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The Daily Light-dark Cycle of Photosynthetic Oxygen Evolution in Three Species of Tropical Calcareous Algae

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Abstract Endogenous coordination between light, temperature and other factors by different species of algae would be vital in the production of several proteins needed for growth and adaptations, and therefore will affect their productivity. Among plant activities that are governed by daily light-dark cycles (i.e. 12L:12D and 12L:12L) among calcareous algae (*Halimeda simulans*, *Mastophora rosea* and *Padina australis*) were conducted by monitoring the dissolved oxygen (DO) concentration inside incubating bottles with algal samples for 24h and the features of the resulting DO curves were determined by measuring the number of pixel under the curve through image analysis. There was no significant difference among algae during the initial 12L:12D cycle suggesting their normal response to light-dark cycles. However, after six days under continuous light, *M. rosea* showed a significant decrease in the DO curve (lesser number of pixel under the curve) compared to DO curves during the initial 12L:12D cycle. The decrease in the DO concentration during the continuous L:L treatments might be attributed to the photoinhibitory effect of the red alga being less adoptive to subsequent high intensities. Although an increase in DO concentrations is expected with continuous light, not all algae responded to it. Only *Padina* exhibited circadian rhythm in our 24h observation under continuous light.

Keywords: *Coral reef, Padina, Halimeda, Mastophora, circadian rhythm*

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1. Introduction

There are behaviors and processes in organisms that are observed to be repetitive and therefore predictable during the course of their existence. The adaptation of living cells to daily fluctuations of light, over a period of about a day, is reflected by endogenous rhythms (circa diem) [3]. These rhythms are thought to be regulated by internal oscillators (circadian clocks) or are endogenous biological clock that governs biochemical phenomenon or behavior which allows organisms to predict environmental changes, such as day/night and seasons that result from the rotation of the earth [19]. Among the many environmental cues, light affects virtually all aspects of plant growth and development like gravitropism, phototropism, floral initiation, germination and vegetative circadian rhythm to daily changes in environmental conditions over a 24h light dark (L:D) cycles (Kendrick and Kroneberg, 1994 in [2]). It is for this reason that studies on the effects of light on circadian rhythm and daily L:D cycles in plant are needed to maximize their productivity potential or their sustainability toother geographic locations.

Similar to land plants, circadian rhythms are also expressed in marine algae under various light regimes and irradiances. The role of circadian rhythm has been very evident on the high productivity of some algae in the initiation and formation of proteins vital in the synthesis of organic compounds needed for growth and other functions. Various activities tested in rhodophytes *Kappaphycus alvarezii* [10], *Gracilaria tenuistipitata* [14] and *Gracilaria chilensis* [5] on nitrate assimilation characteristics revealed that nitrate reductase activity is under circadian control, exhibiting daily rhythm under L:D cycles and constant light. On the other hand, Drew and Abel [7] observed that the segments of *Halimeda* rapidly become very pale after dark, but are green enough by dawn to permit rapid photosynthesis as soon as light is available suggesting that the re-emergence of the chloroplast before dawn and their subsequent withdrawal appears to be controlled by an endogenous rhythm which is independent of light and reportedly work at temperatures ranging between 20°C and 30°C [9]. Photosynthesis and other processes (i.e., calcification in calcareous algae) appear to be under circadian control in some of the algae studied. Larkum et al., [12] concluded that in *Halimeda macroloba*, alkalization of the internal utricular space triggers calcification during photosynthesis and exhibited an endogenous rhythm during a 12:12 hrs L:D treatment but not in continuous light. Unlike *Halimeda*, another green alga, *Acetabularia* exhibited endogenous rhythm even in continuous light [11]. This contradiction indicates that further studies are needed to verify the effects of continuous light on other calcareous or coralline algae.

The polarographic dissolved oxygen (DO) meter can be used to monitor oxygen evolution and consumption of an incubated algal sample. With appropriate methodology

and experimental protocols, the use of this basic laboratory equipment can be further applied to the study of primary productivity, photosynthetic potential, and changes in DO concentrations of incubated algal samples. Mishkind et al. [15] used Fieldlab O_2 analyzer to study the diurnal oscillation of light-saturated photosynthetic O_2 evolution in *Ulva lactuca*. Studies on the circadian photosynthetic rhythm of Synechococcus (a cyanobacteria) and *Kappaphycus alvarezii* (a rhodophyte) were effectively demonstrated using DO meters by Yen et al., [19] and Granboom et al. [10], respectively.

In the laboratory, we altered the L:D cycle of three tropical species of intertidal calcareous algae (*Halimeda simulans*, *Mastophora rosea* and *Padina australis*) and monitored their photosynthetic responses using a portable DO meter. Then we compared the standardized DO curves of the initial 12L:12D cycle against the final 12L:12L cycle after the algae were subjected to continuous light for six days.

