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Effects of ocean warming, eutrophication and salinity variations on the growth of habitat-forming macroalgae in estuarine environments

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ABSTRACT

Global change and coastal eutrophication are affecting macroalgae worldwide. We analyzed the effects of increased water temperature (25, 28 and 32 °C) and eutrophication on the growth of *Bostrychia binderi* and *Bostrychia montagnei* in a range of salinities (18, 24, 30, 36 and 42 PSU) through three independent multifactorial experiments. Both species had higher growth at 25 °C than at 28 and 32 °C (warming scenario projected by IPCC), suggesting a negative effect of ocean warming. The species showed a broad tolerance to the range of salinities tested, with higher growth at 36 and 42 PSU, as a local adaptation strategy. Oligotrophic seawater significantly affected the growth of both species because the lowest growth was found in this condition, whereas highest growth was found with increased availability of nutrients, which is probably because estuaries are nutrient-rich environments due to continental runoff. High temperatures, low salinities and few nutrients had negative interactive effects on the growth of both species. Our results show that ocean warming can be detrimental to the studied macroalgae, and that both species are tolerant to eutrophication, with *B. montagnei* being more sensitive than *B. binderi*. Our results also reinforce the euryhaline characteristic of the genus *Bostrychia*.

Keywords: *Bostrychia*, Bostrychietum, climate change, ecophysiology, estuarine macroalgae, eutrophication, global change, growth, salinity variation, sea level rise

Introduction

The Intergovernmental Panel on Climate Change (IPCC) has demonstrated global increases in anthropogenic emissions of greenhouse gases into the atmosphere, mainly carbon dioxide (CO_2), which are inducing global changes such as continental and oceanic warming, ocean acidification and sea level rise (Collins *et al.* 2013; IPCC 2014; Cornwall & Hurd 2020). The concentration of atmospheric CO_2 has been rising steadily and has already been recorded at a level above 400 ppm (Hurd *et al.* 2020). Models from the IPCC (2014) project a continuous increase in temperature on all continents and ocean surfaces until the end of the 21st century (2081–2100). These models are projected future scenarios called Representative Concentration Pathways (RCPs). According to the most optimistic scenario (RCP2.6), mean global warming will be approximately 1 °C, while moderate scenarios (RCP4.5 and RCP6.0) predict a warming around 2 °C and the critical scenario (RCP8.5) predicts approximately 4 °C.

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Climate change is causing sea level rise due to thermal expansion of water and the melting of glaciers and ice caps (Collins *et al*. 2013). Sea level rise will lead to the expansion of coastal flooding areas, resulting in estuary level rise and physical and chemical changes in estuarine waters (*e.g.* changes in: temperature, pH, luminosity, salt wedge affecting salinity gradients), because the estuarine systems are strongly affected by the surrounding sea (Rybczyk *et al*. 2012; Couto *et al*. 2014).

Besides warming and sea level rise, another process that has been impacting coastal ecosystems worldwide is anthropogenic eutrophication. Human societies have dramatically increased nitrogen (N) and phosphorus (P) exports into aquatic environments across the globe (Smith *et al*. 1999; Fowler *et al*. 2013), causing the eutrophication process (Drljepan *et al*. 2014; Smith *et al*. 2014). Urbanization and use of coastal zones are increasing nutrient inputs into coastal waters, causing eutrophication in coastal ecosystems (Smith *et al*. 1999; Gao *et al*. 2017). Eutrophication has been a growing threat for many coastal ecosystems as estuaries and salt marshes (Bricker *et al*. 2008; Deegan *et al*. 2012; Paerl *et al*. 2014). Agriculture (mainly from fertilizer use), aquaculture (*e.g.* shrimp and fish farming), wastewater inputs and runoff from urban and industrial areas are all sources of eutrophication in coastal ecosystems (Marinho-Soriano *et al*. 2011; Paerl *et al*. 2014; Tavares *et al*. 2014; Gao *et al*. 2017).

