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The effect of light on bacterial activity in a seaweed holobiont

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Abstract

Holobionts are characterized by the relationship between host and their associated organisms such as the biofilm associated with macroalgae. Considering that light is essential to macroalgae survival, the aim of this study was to verify the effect of light on the heterotrophic activity in biofilms of the brown macroalgae *Sargassum furcatum* during its growth cycle. Measurements of heterotrophic activity were done under natural light levels at different times during a daily cycle and under an artificial extinction of natural light during the afternoon. We also measured *Sargassum* primary production under these light levels in the afternoon. Both measurements were done with and without photosynthesis inhibitor and antibiotics. Biofilm composition was mainly represented by bacteria but diatoms, cyanobacteria and other organisms were also common. When a peak of diatom genera was recorded, the heterotrophic activity of the biofilm was higher. Heterotrophic activity was usually highest during the afternoon and the presence of a photosynthesis inhibitor caused an average reduction of 17 % but there was no relationship with *Sargassum* primary production. These results indicate that autotrophic production in the biofilm was reduced by the inhibitor with consequences on bacterial activity. Heterotrophic activity was mainly bacterial and the antibiotics chloramphenicol and penicillin were more effective than streptomycin. We suggest primary producers in the biofilm are more important to increase bacterial activity than the macroalgae itself because of coherence of the peaks of heterotrophic and autotrophic activity in biofilm during the afternoon and the effects of autotrophic inhibitors on heterotrophic activity.

Keywords: prokaryotic-eukaryotic interaction; sun light; autotrophic-heterotrophic production; leucine incorporation; specific metabolic inhibitors; Brazilian upwelling

Introduction

Biofilms are ubiquitous features of any immersed surface in aquatic environments including the surfaces of living organisms [35]. Macroalgae or seaweeds provide a highly attractive surface for the development of microbial biofilms; not only do they provide a solid surface for attachment but they also release large amounts of organic carbon which can be utilised by microorganisms [1]. Hence macroalgae support complex and highly dynamic microbial communities. Understanding of the interaction between these predominantly prokaryotic communities and the seaweeds on which they grow is relatively poor [13]. This is surprising given that the seaweed/biofilm system potentially provides an ideal model to explore the interface between eukaryotic and prokaryotic ecology and specifically to examine the bi-directional relationship between holobiont partners [32].

The relationship between seaweeds and prokaryotes can operate in a positive and negative sense for both partners [13]. For example, algal products such as dissolved organic matter can stimulate prokaryotic activity [15] but algae can also produce compounds which are toxic to prokaryotes [21]. Products derived from epiphytic prokaryotes such as CO_2, fixed nitrogen and specific growth factors can benefit macroalgal photo-autotrophy and growth [9; 19]. In this holobiont model, biological, chemical and physical surface properties of seaweeds, which may be determined by factors such as environmental conditions and seaweed age, have an important role in determining the composition and activity of prokaryotic communities [2].

In addressing the nature and direction of the relationship between prokaryote biofilms and eukaryotic seaweeds, one profitable approach can be through

measurements of prokaryotic heterotrophic activity by the ^{3}H-leucine incorporation technique [5]. Using *Sargassum* as a biological model, the use of specific inhibitors showed that biofilm heterotrophic activity was mainly bacterial. However, the effect of different antibiotics were tested only in laboratory conditions. In addition, bacterial activity was also inhibited by an eukaryotic inhibitor in dark conditions, suggesting a symbiotic relationship between eukaryotes and prokaryotes in the *Sargassum* model. Finally, bacterial activity was higher when incubations were done in field conditions than in the laboratory, suggesting that natural solar light could be also important to the heterotrophic prokaryotic production. Thus, it is possible that bacterial activity is associated with algal autotrophic production that could change during the daily cycle and by light intensity.

Using *Sargassum furcatum* as a seaweed holobiont model, the aim of this work was to determine if: 1) bacterial activity in the biofilm is associated with algal autotrophic production; 2) varies seasonally; 3) varies over the day-night cycle as well as 4) under different light intensities in the afternoon; and 5) different antibiotics have different effects on the bacterial activity. In a series of experiments performed monthly using different ages of *Sargassum*, we also evaluated environmental conditions, observed biofilm by microscopy and compared biofilm bacterial activity with or without a photosynthesis inhibitor.

Materials and Methods

Sargassum Sampling

Experiments were done in Arraial do Cabo (23°S, 42°W), the main upwelling

area on the Brazilian coast located in the Cabo Frio region of Rio de Janeiro state [details in 7]. Sampling was undertaken monthly in a *Sargassum* bed at 4-6 m depth at the Farol beach station, Cabo Frio Island, between November 2007 and April 2008. Physico-chemical water variables (nutrients, chlorophyll, temperature and salinity) were available over the period of study through the weekly monitoring of the Brazilian navy (Instituto de Estudos do Mar Almirante Paulo Moreira – IEAPM) and methods used are described elsewhere [6]. Solar light radiation was measured every 5 minutes during all experimental periods using a LICOR LI-1.000 Datalogger/Li-193 SA spherical quantum sensor.