2. Materials and Methods

The idea underlying the experiment is based on the normal response of plants subjected to light. The alga is considered to behave "normally" if photosynthesis (oxygen evolution) proceeds under light treatment. Conversely, when the alga is incubated in the dark, a decreasing DO concentration should be expected regardless of whether it is night or day.

Evaluating light-dark cycles requires continuous monitoring of oxygen evolution/consumption by the alga over 24 hours (or 1 day) using a DO meter. Three species of algae, *Halimeda simulans* (Mean±SD, N=5;3.6±1.6 g wet wt), *Mastophora rosea* (5.4±1.8 g) and *Padina australis* (2.2±0.3 g), were collected and washed thoroughly with seawater to remove sediments and other debris. Each individual specimen was acclimated under 12L:12D cycle for 24h at 20°C in an aerated 1L glass jar inside an environmental chamber. After the acclimation, the alga was placed in the BOD bottle (250ml capacity) and monitored for oxygen evolution by exposing it to an altered light regime of 12L:12D (12h of subjected light at night: 12h subjected dark during the day) using cool white T5 tubes (Firefly® 8Wx8 pcs) mounted inside the box (Figure 1). The DO concentration was measured every 30 minute for 24h. The total irradiance inside the box was 150μmol photons $m^{-2}s^{-1}$ as measured by a Li-COR light meter with a cosine corrected quantum sensor. After the 24h measurements, the same algal specimen was then exposed to continuous light for six days inside the environmental chamber, changing the seawater daily. Finally the algae was incubated again, this time, for continuous subjective light (12L:12L) and the DO concentration inside the BOD bottle was again measured every 30 minutes for 24h.

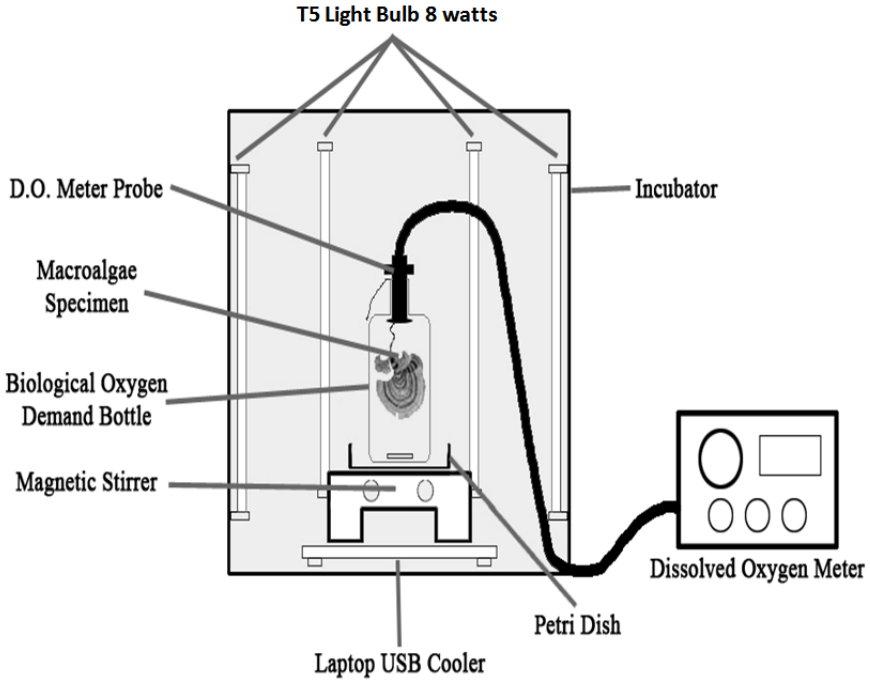

Figure 1. Schematic diagram of the incubating chamber

The 24h data for each replicate for each alga was encoded in Microsoft Excel to generate X-Y graphs (Y-axis DO concentration; X-axis time). But before generating the graphs, all data were standardized by subtracting each 24h data set with the initial DO concentration, thereby making all DO curves to start at zero. This was necessary so that the line graphs that were generated will have a uniform scale before they were scanned as image files in an image processing software, ImageJ. The software provided a faster, hassle-free and a more reliable number of pixel under the curve of all the image data (X-Y graphs). The number of pixels under the curve of each graph was then subjected to statistical tests.

Analysis of variance with repeated measure (ANOVAR) was used to compare the effect of before and after treatments (12L:12D and 12L:12L cycles) and among species of algae on the number of pixels under the curve of the X-Y graphs. If main treatments (i.e., algal species and cycles) were significantly different, then the feature (in terms of number of pixels under the curve) of the

standardized DO curve should be different. Levene's test was used to test for the homogeneity of variance to comply with the assumption of the test. LSD test was used as post-hoc comparison if a significant difference was detected. Significant level was set at 95%.