Costal eutrophication and global change are impacting macroalgae worldwide (Smale & Wernberg 2013; Ji *et al*. 2016; Duarte *et al*. 2018). Substantial loss of macroalgae biodiversity and considerable changes in macroalgae assemblages have already been recorded in the Southwest Atlantic caused by coastal urbanization (Scherner *et al*. 2013). Several ecophysiological studies performed in the field or mesocosm systems (*e.g.* Figueroa *et al*. 2014; Burdett *et al*. 2015; Celis-Plá *et al*. 2015; Kim *et al*. 2016; Scherner *et al*. 2016; Gouvêa *et al*. 2017; Sampaio *et al*. 2017; Kumar *et al*. 2018; Rich *et al*. 2018; Al-Janabi *et al*. 2019) and in the laboratory (*e.g.* Sinutok *et al*. 2012; Johnson *et al*. 2014; Fernández *et al*. 2015; Kram *et al*. 2016; Young & Gobler 2016; Gao *et al*. 2017; Phelps *et al*. 2017; Muñoz *et al*. 2018; Piñeiro-Corbeira *et al*. 2018; Zweng *et al*. 2018; Graba-Landry *et al*. 2018; Britton *et al*. 2019; McNicholl *et al*. 2019; Pajusalu *et al*. 2019; Cornwall & Hurd 2020) have experimentally analyzed the possible effects of global change and eutrophication on the physiological and biochemical responses of macroalgae from different morpho-functional groups.

Other studies have created species distribution models to predict possible effects of global change on the climate niche of some macroalgae (*e.g.* Wernberg *et al*. 2011; Smale & Wernberg 2013; Martínez *et al*. 2015; Khan *et al*. 2018; Martínez *et al*. 2018). However, data reporting possible effects of global change on habitat-forming macroalgae in estuarine environments are still scarce in the scientific literature, which is recognized in the review by Koch *et al*. (2013) (a review of > 100 marine plant species), in which they highlighted the possible ability of *Bostrychia scorpioides* to assimilate bicarbonate (HCO_3^-) under ocean acidification.

The *Bostrychia* genus (Rhodomelaceae, Rhodophyta) includes macroalgae distributed in tropical and temperate regions, which can be found in marine environments as rocky shores (Machado *et al*. 2011) and continental aquatic environments, but are predominant in mangrove swamps and salt marshes (King & Puttock 1989). *Bostrychia* species are the main constituents of the mangrove community known as Bostrychietum (Yokoya *et al*. 1999a; Fontes *et al*. 2007; Jesus *et al*. 2015). This term was proposed by Post (1936) and includes mainly rhodophytes as *Bostrychia*, *Caloglossa* and *Catenella*, as well as cyanobacteria and chlorophytes that associate themselves to pneumatophores of *Avicennia*, and to rhizophores and stems of *Rhizophora* and *Laguncularia* (West 1991; West *et al*. 1993; Pedroche *et al*. 1995; Yokoya *et al*. 1999a; Fontes *et al*. 2007; Jesus *et al*. 2015). These macroalgae, along with microalgae, represent a major source of primary productivity in mangrove ecosystems (Karsten *et al*. 1994a), as they produce organic matter and participate in nutrient cycling (McClusky & Elliot 2004). Furthermore, they act as microhabitats for several organisms, mainly protists and invertebrates (García *et al*. 2016; Borburema 2017; Vieira *et al*. 2018) and can be indicators of environmental quality (Melville & Pulkownik 2006; Fontes *et al*. 2007; Melville & Pulkownik 2007) and food resource for consumers (Campos *et al*. 2015).

Ecophysiological studies of *Bostrychia* species with salinity variations have demonstrated their physiological ability to tolerate salinity ranges, showing in some works an optimum growth at low salinities (Karsten & Kirst 1989a; b; Karsten *et al*. 1990; Karsten *et al*. 1992; Karsten *et al*. 1993; Karsten *et al*. 1994a; b; Karsten *et al*. 1996). However, effects of increasing temperature on *Bostrychia* species have still not been well investigated (*e.g.* Davis & Dawis 1981; Mann & Steinke 1988) and more current studies have investigated physiological responses of *Bostrychia* species under different light conditions (Cunha & Duarte 2002; Pedro *et al*. 2014; Pedro *et al*. 2016) and their mechanisms of inorganic carbon acquisition (Ruiz-Nieto *et al*. 2014). Ryder *et al*. (1999) analyzed the growth of *Bostrychia moritiziana* in various N and P conditions, showing that non-enriched treatments by nitrogen resulted in the lowest growth.

Such studies with *Bostrychia* species investigated the effects of the abiotic variables independently, and the interactive effects of abiotic variables on *Bostrychia* species are poorly understood. Muangmai *et al*. (2015) had a two-factor approach, investigating the growth of cryptic species of *Bostrychia intricata* in various salinity and temperature conditions, nevertheless, it had a mainly taxonomic purpose. Ecological impacts of global change are generated by multiple synchronous or asynchronous drivers which interact with each other (Al-Janabi *et al*. 2019) and recent reviews have highlighted the need for global change research to consider how stressors may interact and affect species (Rich *et al.* 2018). Field evidence is essential to assess the consequences of global change on macroalgae, but finding a solid causal link often requires obtaining additional information under controlled laboratory conditions (Piñeiro-Corbeira *et al.* 2018).