Specimens of *Sargassum furcatum* were collected and selected by size over its growth cycle from November to April [26]. Therefore, individuals up to 5 cm were sampled in November; between 5 and 15 cm in December; between 10 and 20 cm in January; between 15 and 25 cm in February; between 15 and 30 cm in March and; up to 10 cm in April, representing the end of its life cycle. Five individuals in the size classes described above were collected at random within the *Sargassum* bed and transferred in polyethylene pots (500 mL) to the laboratory approximately 200 m away. Biofilm composition was observed by epiflourescence and scanning electron microscopy. Blades of each *Sargassum* individual were used for microscopic observations (n=5) as well as for measurements of bacterial activity (n=5). A pilot study showed no statistical difference in bacterial activity using 5 or 15 replicates.

Experimental conditions

Bacterial activity was measured at all six sampling dates. To understand the daily pattern of heterotrophic production, measures were made at different times of the

day during the afternoon (12:00 – 15:00), evening (18:00 – 20:00), night (23:00 – 01:00) and morning (07:00 – 09:00) at each sampling event. Light levels vary over this 24 hour cycle but co-vary with other factors including temperature and internal diel rhythms. Thus to unambiguously determine the effect of light, measures were made at the time of peak light levels during the afternoon (12:00 – 15:00) over a light extinction sequence using an artificial reduction of natural light (100 %, 75 %, 50 %, 25 %, 10 %, 1 % and 0 %) using different levels of a black mesh.

All measures of activity were made in the sea at a depth of approximately 0.5 m, using a submerged platform supporting an open ended acrylic tube into which algal material was placed. One blade of approximately 1 cm^2 from each plant (n=5) were incubated in separate 2-mL microcentrifuge tubes (eppendorf) with local 0.22-µm filtered water held within a 125-ml Winkler bottle. The dry weight in each cm^2 of *Sargassum* blade was 0.004 ± 0.001 g.

For autotrophic production, five blades were inserted into one 125 mL glass Winkler bottle with local 0.22-µm filtered water to have an amount of algal biomass that could be detected by the method used. In November, the measurement was determined by the mean of three Winkler bottles since there was no experimental treatment. In the other months, two treatments were tested (with and without specific inhibitors) and photosynthesis incubation was done in three bottles (n=3).

To directly test the relationship between autotrophic production and bacterial activity a photosynthesis inhibitor (10 µM diuron) was used at four of the sampling dates at each of the four times of day (Table 1). Bacterial activity was thus compared when photosynthesis was naturally occurring and when inhibited. We also tested different antibiotics in November, January and March (through comparing bacterial activity with and without the use of antibiotics at the sampling dates): 5 $\mu g.L^{-1}$

141 streptomycin + 100 units.L^{-1} penicillin were used in November, 5 µg.L^{-1} streptomycin
142 were used in January and 0.2 nM chloramphenicol in March samplings (Table 1).
143
144 *Bacterial activity*
145
146 Bacterial activity was determined using an adaptation of the ^{3}H-leucine
147 incorporation technique [5] following a methodology for periphyton associated with
148 macrophyte roots [23]. Briefly, blades of approximately 1 cm^2 were incubated in 2.0
149 mL eppendorf with 35 nM ^{3}H-leucine in 1.5 mL of 0.22-µm filtered water (n=5). The
150 incubation time was 1.5 – 3 h and 80 µL of 100 % trichloroacetic acid (TCA) was used
151 to stop the incubation. In addition all experiments were performed using a killed control
152 where production was terminated at the start by adding 5 % TCA just before the input
153 of ^{3}H-leucine. Following incubation, samples were frozen until protein extraction.
154 Extraction started with a 5-min sonication bath, and then the blade was removed.
155 Samples were centrifuged at 2,500 g for 15 min, and the supernatant was transferred to
156 a new microcentrifuge tube before being centrifuged again at 13,000 g for 10 min, after
157 which time the supernatant was discarded; 1.5 mL of cold TCA (5 %) was added, and
158 the centrifugation process was repeated. One milliliter of 80 % ethanol was added, and a
159 new centrifugation was done. Finally, 1 mL of scintillation cocktail (Cytoscint) was
160 added and, after an overnight period, radioassayed by scintillation counting (TRICARB
161 PACKARD 1600) for 30 min or after the accumulation of 10,000 counts [as determined
162 by 6]. Leucine incorporation rate (in moles per squared meter per hour) was calculated
163 considering net disintegrations per minute (DPM), sample area (1 cm^2), leucine
164 concentration, ^{3}H-leucine specific activity (72 $Ci.mmol^{-1}$) and time of incubation.
165

Sargassum Autotrophic production

To measure the *Sargassum* primary production, five blades (1 cm^2) were incubated in 125 mL glass Winkler bottles with local 0.22-µm filtered water. It is important to mention that blades were not axenic since they were sampled from the natural bed. Hence both microautotrophs and heterotrophs were included in both production and consumption of carbon. Oxygen was fixed to determine its initial concentration. A dark control was used to determine oxygen consumption, and a glass with no blade of *Sargassum* was used to verify phytoplankton production. Then, primary production was determined using the Winkler method after 2-3 h of incubation stopped with oxygen fixation [27]. The results were calculated considering the difference of oxygen between the end and the beginning of the experiment divided by the total dry weight of the five blades and the time of incubation ($ngC.gdw^{-1}.h^{-1}$). The photosynthetic coefficient was between 0.98 – 1.01 [31].

