série Botânica 62: 31-38.

García, A.F., Bueno, M. & Leite, F.P.P. 2016. The Bostrychietum community of pneumatophores in Aracá Bay: an analysis of the diversity of macrofauna. Journal of the Marine Biological Association of the United Kingdom 96: 1617-1624.

Holdt, S.L., Christensen, L. & Iversen, J.J.L. 2014. A novel closed system bubble column photobioreactor for detailed characterisation of micro- and macroalgal growth. Journal of Applied Phycology 26: 825-835.

Kawai, H., Motomura, T. & Okuda, K. 2005. Isolation and Purification Techniques for Macroalgae. *In*: R.A. Andersen (ed.). Algal culturing techniques. Elsevier Academic Press, San Diego, pp. 133-144.

Kerrison, P.D., Le, H.N., Twigg, G.C., Smallman, D.R., MacPhee, R., Houston, F.A.B. & Hughes, A.D. 2016. Decontamination treatments to eliminate problem biota from macroalgal tank cultures of *Osmundea pinnatifida*, *Palmaria palmate* and *Ulva lactuca*. Journal of Applied Phycology 28: 3423-3434.

King, R.J. & Puttock, C.F. 1989. Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Rhodophyta). Australian Systematic Botany 21: 1-73.

Lewin, J.C. 1966. Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. Phycologia 6: 1-12.

Markham, J.W. & Hagmeier, E. 1982. Observations on the effects of germanium dioxide on the growth of macro-algae and diatoms. Phycologia 21: 125-130.

Miranda, G.E.C.D., Yokoya, N.S. & Fujii, M.T. 2012. Effects of temperature, salinity and irradiance on carposporeling development of *Hidropuntia caudata* (Gracilariales, Rhodophyta). Revista Brasileira de Farmacognosia 22: 818-824.

Oliveira, E.C., Paula, E.J., Plastino, E.M. & Petti, R. 1995. Metodologias para cultivo no axenico de macroalgas marinas in vitro. *In*: E.C. Oliveira, E. Sar (eds.). Manual de Métodos Ficológicos. Universidad de Concepción, Concepción, pp. 429-447.

Pedroche, F.F., West, J.A., Zuccarello, G.C., Sentíes, A.G. & Karsten, U. 1995. Marine red algae of the mangroves in Southern Pacific México and Pacific Guatemala. Botanica Marina 38: 111-119.

Post, E. 1936. Systematische und pflanzengeographische Notizen zur *Bostrychia-Caloglossa* Assoziation. Revue Algologie 9: 1-84.

Saminathan, K.R., Ashok, K.S., Veeragurunathan, V. & Mantri, V.A. 2014. Seedling production in the industrially important agarophyte *Gracilaria dura* (Gracilariales, Rhodophyta). Journal of Applied Phycology 27: 1541-1548.

Shea, R. & Chopin, T. 2007. Effects of germanium dioxide, an inhibitor of diatom growth, on the microscopic laboratory cultivation stage of the kelp, *Laminaria saccharina*. Journal of Applied Phycology 19: 27-32.

Vieira, E.A., Filgueiras, H.R., Bueno, M., Leite, F.P.P. & Dias, G.M. 2018. Co-occurring morphologically distinct algae support a diverse associated fauna in the intertidal zone of Araçá Bay, Brazil. Biota Neotropica 18: e20170464.

West, J.A. 1991. New records of marine algae from Perú. Botanica Marina 34: 459-464.

West, J.A., Zuccarello, G., Karsten, U. & Calumpong, H.P. 1993. Biology of *Bostrychia, Stictosiphonia* and *Caloglossa* (Rhodophyta, Ceramiales). Proc. of the 2nd RP-USA Phycology Symposium, Cebu and Dumaguete, pp. 145-162.

West, J.A., Hommersand, M. & Zuccarello, G.C. 2001. Morphology and reproduction of *Bostrychia pinnata* (Rhodomelaceae, Ceramiales) in laboratory culture. Phycological Research 49: 285-297.

Yokoya, N.S., Plastino, E.M., Braga, M.R.A., Mutue, T.J., Cordeiro-Marino, M., Eston, V.R. & Harari, J. 1999. Temporal and spatial variations in the structure of macroalgal communities associated with mangrove trees of Ilha do Cardoso, São Paulo state, Brazil. Revista Brasileira de Botânica 22: 195-204.

Yokoya, N.S. 2000. Apical callus formation and plant regeneration controlled by plant growth regulators on axenic culture of the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). Phycological Research 48: 133-142.

Yong, S.Y., Yong, W.T.L. & Anton, A. 2013. Analysis of formulae for determination of seaweed growth rate. Journal of Applied Phycology 25: 1831-1834.

Yong, W.T.L., Ting, S.H., Yong, Y.S., Thien, Y.V., Wong, S.H., Chin, W.L., Rodrigues, K.F. & Anton, A. 2014. Optimization of culture conditions for the direct regeneration of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae). Journal of Applied Phycology 26: 1597-1606.

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