Growth experiments of *Bostrychia* species in the laboratory under controlled conditions that simulate ocean warming and eutrophication can elucidate the possible effects of these global changes on their growth, because the physiological effects caused by the environmental stressors affect algal growth (Gouvêa *et al.* 2017). Experiments with estuarine organisms that consider various salinity conditions are relevant because the salinity varies naturally in estuaries. Lourenço *et al.* (2006) evaluated the tissue N and P in macroalgae from a tropical eutrophic environment and found high percentages of these in *Bostrychia radicans*. For these authors macroalgae function very well as monitors of environmental changes and experimental data are needed to identify the environmental processes that promote changes in macroalgae.

In this context, we analyzed the growth of *Bostrychia binderi* and *Bostrychia montagnei* cultivated in three independent multifactorial experiments under three water temperature conditions: average winter temperature, average summer temperature and a warming scenario (RCP8.5) projected by the IPCC until the end of the 21st century combined with various salinity (five values) and nutrient (four levels) conditions. We hypothesized that (1) the analyzed species would have highest growth in high nutrient availability, low salinities and temperature, (2) the species would have the lowest growth under a warming scenario (RCP8.5), showing that future ocean warming conditions can negatively affect the species. This study is the first to evaluate the growth of *Bostrychia* species in a context of global change.

Materials and methods

Algal collection, establishment and maintenance of cultures

The adult thalli of *B. binderi* and *B. montagnei* were collected from the mangrove swamp within the Barra do Rio Mamanguape Environmental Protection Area of, northeastern Brazil, in August 2016 (last winter month/rainy month) and February 2017 (last summer month/dry month) (CPTEC/INPE 2016). Specimens collected in August 2016 were submitted to the first experiment (with average winter temperature), and specimens collected in February 2017 were submitted to the second (with average summer temperature) and third experiment [a warming scenario - RCP8.5 - projected by the IPCC (2014) until the end of the 21st century]. More details about set temperatures are provided below. The collection point (6°46'23.24" S, 34°56'20.55" W) was established near the estuary's mouth because mainly the downstream estuarine populations will be impacted by future ocean warming conditions and sea level rise.

Voucher specimens were deposited in the Lauro Pires Xavier Herbarium (Universidade Federal da Paraíba, Brazil) with the accession numbers JPB 63215 for *B. binderi* and JPB 63216 for *B. montagnei*. The Lauro Pires Xavier Herbarium is registered in the *Index Herbariorum* with acronym JPB.

During the field collections, most of the estuarine sediment was removed from the thalli with estuary water. In the laboratory, the remaining sediment adhered to the thalli was removed by washing and spraying them with sterilized seawater and the associated macrofauna individuals were removed with tweezers under a stereoscopic microscope. The thalli were immersed in liquid detergent based on 5 % sulfonic acid (w/w) for 60 seconds, afterwards the detergent was completely removed from the thalli by several washings with sterilized seawater. The thalli were then immersed in sodium hypochlorite (0.2 % active chlorine L^{-1} of deionized water) for two minutes, which was completely removed from the thalli with sterilized seawater. These procedures were performed following Borburema (2017) to eliminate contaminating organisms.

The seawater used in all procedures and growth experiments was sterilized through filtering using cellulose membrane filters (Millipore® HAWP 0.45 µm pore), followed by heating in a laboratory drying oven at 90 °C for two hours (after cooling, it was heated up again).

In the laboratory, macroalgae (± 1 g L^{-1}) were maintained in aquariums containing sterilized seawater (35 PSU ± 1) enriched with von Stosch's solution (VSES) (8 mL L^{-1}), which was prepared as described by Edwards (1970) and modified by reducing vitamin concentrations by 50 % (Yokoya 2000). The culture medium was continuously aerated and replaced weekly for nutrient renovation, water temperature was maintained at 24 °C (± 0.5) (Karsten & Kirst 1989a), photonic flux density at 60 - 80 µmol photons $m^{-2} s^{-1}$ (Karsten *et al.* 1994a adapted) and the photoperiod was 12 h: 12 h (light: dark cycle).

Growth experiments

For the growth experiments, apical segments (3 - 3.5 cm in primary axis length, weighing 230 mg ± 10) with lateral branches were cut from female plant thalli using scalpels under a stereoscopic microscope. Female plants were used in the experiments because they did not release reproductive products that would have interfered with growth measurements in cultures. Sporophytes and male phases usually released reproductive products.