Statistical analysis

Two way ANOVA was done considering all factors as fixed and orthogonal and a 0.1 significance level. 4 levels were used for the factor time of day (morning, afternoon, evening and night), 7 levels for the factor light incidence (100, 75, 50, 25, 10, 1, 0 %) and two levels for the factor inhibitor (with or without). When necessary, data were log transformed and analyses run using WinGmav software (version 5.0). In the experiments at different times of the day, November was omitted owing to lack of measurement in the morning time. Multiple comparisons of levels within significant factors were made using Student Newman Keuls (SNK) tests.

Results

Environmental conditions during the experiments

All environmental conditions during sampling can be found in Tables 2 and 3. Light intensity (at approximately 0.5 m depth) was high during the afternoon and morning experiments except in December and January when it was approximately one thousand times lower compared to the other months. During this time, a bloom of phytoplankton was visually observed and the *Sargassum* bed was not visible from the boat (4 m above) during arrival at the experimental area in the morning before the experiment began. In addition, pheophytin concentrations peaked in December and January (2.8 and 1.3 mg/m^3, respectively) and chlorophyll *a* in December (1.7 mg/m^3). For nutrients, nitrate was highest in February (3.1 µmol/L) and below the detection limit in April. Peak of ammonia was observed in November (1.8 µmol/L) and, of phosphate and of nitrite in December (0.6 and 0.4 µmol/L, respectively).

Surface water temperatures over the duration of the experiments varied by a few degrees among months (Table 2). Surface water temperatures over 24 hours were around 20.6 °C during the experiments of November and around 23.3 °C in December but ranged from 25.3 to 23.0 °C in February, 27.2 to 24 °C in March and 25.5 to 24.4 °C in April. In January, upwelling was more intense during sampling since water temperature was below 18 °C in the *Sargassum* bed at 4-6 m depth.

Biofilm composition includes both autotrophic and heterotrophic organisms such as bacteria, phytoflagellates, diatoms, hydrozoans, cyanobacteria and eukaryotic algae. Diatom abundance peaked in January and the main taxa were *Cocconeis*, *Navicula*,

Nitzschia, *Licmophora* and *Striatella* (Baeta-Neves pers. obs.). SEM images showed high abundance of bacterial rods and *Cocconeis* diatoms as well as EPS production (Supplementary material).

Daily and seasonal bacterial activity

Bacterial activity showed little obvious seasonal variation when measured in the evening, night-time and morning. When measured in the afternoon it showed a rapid increase from very low levels in November to a peak of over 300 $pgC.cm^{-2}.h^{-1}$ in January followed by a gradual decline over the following months (Fig. 1). Two way ANOVA revealed a significant interaction between the factors month and time of experiment ($p=0.04$) and SNK confirmed that bacterial activity was significantly highest during the afternoon of January. However, bacterial activity in January was only significantly higher than April ($p=0.014$) and independently of season, activity was significantly highest during the afternoon ($p\leq0.001$).

Bacterial activity in a simulated light extinction during the afternoon and its relationship with Sargassum autotrophic production

To check if bacterial activity is influenced by light intensity in the afternoon a series of experiments simulating different degrees of light intensity was done in each month during the *Sargassum* growth cycle. Considering each month no obvious pattern was observed among treatments (Fig. 2). However, ANOVA and SNK tests revealed that heterotrophic activity was highest under low light conditions in the experiments of November ($p\leq0.001$) and February ($p=0.01$). Pooling all data in function of light

intensity, the highest activity was also observed under low light conditions (Fig. 2). In addition, there was no evident relationship between *Sargassum* primary production and the bacterial activity in the biofilm.

The effect of specific metabolic inhibitors

The use of 10 µM diuron in February and April inhibited *Sargassum* autotrophic production completely (data not shown) and its effect on bacterial activity was variable (Table 4). In the experiments conducted at different times of day done in November, December, February and April, the presence of diuron reduced heterotrophic activity between 0 to 66 % (in average 17 ± 11 %). ANOVA revealed a significant interaction in December ($p=0.002$) when the presence of diuron reduced bacterial activity significantly during the afternoon and night-time; and in April ($p=0.008$) when heterotrophic activity reduced significantly during the night-time and in the morning (Fig. 4). The effect of diuron on heterotrophic activity in the experiments simulating light extinction during the afternoon was also variable ranging from 0 to 81 % (in average 16 ± 13 %) and its effect was only significant ($p=0.02$) under 50 % of natural light in November (Table 4).