3. Results

During the initial 12L:12D cycle, the DO evolution rate of the three algae exhibited the normal response when subjected to light. On the average, DO increased when exposed to subjective light for the first 24h and decreased after 12h when light were switched off (subjected dark) inside the box (Figure 2). A post-hoc comparison on the number of pixel under the curve of the 12L:12D cycle showed no significant difference among the three algal species despite differences in wet weight at the start of the experiment. However, there was a significant difference between 12L:12D and 12L:12L cycles (Table 1). Among the three species of algae, *M. rosea* showed greatest DO curve differences. The number of pixel under the curve before and after the continuous light treatment was statistically comparable for *H. simulans* and *P. australis*.

Table 1. Analysis of Variance (ANOVAR) with repeated measures on the different algae (*Padina australis, Halimeda simulans* and *Mastophora rosea*) on different light treatments: light-dark (L:D) and continuous light (L:L) based on the number of pixels under the curve of the X-Y graphs. Levene's test was used to test for the homogeneity of variance to comply for the assumption of the test. LSD test was used as post-hoc comparison if a significant difference was detected. Significant level was set at 95%

	SS	Df	MS	F	p-value
Effect on Species	1.06E+12	1	1.06E+12	57.317	0.000*
Intercept	1.06E+11	2	5.30E+10	2.855	0.100
Error	2.04E+11	11	1.85E+10		
Effect on Light regime	6.04E+10	1	6.04E+10	6.590	0.026*
Effect on Light regime x species	2.89E+10	2	1.44E+10	1.578	0.250
Error	1.01E+11	11	9.17E+09		

SS-sum of squares; Df-degrees of freedom (N-1); MS- ;F- frequency; (*) – significant difference