The apical segments of *B. binderi* and *B. montagnei* were experimentally cultivated in 150 mL transparent glass containers containing 100 mL of culture medium, which was replaced weekly. Apical segments were cultivated in continuous immersion because highest photosynthetic activity was recorded in *Bostrychia* sp. under submersed conditions (Peña *et al.* 1999). This was considered due to the tidal regime in estuarine ecosystems. Growth experiments were performed in an environmental control chamber through three independent multifactorial experiments. All experiments were carried out for 28 days (Karsten *et al.* 2000; Muangmai *et al.* 2015). Each experiment had 40 treatments (two species x one temperature x five salinities x four nutrient levels) and each treatment had five replicas. The water temperature of each experiment was 25, 28 and 32 °C (± 0.5) combined with various salinity and nutrient conditions (described below). Photoperiod and photonic flux density conditions described above (for maintenance of cultures) were applied in the experiments.

Temperatures

A temperature of 25 °C was established according to the average surface temperature of water during the winter, 28 ºC according to the average surface temperature during the summer, and 32 ºC was the maximum average temperature scenario (RCP.8.5) projected by the IPCC for the marine area near the algal collection site (Tyberghein *et al.* 2012; Assis *et al.* 2018). Temperatures of 25 and 28 ºC were established with reference to the area where the macroalgae were collected, based on temperature data (NSST - Night Sea Surface Temperature) from a ten-year temporal series (monthly averages, January 2007 - December 2016). Temperature data were obtained by the MODIS (Moderate Resolution Imaging Spectroradiometer) sensor coupled to the AQUA satellite from the NASA. NSST data were used to prevent major errors in relation to solar reflectance and discrepancies in SST (Sea Surface Temperature) values for the region (Telles & Delcourt 2015).

Salinities

Salinities used in each experiment were 18, 24, 30, 36 and 42 PSU. The salinity values were obtained by freezing seawater (35 PSU) and gradually melting it to produce seawater with different salinities. Afterwards, mixtures of seawater with different salinities produced the desired salinities (Yokoya *et al.* 1999b). This salinity range was established according to Campos *et al.* (2015), who recorded a salinity range between 10 and 42 PSU in the estuary from the Barra do Rio Mamanguape Environmental Protection Area. Salinity was measured with a handheld refractometer.

Nutrients

Sterilized seawater was enriched to obtain different nutrient levels in the experiments by adding VSES. The nutrient levels established were: non-enriched sterilized seawater (N0), sterilized seawater enriched with VSES/2 - 4 mL L^{-1} (N1), sterilized seawater enriched with VSES - 8 mL L^{-1} (N2), and sterilized seawater enriched with 2VSES - 16 mL L^{-1} (N3). VSES corresponds to 8 mL of von Stosch's solution diluted in 1 L of sterilized seawater (Edwards 1970). Nitrate concentrations at N1, N2 and N3 were around 1.95, 3.9 and 7.8 g L^{-1}, respectively, whereas phosphate concentrations were around 0.15, 0.3 and 0.6 g L^{-1}, respectively.

The concentrations of nitrate, nitrite, phosphate, ammonia and total phosphorous in sterilized seawater used in the experiments were analyzed (n = 3). The dissolved nitrate and nitrite were quantified following the methods proposed by Grasshoff *et al.* (1983). For dissolved phosphate, the methods suggested by Strickland & Parsons (1972) and Grasshoff *et al.* (1983) were followed. Ammonia concentration was determined using a phenol spectrophotometry method and total phosphorous was determined by the persulphate digestion method (APHA *et al.* 2005). The nutrient concentrations of non-enriched sterilized seawater (N0) are in Table 1.

Table 1. Nutrient concentration in sterilized seawater no enrichment with VS. The values are averages and standard deviations referent to three replicas.

	Concentration	
Nutrients	mg L^{-1}	mg m^{-3}
Nitrate	0.02 ± 0.001	20
Nitrite	0.01 ± 0.002	10
Phosphate	0.07 ± 0.001	70
Ammonia	0.01 ± 0.003	10
Phosphorus total	0.08 ± 0.005	80

Considering the total-N concentration (mg m^{-3}) in sterilized seawater, the N0 treatments can be categorized as oligotrophic (Hakanson 1994; Smith *et al.* 1999). All treatments were performed with sterilized seawater from only one collection.

Growth rate

Apical segments were weighed weekly on an analytical balance when the culture medium was replaced and were gently blotted dry with paper tissue to remove excess water before weighing. At the end of the experiments relative growth rates (RGRs) of the apical segments were estimated following the formula recommended by Yong *et al.* (2013): $RGR = [(W_t/W_i)^{1/t} - 1] \times 100$, where W_t is the fresh weight after t days, W_i is the initial fresh weight, and t is the cultivation period.