Antibiotics had different actions on both autotrophic and heterotrophic activities. While penicillin with streptomycin completely inhibited photosynthesis of *Sargassum* and the heterotrophic activity in biofilm (data not shown), the effect of streptomycin alone was light-dependent since the significant reduction of both measurements were observed only under 50 % of light in the experiment conducted in January (Fig. 6). Chloramphenicol inhibited bacterial activity significantly ($p\leq0.001$) and also had a light-dependent effect on *Sargassum* photosynthesis; stopping its activity under high

lights conditions (Fig. 4).

Discussion

Studies on the ecology of seaweed holobionts aim to understand the mechanism and function of all microbial members and their ecological role in the alga's life cycle. However, manipulative experiments in field conditions are challenging [11]. Our observations of the *Sargassum furcatum* holobiont model in its natural environment showed that biofilm composition varied with algal age and with environmental conditions; such variation potentially plays an important role in determining bacterial activity. However, we could not separate the effects of specific environmental conditions from the plant size and age. Observations at different times of the day showed that bacterial activity consistently peaked in the afternoon, when light levels were highest suggesting an association of heterotrophic and autotrophic productions. However, there was no relationship between bacterial activity and light intensity during the afternoon and no obvious correlation between *Sargassum* primary production and the bacterial activity in its biofilm. More studies are need to check if *Sargassum* also incorporate leucine. Nevertheless, our results showed some evidence of a negative effect of a photosynthesis inhibitor on bacterial activity and the highest activity occurred when diatoms were more abundant suggesting that heterotrophs may be stimulated by the autotrophic production in biofilm. Such prokaryotic-eukaryotic coupling is expected in a healthy holobiont system highlighted herein by the effect of antibiotics on both heterotrophic and autotrophic activities.

In the studied region, upwelling events are seasonal and *Sargassum* growth occurs as a function of its intensity [14]. Thus, upwelling should be considered an

important factor causing turnover in community composition of the benthic environment but also of planktonic prokaryotes [4] and eukaryotes [34] which are able to colonize the surface of macroalgal blades [5]. It is expected that *Sargassum* also selects its biofilm composition since *Sargassum* produces compounds with antioxidant, antibacterial, antitumoral, antimalarial, antiherbivory and antifouling properties [e.g. 28] but the concentrations of these compounds may reduce as algae age [10]. We also observed using SEM images the presence of holes indicating that settled organisms detached early increasing spatial heterogeneity and forming new habitats and niches [2] that could enhance productivity [3]. We suggest a higher niche overlap on the youngest blades when higher competition decreased bacterial activity [12] and the peak of activity in January could be associated not only with a more stable habitat but also with the subsequent increase in diatom abundance and diversity. Such increases could enhance bacterial activity by provision of diatom metabolic products. The reduction in bacterial activity after the peak in January could be a function of both diatom detachment and the regressing of *Sargassum* blades (increasing polymer degradation) leading to a reduction in autotrophic enhancement.

Bacterial activity peaked in the afternoon and we suggest its association with bioactive secondary metabolites as a consequence of photosynthesis [11] as well as with microalgal-(nutrient)-leakage after a day of photosynthesis [22] since it peaked during a phytoplankton bloom under an upwelling event. It is known that biofilm conditions vary in the contrasting light circumstances over a day [31] and seasonal variations in the biofilm conditions are strictly associated with light [18]. In addition, bacterial growth is usually higher during daylight hours [17] and the effect of antibiotics showed herein point to Bacteria being the major group incorporating leucine in our measurements, confirming observation in laboratory assays [5]. Similar observations have been made

316 in freshwater epiliphic biofilms, where bacterial biomass and activity was highest in the
317 presence of light as a consequence of organic substrates produced by algae [29]. Thus,
318 we suggest that exudation is an important factor for coupling algal primary productivity
319 and bacterial activity in a seaweed holobiont model.

320 We cannot affirm if diatoms increased bacterial activity because of facilitation or
321 by character displacement, since both enhance productivity over time [12; 20] and we
322 did not measure important biotic factors such as competition, predation and herbivory
323 [22; 25; 30]. In contrast, the importance of microalgal exudation is partially supported
324 by the effect of photosynthesis inhibitor on bacterial activity. The wide variation in the
325 effect of diuron could be associated with the ability of biofilms to decrease the action of
326 many compounds [8] but also with the direct relationship between grazing pressure on
327 bacteria and the concentration of exudates [22]. However, our results support the
328 hypothesis that on average 17 % of the autotrophic production in the biofilm is used by
329 bacteria in the *Sargassum* holobiont model.

330 Our results, showing different degrees of antibiotic effects, corroborate the work
331 of Nair et al. [24] that showed marine bacteria to be less sensitive to streptomycin than
332 to penicillin and chloramphenicol. Additionally, our results showed a light-dependent
333 effect of streptomycin and chloramphenicol on photosynthesis; such effects may not
334 only be related to their antibiotic properties. Both are directly associated with
335 photoinhibition since they inhibit photosystem II photosynthetic efficiency. Hader et al.
336 [16] showed inhibition of D1 protein biosynthesis in chloroplast of seaweeds, using
337 almost the same concentrations of both substances as in our study (500 μg/mL and 2
338 mg/mL, for streptomycin and chloramphenicol respectively). D1 protein controls
339 electron transport after primary photon absorption and is inhibited by visible and UV
340 lights.

In future studies, we recommend the determination of 1) the importance of periphytic algae to the attached bacterial production and of 2) the specific effect of plant size and environmental conditions in the experimental design. In the first case, it is important to measure exudation rates by both seaweed and periphytic algae to estimate the relationship between bacterial production and primary production of both macroalgae and periphyton. Our photosynthesis measurements included both components and its inhibition resulted in a mean reduction of bacterial activity potentially caused by a reduction in carbon uptake by bacteria from periphyton or macroalgal exudate [22]. In addition, primary production is also affected by plant age and environmental conditions. Although there are benefits of measuring bacterial activity under natural conditions [5], it is important to check the effect of biotic and abiotic factors in laboratory conditions controlling variables such as secondary compounds and polymer concentrations of seaweed, periphytic algae and phytoplankton as well as UV intensity, temperature and substrate concentrations.

In conclusion solar light is likely important to bacterial activity in the biofilm but not necessarily as a function of the photosynthetic activity of *Sargassum furcatum.* Despite bacterial activity being highest during the afternoon it was not correlated to light intensity. Bacterial activity peaked during the intermediate age of *Sargassum* when diatoms were abundant in the biofilm, suggesting the importance of periphytic algae.

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512 FIGURE LEGENDS
513
514 Figure 1: Seasonal bacterial activity (mean ± SE) at different times of the day during
515 *Sargassum* growth between November/2007 and April/2008 (n=5).

516 Figure 2: Mean of bacterial activity at different levels of light during *Sargassum* growth
517 in the experiments done between November/2007 and April/2008 (top) (n=5); and its
518 relationship with light intensity pooling all data (bottom) (n=3).

519 Figure 3: Mean ± SE of bacterial activity ($pgC.cm^{-2}.h^{-1}$) at different times of the day
520 under the presence or absence of diuron in the experiments undertaken in
521 December/2007, February and April/2008 (n=5).

522 Figure 4: Mean ± SEM of biofilm heterotrophic activity in different times of a day and
523 under different levels of light during the afternoon (n=5) as well as in *Sargassum*
524 autototrophic production under different levels of light during the afternoon (n=3) with
525 and without the antibiotic streptomycin (left) and chloramphenicol (right) during the
526 experiments conducted in January/08.

Figure

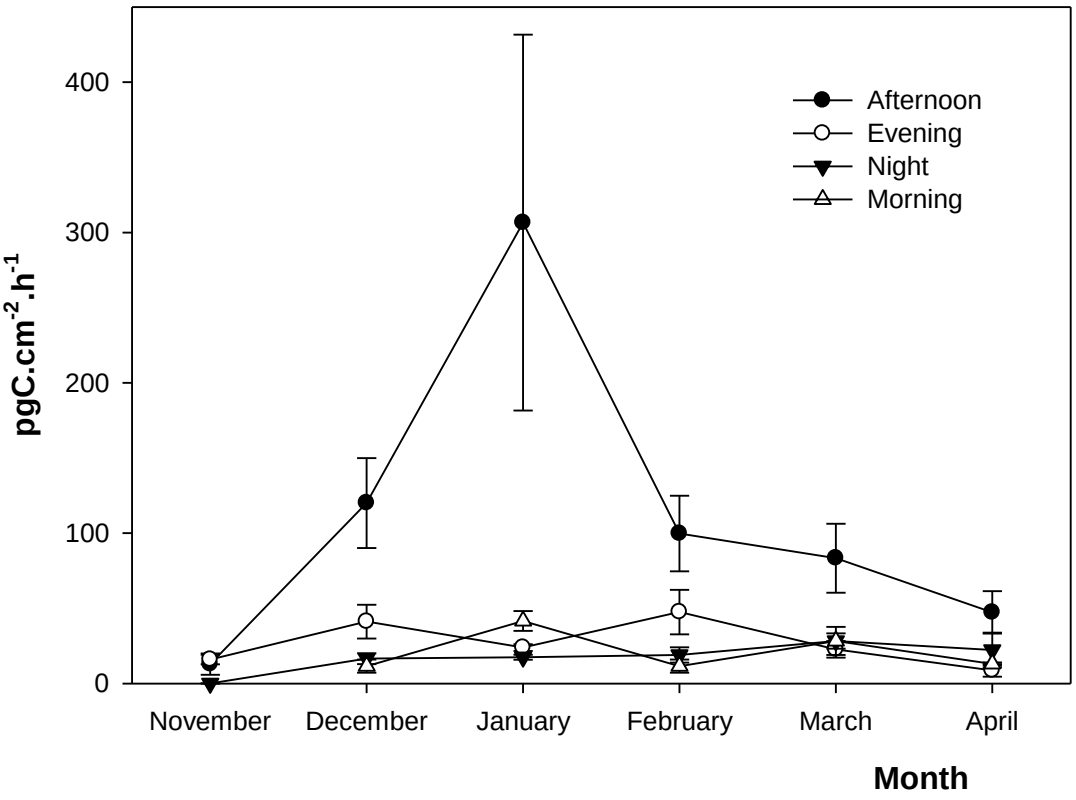

Figure 1: Seasonal bacterial activity (mean ± SE) at different times of the day during *Sargassum* growth between November/2007 and April/2008 (n=5).