Statistical analyses

The following descriptive statistics referent to RGRs were calculated: average, minimum, maximum, standard deviation and error (SD and SE, respectively). The graphic of the average RGRs from the treatments was plotted. RGRs of *B. binderi* and *B. montagnei* were compared by a linear model (LM). The effects of the treatments on the RGRs of *B. binderi* and *B. montagnei* were assessed using analysis of variance (multifactorial ANOVA) and post hoc Tukey's tests. The statistical analyses were performed using the R program (4.0.0 version) and the significance value adopted was 5 % (0.05).

Results

Bostrychia binderi had the highest RGR (1.80 % day^{-1}) in the treatment where the apical segments were cultivated at 25 °C, 30 PSU and highest concentration of nutrients (N3), whereas the lowest RGR (0.03 % day^{-1}) was at 32 ºC, 42 PSU and lowest nutrient availability (N0). In treatments without nutrient enrichment (N0) at 28 ºC and 18 PSU, as well as at 32 ºC and 24 PSU, some replicas had no growth.

Bostrychia montagnei showed the highest RGR (2.19 % day^{-1}) in the treatment which was cultivated at 25 °C, 36 PSU and N2 nutrient level. The lowest RGR (0.03 % day^{-1}) was at 32 ºC, 24 PSU and N0. Some replicas of the treatments at 28 ºC: 24 and 36 PSU; 32 ºC: 18, 24, 36 and 42 PSU had no growth, independent of the nutrient level.

Bostrychia montagnei had a higher average RGR (0.83 % day^{-1} ± 0.02 SE) than *B. binderi* (0.67 % day^{-1} ± 0.02 SE), differing significantly (Fcal = 22.14, $p < 0.01$). Both species showed higher average RGRs at 25 ºC (*B. binderi* 1.06 % day^{-1} ± 0.03 SE; *B. montagnei* 1.21 % day^{-1} ± 0.04 SE) than at 28 ºC (*B. binderi* 0.51 % day^{-1} ± 0.02 SE; *B. montagnei* 0.75 % day^{-1} ± 0.04 SE) and 32 ºC (*B. binderi* 0.43 % day^{-1} ± 0.01 SE; *B. montagnei* 0.54 % day^{-1} ± 0.03 SE), and were statistically different among the three temperatures (Tab. 2, Fig. 1A-B).

The salinity variation significantly affected the average RGR of *B. binderi* and *B. montagnei* (Tab. 2). *B. binderi* at 18 PSU had an average RGR of 0.67 % day^{-1} ± 0.16 SE; at 24 PSU 0.59 % day^{-1} ± 0.18 SE; at 30 PSU 0.64 % day^{-1} ± 0.18 SE; at 36 PSU 0.75 % day^{-1} ± 0.15 SE and at 42 PSU 0.72 % day^{-1} ± 0.17 SE. The average RGR at 24 PSU was statistically different from that observed at 36 and 42 PSU and the RGR at 36 PSU was different from 30 PSU ($p < 0.01$). *B. montagnei* at 18 PSU showed an average RGR of 0.70 % day^{-1} ± 0.22 SE, at 24 PSU was 0.65 % day^{-1} ± 0.22 SE, at 30 PSU was 0.77 % day^{-1} ± 0.19 SE, at 36 PSU was 0.90 % day^{-1} ± 0.24 SE and at 42 PSU was 0.96 % day^{-1} ± 0.19 SE. For *B. montagnei*, the average RGR at 24 PSU was statistically different from 36 and 42 PSU and at 30 PSU was different from 42 PSU ($p < 0.01$). Regarding salinity, the lowest average RGR was found at 24 PSU for both species.

For both species, the lowest average RGRs were found in N0. The average RGR of *B. binderi* and *B. montagnei* at N0 was statistically different from the other nutrient levels (N1, N2 and N3) ($p < 0.01$). The average RGR of *B. binderi* at N1 was also different from N3. The maximum enrichment resulted in increased growth of *B. binderi* in most treatments, except that at 25 °C and 36 PSU, 28 °C and 24 PSU, 30 °C and 42 PSU; and at 32 °C and 18 PSU, in which the species showed lower RGRs at N3 than at N2 and/or N1. At 32 °C and 18 PSU, the highest average RGR was at N1 (VSES/2). RGRs observed in the treatments at 25 °C: 18, 24, 30 and 42 PSU; at 28 °C: 18 and 36 PSU; and at 32 °C: 24 - 42 PSU had similar patterns in relation to the nutrient levels (Fig. 1A).