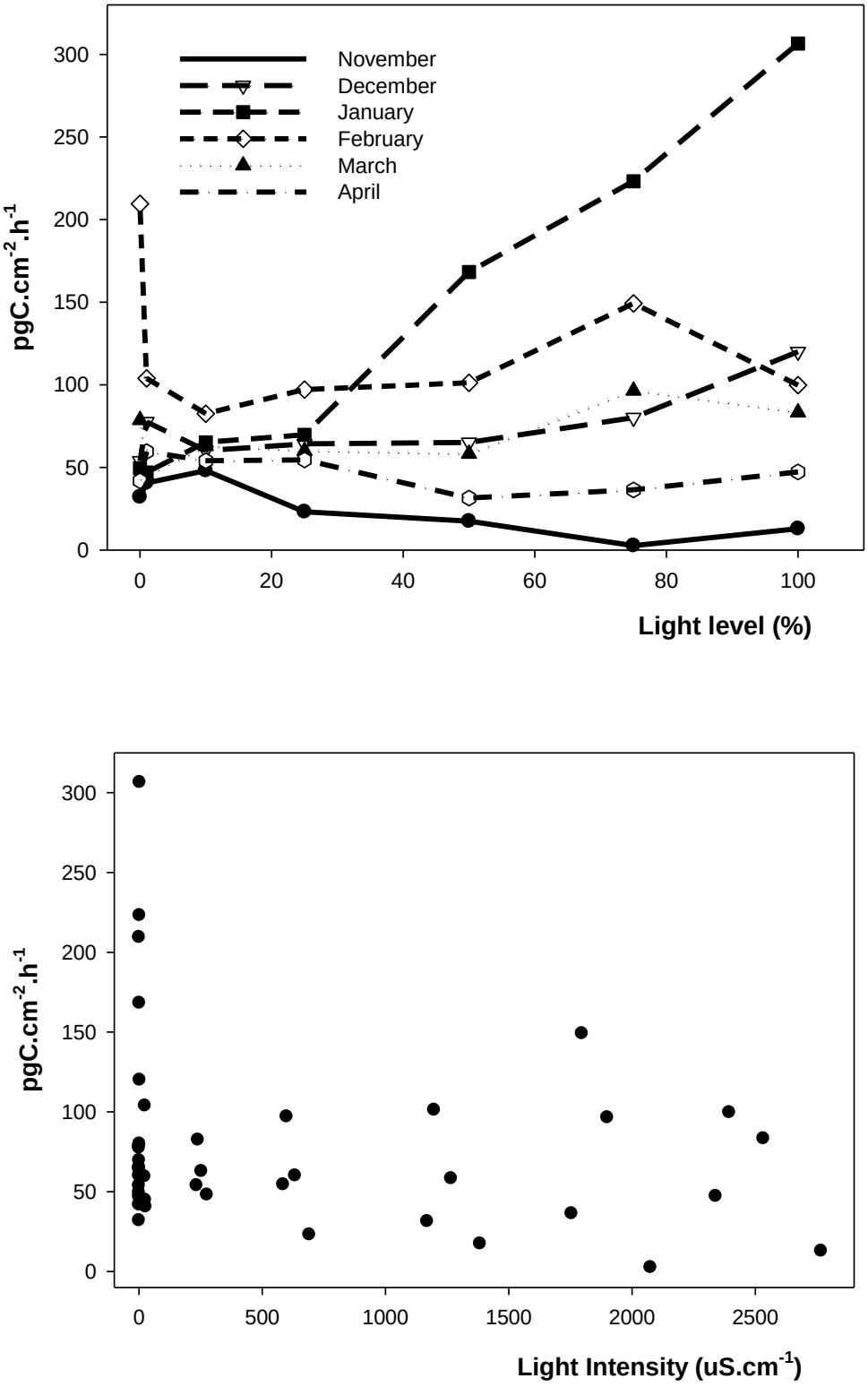

Figure 2: Mean of bacterial activity at different levels of light during *Sargassum* growth in the experiments done between November/2007 and April/2008 (top) (n=5); and its relationship with light intensity pooling all data (bottom) (n=3).

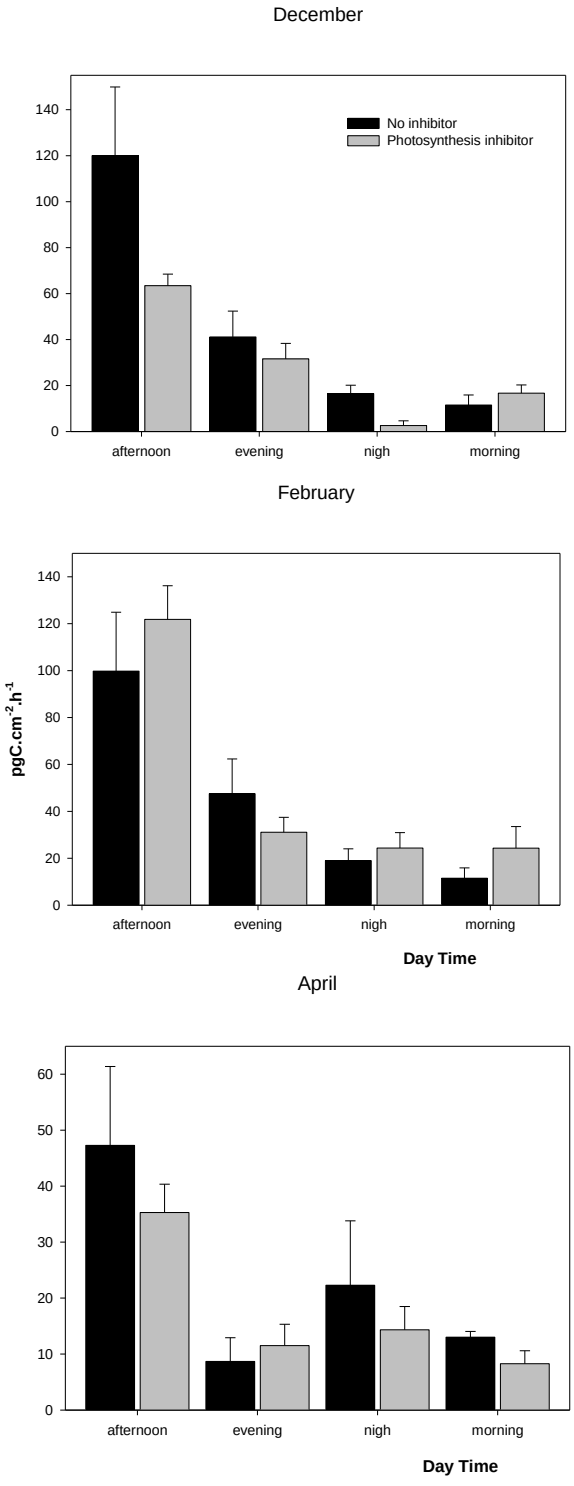

Figure 3: Mean ± SE of bacterial activity ($pgC.cm^{-2}.h^{-1}$) at different times of the day under the presence or absence of diuron in the experiments undertaken in December/2007, February and April/2008 (n=5).