At 25 °C, *B. montagnei* had the highest average RGRs at intermediate levels of nutrients (N1 and / or N2), decreasing growth at N3, except at 42 PSU. At 28 °C, the species had highest average RGRs at high nutrient availabilities, with no significant decrease in growth at N3. In this temperature there was a noticeable decrease in growth at 36 PSU and N2. At 32 °C, the highest average RGRs were also found at intermediate levels of nutrients, with a decrease in growth at N3 as well (Fig. 1B).

The interaction among temperature, salinity and nutrient showed significant effects (Tab. 2) on the growth of both species. High temperatures (28 and 32 °C), low salinity and oligotrophic conditions (N0) resulted in the lowest growth of *B. binderi* and *B. montagnei* (Fig. 1A-B).

Discussion

Data obtained in this study suggest that the growth of *B. binderi* and *B. montagnei* could be negatively affected in future warming scenarios since the lowest average RGRs were observed in the warming scenario (32 °C - RCP8.5). Macroalgal growth decreased with increasing temperature (25° > 28 °C > 32 °C). Thermal stress affects metabolic activities and membrane-associated processes. The increasing temperature causes a decrease in enzymatic activity, affects the antioxidant systems by stimulating the production of reactive oxygen species (ROS) (Larkindale *et al.* 2005; Bischof & Rautenberger 2012) and causes changes in resource allocation (*e.g.* for biosynthesis of antioxidant proteins and detoxifying enzymes) (Collén *et al.* 2007; Gouvêa *et al.* 2017), all of which reduce algal growth (Gouvêa *et al.* 2017). This result also indicates that female phases of *B. binderi* and *B. montagnei* probably grow better in the rainy season. However, field data are need to better understand seasonal effects on the life phases of these species.

For tropical marine macroalgae, lethal and sublethal temperatures have been recorded between 32 °C and 38 °C (Koch *et al.* 2013) (*e.g.* Miranda *et al.* 2012; Araújo *et al.* 2014; Castro & Yokoya 2019). The maximum temperature tested in this study (32 °C) was not lethal for either species, although their lowest growth was recorded at this temperature. Davis & Dawes (1981) and Mann & Steinke (1988) evaluated photosynthetic and respiratory responses of *B. binderi* and *B. radicans* (respectively) under temperature variation (12 to 42 °C and 12 to 37 °C, respectively) in short-term experiments (2 - 3 days) and found that the species had high photosynthetic activity at high temperatures (30 to 42 °C and 32 to 37 °C, respectively). Although the thermal tolerance recorded by these authors should be considered, it is possible that the high photosynthetic activity they recorded was indicative of physiological stress since under stress conditions macroalgae can have the metabolism stimulated to synthesize metabolites associated with cell protection against detrimental environmental factors (Hargrave *et al.* 2016). McCoy *et al.* (2020) suggest that increased photosynthetic rates may be a consequence of the energy expenditures related to strong chemical defenses. Nevertheless, biochemical and ecophysiological studies are needed to elucidate the protective strategies of *Bostrychia* species at high temperatures and to identify their maximum tolerance level.

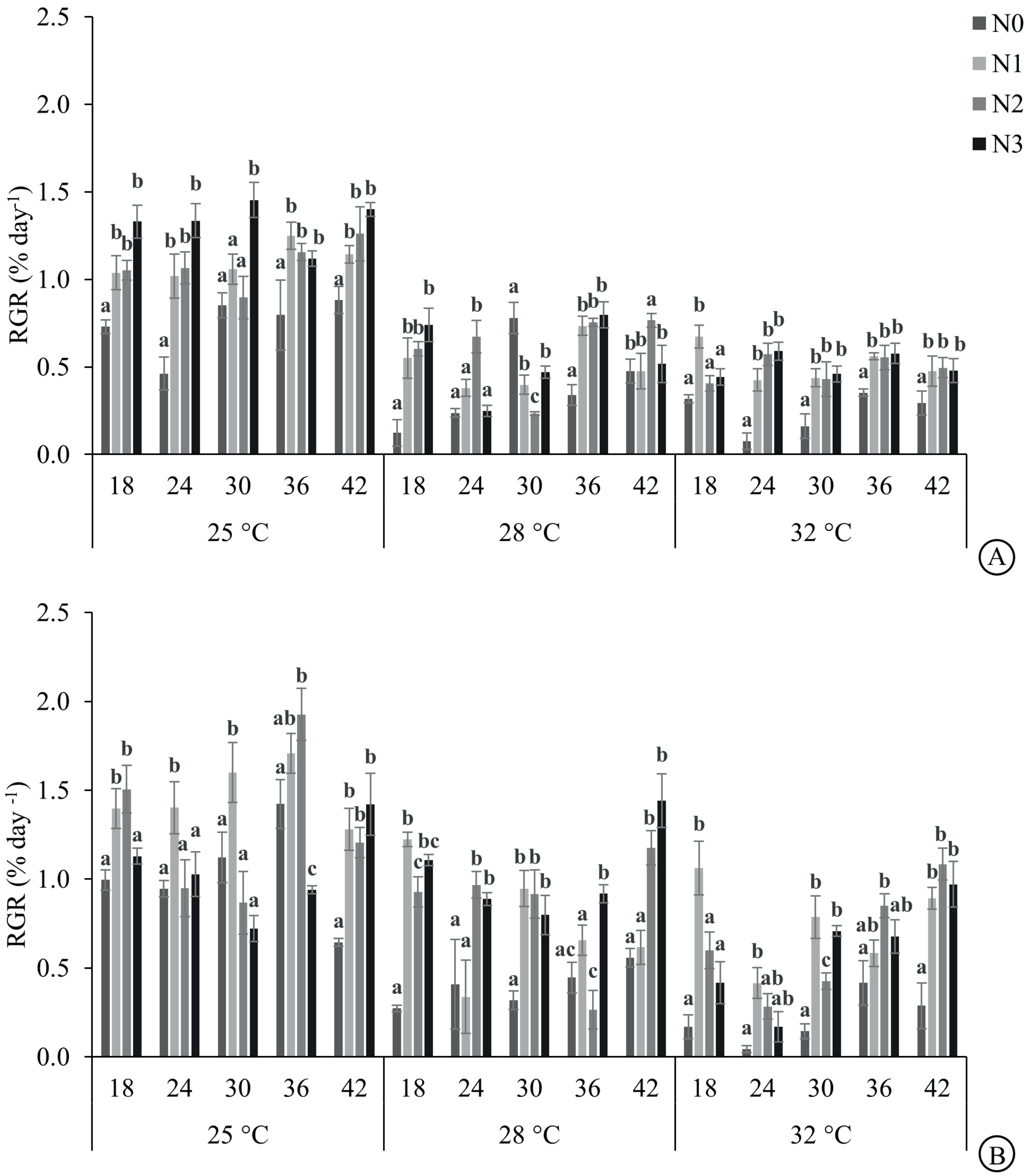

Figure 1. RGRs of *B. binderi* (**A**) and *B. montagnei* (**B**) cultivated in three independent multifactorial growth experiments with temperature (25, 28 and 32 °C), salinity (18, 24, 30, 36 and 42 PSU) and nutrient (N0, N1, N2 and N3) variations. Columns are averages and bars are standard errors of the five replicas. Different letters indicate statistical difference among nutrient levels at the same salinity and temperature.

Table 2. Results of the multifactorial ANOVA tests from the growth experiments of *B. binderi* and *B. montagnei* cultivated in treatments with temperature (25, 28 and 32 °C), salinity (18, 24, 30, 36 and 42 PSU) and nutrient (N0, N1, N2 and N3) variation. F is referent to the F calculated and df to the degrees of freedom.

Species	Variables	df	F	P value (ANOVA)
B. binderi	Temperature	2	397.48	< 0.01
	Salinity	4	8.41	< 0.01
	Nutrient	3	55.92	< 0.01
	Temperature: Salinity	8	1.99	0.05
	Temperature: Nutrient	6	7.78	< 0.01
	Salinity: Nutrient	12	5.20	< 0.01
	Temperature: Salinity: Nutrient	24	3.50	< 0.01
	Residuals	240		
B. montagnei	Temperature	2	177.64	< 0.01
	Salinity	4	14.36	< 0.01
	Nutrients	3	46.87	< 0.01
	Temperature: Salinity	8	8.47	< 0.01
	Temperature: Nutrient	6	9.86	< 0.01
	Salinity: Nutrient	12	6.13	< 0.01
	Temperature: Salinity: Nutrient	24	4.14	< 0.01
	Residuals	240		

Both species used in this study tolerated the range of salinities tested, showing highest average RGRs at 36 and 42 PSU. Other ecophysiological studies have shown the euryhaline characteristic of *Bostrychia* species. Karsten & Kirst (1989a) evaluated the growth of *B. radicans* in various salinities (9.9 - 37.4) and Karsten *et al.* (1994a) evaluated the growth of *B. simpliciuscula* at salinities of 5 to 70 PSU, observing that increased salinity was accompanied by a decrease in growth rates. In both works, the highest average growth occurred in low salinities (5 - 10) and the temperatures established in these works were 24 and 25 °C, respectively. The results found by these authors differ from those found herein.