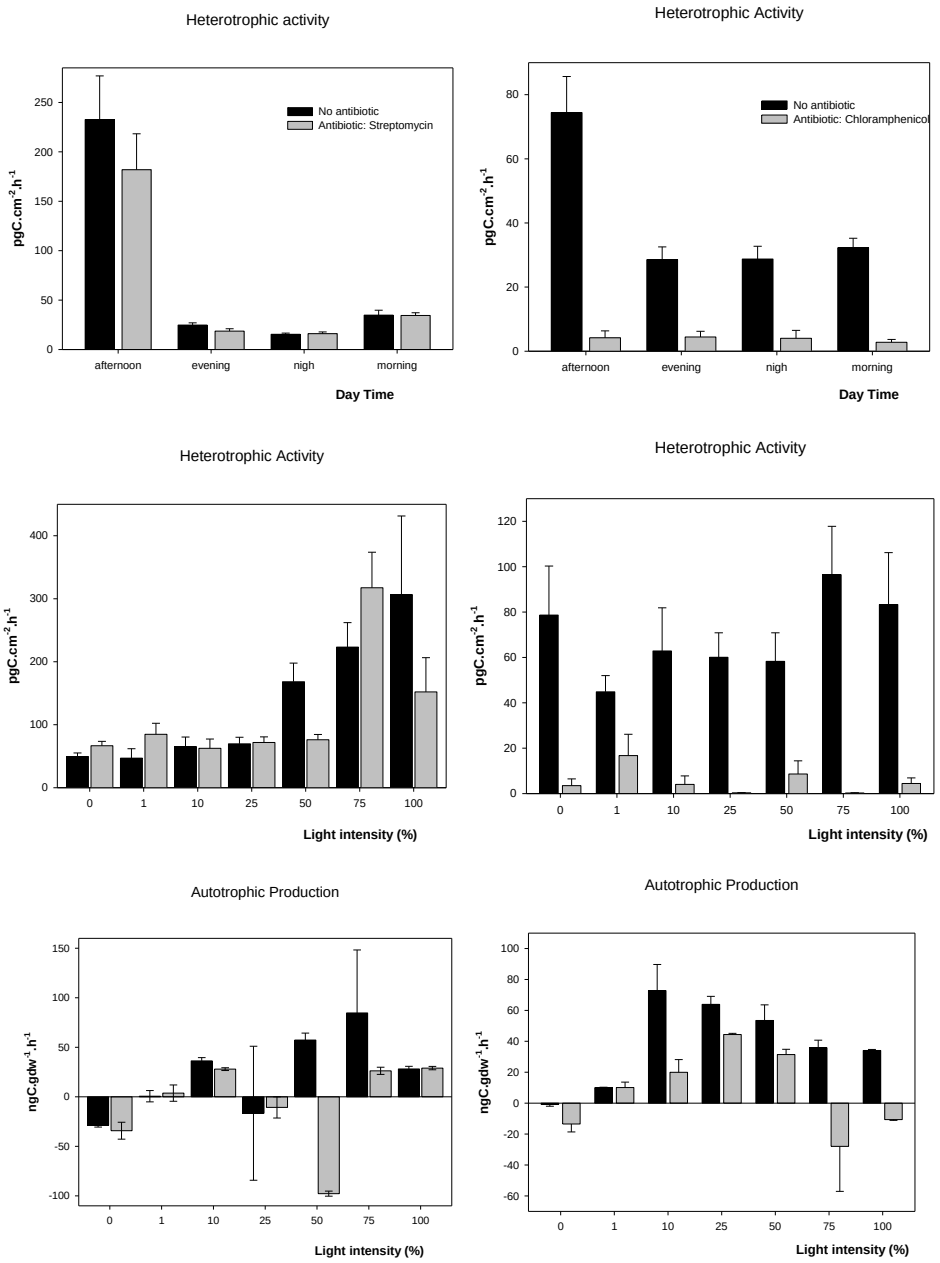

Figure 4: Mean ± SEM of biofilm heterotrophic activity in different times of a day and under different levels of light during the afternoon (n=5) as well as in *Sargassum* autototrophic production under different levels of light during the afternoon (n=3) with and without the antibiotic streptomycin (left) and chloramphenicol (right) during the experiments conducted in March/08.

Table

Table 1: The use of antibiotics (penicillin, streptomycin and chloramphenicol) and photosynthesis inhibitor (diuron) in both autotrophic production and bacterial activity for the experiments realized during the *Sargassum* growth cycle.

Month of the Experiment	Autotrophic production	Bacterial Activity
November	No inhibitor	Penicillin + Streptomycin
December	Penicillin + Streptomycin	Diuron
January	Streptomycin	Streptomycin
February	Diuron	Diuron
March	Chloramphenicol	Chloramphenicol
April	Diuron	Diuron

Table 2: Physical-chemical variables measured in surface and deep waters during the sampling and experimental dates.

Month of the Experiment	Temp. (°C)		OD (mg/L)		P-PO_4^{3-} (µMol.L-1)		N-NO_2^- (µMol.L^{-1})		N-NO_3^{2-} (µMol.L^{-1})		N-NH_4^+ (µMol.L^{-1})		pH	
	sup	dp	sup	dp	sup	dp	sup	dp	sup	dp	sup	dp	sup	dp
November	21.0	20.0	5.6	5.7	0.2	0.3	0.2	0.2	0.8	0.6	1.3	1.8	8.5	8.5
Dezember[a]	23.4	21.5	5.4	5.0	0.1	0.6	0.0	0.4	0.1	1.0	1.1	1.7	8.8	8.7
January	23.4	17.8	5.4	5.1	0.1	0.1	0.0	0.1	0.5	2.2	1.4	1.2	8.4	8.5
February[b]	22.0	21.3	5.2	5.3	0.2	0.3	0.0	0.1	3.1	3.0	0.7	1.1	8.8	8.9
March	23.5	23.2	4.9	4.9	0.3	0.3	0.1	0.0	2.0	1.7	1.3	1.1	8.2	8.3
April	24.3	23.8	5.1	5.1	0.1	0.1	0.0	0.0	0.0	0.0	1.1	1.1	8.4	8.4

[a] 2 days after experimen; [b] 1 day after experiment

Table 3: Concentration of chlorophylls in surface and deep waters during the sampling and experimental dates.

Month of the Experiment	Chlorophyll-*a*		Chlorophyll-*b*		Chlorophyll-*c*		Pheofitin	
	sup	dp	sup	dp	sup	dp	sup	dp
November	0.9	0.9	0.2	0.2	0.3	0.1	0.3	0.9
Dezember[a]	1.8	1.6	0.0	0.0	0.0	0.6	2.8	0.0
January	0.6	0.5	0.0	0.0	0.0	0.0	1.3	0.3
February[b]	1.0	0.7	0.1	0.2	0.5	0.3	0.0	0.0
March	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1
April	0.6	0.3	0.0	0.0	0.0	0.0	0.6	0.0

[a] 2 days after the experiment; [b] 1 day after the experiment

Table 4: Range in the reduction of bacterial activity (%) under the presence of photosynthesis inhibitor (diuron) in experiments in the daily cycle and simulating light extinction during the afternoon.

Month	Daily cycle	Afternoon	Significant effect
November	13 - 66	0 - 81	50 % of light
December	0 - 59	0 - 47	afternoon and night
February	0 - 40	0 - 44	ns
April	0 - 39	0 - 25	morning and night