However, Karsten *et al.* (1994b) evaluated the growth of nine isolates of *B. radicans* from the eastern coast of the USA, and found different physiological ecotypes in the species in relation to different salinities (5.3, 15, 30, 50 and 70). Six isolates exhibited optimum growth at 30 PSU. Intraspecific (ecotypic differentiation) and interspecific differentiation is important to explain local adaptations to different habitats (Thomas & Kirst 1991; Piñeiro-Corbeira *et al.* 2018). The fact that *B. binderi* and *B. montagnei* specimens analyzed in this study were collected near the estuary's mouth could explain the highest average RGRs in high salinities. *Aconthophora spicifera*, a species that also belongs to Rhodomelaceae, exhibited high tolerance from 25 to 40 PSU, with little changes in its physiology, which favors the occurrence of this species in diverse environments as the supratidal region (Pereira *et al.* 2017).

Osmotic acclimation in *Bostrychia* species has been well documented in the scientific literature. It occurs by increasing the intracellular concentrations of organic osmolytes, D - Sorbitol and D - Dulcitol polyols to maintain Turgor pressure (Karsten & Kirst 1989b; Karsten *et al.* 1990; Karsten *et al.* 1992; Karsten *et al.* 1994a; b; Karsten *et al.* 1996; Pedro *et al.* 2016). This physiological property of *Bostrychia* species explains their success in estuarine environments. In future conditions of sea level rise and possible changes in salinity gradients of estuaries, there is a strong possibility that *Bostrychia* species will adapt because of such physiological property (Duarte *et al.* 2018).

In general, *B. binderi* and *B. montagnei* tolerated eutrophic levels and had lowest average RGRs at the oligotrophic level (N0), especially in high temperatures (28 and 32 °C), due to interactive effects. *B. moritziana* showed a similar growth pattern in relation to nutrient levels. Furthermore, this species presented lowest growth in the culture medium without nitrogen enrichment (only sterilized seawater), while at the other three enrichment levels it showed high and similar growth (Ryder *et al.* 1999). This characteristic of *Bostrychia* species can be related to the fact that estuaries receive considerable concentrations of nutrients from continental runoff (Hitchcock & Mitrovic 2015).

However, in most treatments, *B. montagnei* showed lower average RGRs at N3 than at N1 and/or N2. Such data suggest that *B. montagnei* could be more sensitive to hypereutrophic levels than *B. binderi*. Studies have shown that increasing nutrients can decrease growth of macroalgae (Martins & Yokoya 2010; Faveri *et al.* 2015; Portugal *et al.* 2016). High concentrations of ammonium ions in seawater, for example, can cause inhibition of photosynthetic activity and significant variation in chlorophyll *a* and carotenoid contents (de Faveri *et al.* 2015). Marinho-Soriano *et al.* (2006) found an inverse relationship between the carbohydrate content and nutrient concentrations in macroalgae. As mentioned above, D-Sorbitol and D-Dulcitol (low molecular weight carbohydrates) are important for osmotic acclimation in *Bostrychia* species. Detrimental interactive effects of increasing temperature and nutrients were also recorded for the growth of *Laurencia catarinensis* (Rhodomelaceae) (Gouvêa *et al.* 2017).

B. binderi at 28 °C: 24 and 30 PSU and at 32 °C: 18 PSU showed a decrease in growth with increasing nutrients, as well as *B. montagnei* at 28 °C: 36 PSU and N2. In these treatments we observed the proliferation of cyanobacteria and microalgae (mainly diatoms). Usually on the algal surface there are some cyanobacteria, microalgae and bacteria which on ideal conditions proliferate (Fernandes *et al.* 2011). These organisms in macroalgae culture medium grow and proliferate faster than macroalgae, competing for nutrients, light and may release substances into the culture medium that inhibit algal growth (Berland *et al.* 1972).

In conclusion, our study shows that *B. binderi* and *B. montagnei* could be negatively affected by future ocean warming conditions, confirming our second hypothesis. Both species showed a broad tolerance to salinity variations, growing better at 36 and 42 PSU, which could be a local adaptation strategy. Due to the species' tolerance to different salinities, they will likely adapt to future conditions of sea level rise and changes in salinity gradients in estuaries. Oligotrophic waters can negatively affect the growth of the species, especially in high temperatures. Both species showed highest growth at eutrophic levels, probably because estuarine environments are rich in nutrients. However, *B. montagnei* was more sensitive to eutrophication than *B. binderi*. Our first hypothesis was not completely confirmed since the highest growth was recorded at high salinities. Interaction analyses of the variables confirmed this observation because high temperatures, low salinities and few nutrients caused the lowest algal growth.

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