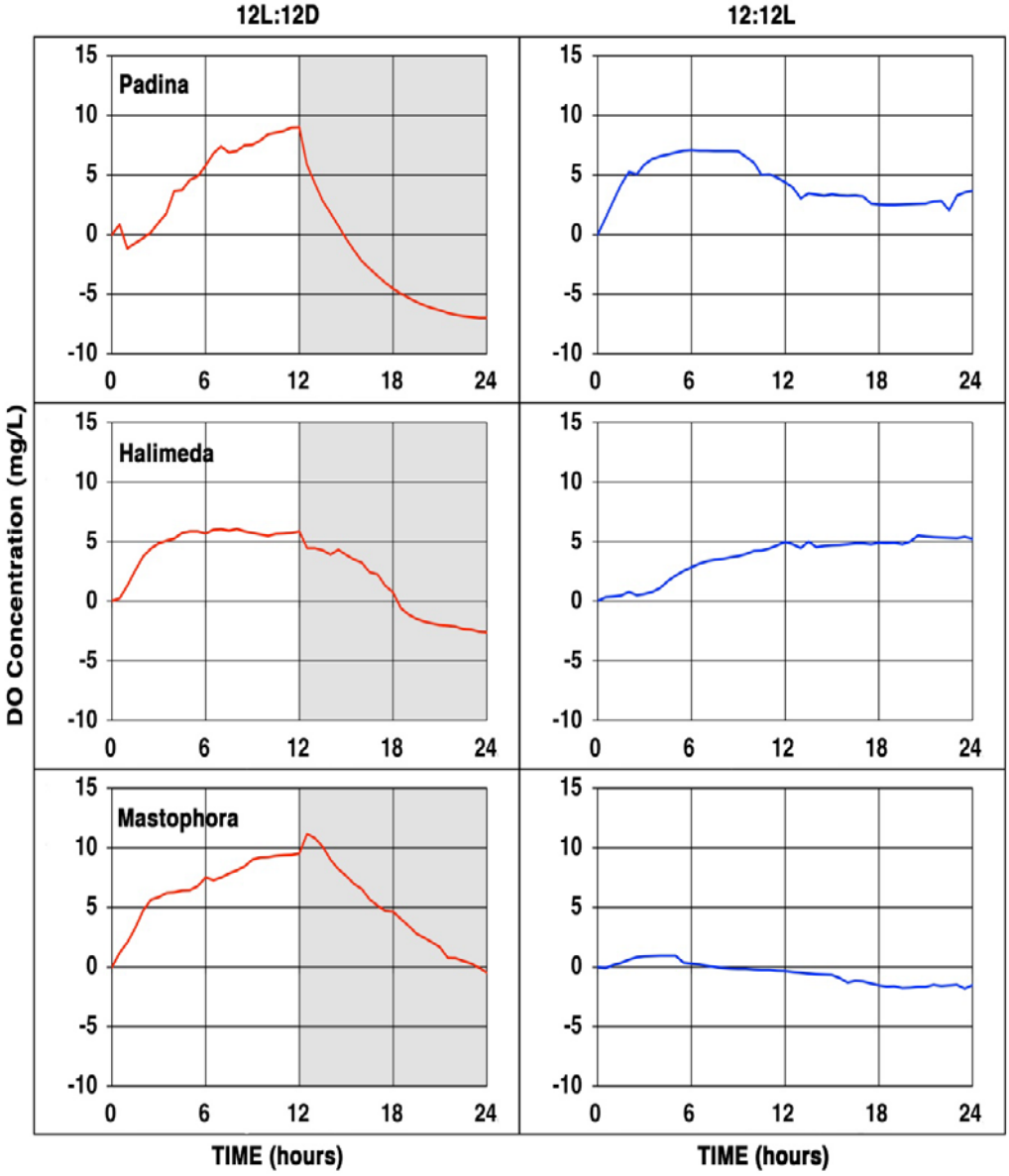

Figure 2. Oxygen evolution rate of the different algae: A. *Padina australis*, *Halimeda simulans* and *Mastophora rosea* subjected to different treatments 12:12 LD and continuous light 12:12 LL

However, by comparing the average DO curves in the 12L:12L cycle, one can see that they were different for the three species of algae (Figure 2). For example, in *H. simulans*, DO concentration increased albeit slowly until the end of the experiment. In *P. australis*, DO concentration increased at the start to reach the peak, decreased after and stabilized till the end of the experiment. In *M. rosea* the DO concentration varyslightly increased at the start but progressively decreased despite the presence of light. From these observations it can be said that although an increase in the DO concentration is expected with continuous light, not all algae responded to it. Only *Padina* exhibited circadian photosynthetic rhythm in our 24h observation. In *Halimeda*, we only observed an increase in the DO concentration and decrease in *Mastophora* (Figure 2).

4. Discussion

Photosynthetic production of the three species of calcareous algae is thought to increase with increase light intensities. However, a confounding effect had resulted when they were exposed to continuous light L:L cycle. The continuous illumination may have altered their photosynthetic circadian rhythm. The changes in irradiance and light quality during photosynthesis inhibit many biological processes if irradiation becomes excessive [4].

Exposure of the algae to continuous light (12L:12L cycle) had a diminishing effect on the photosynthetic activity compared to the same algae exposed to 12L:12D cycle at the beginning of the experiment. Our results were similar to Aguilera et al. [1] when the red algae *Porphyra umbilicalis* was exposed to a continuous PAR+UV radiation during dark periods. They opined that the dark period is supposed to lead to recovery of photosynthetic parameters and a reorganization of pigments in the photosynthetic apparatus as a form of maintenance of plant growth, similar to organism that sleep/rest at night. The same study by Yen et al., [19] in the cyanobacteria, *Synechocytis* sp. also revealed a decrease in the DO peak amplitude under the 12L:12L cycle.

The intertidal habitat of the tropics is described as a heavily stressed environment for many different species of organisms. Supplementary mechanism and processes have evolved to protect vital functions needed for survival, growth and proliferation of species from desiccation and increased solar radiation. Although the three collected species of calcareous algae occupy specific location in the intertidal area, varying photosynthetic circadian rhythms were observed suggesting that different species react uniquely to different quality and intensity. This distinctive adaptation of each species is probably necessary to avoid direct competition and thereby insuring survival in the otherwise diverse environment in the tropics.

A continuous light (12L:12L cycle) regime for a red alga like *M. rosea* could be too much for a species that thrives in the lower part of the intertidal habitat. For example, low-light grown *Ulva rotundata* had a lower capacity for non-photochemical quenching, and showed greater photoinhibitory damage, after light stress treatment than high-light grown thalli [16]. This finding suggests that the ability of an alga to withstand continuous light as a form of stress was lower for algae living in the subtlittoral zone. Induction of a stronger non-photochemical quenching in upper-shore red algae has been associated with a higher zeaxanthin content (Rmiki et al., 1996 in [17]). However, other studies [6,17] have shown that the zeaxanthin content of red algae does not correlate well with irradiance. The zeaxanthin content of *Mastophora rosea* has yet to be determined in future studies. Extremely sensitive responses and growth impairment was observed on the red alga *Delesserria sanguine*, a deep sublittoral species, when exposed to surface solar radiation indicating a lack of protective mechanisms on excessive radiations that may lead to reduced photosynthetic and metabolic activity, and a decrease in biomass production [4].

The increase DO concentrations in the *H. simulans* and *P. australis* incubations at any time during the 12L:12L cycle showed the algae's tolerance to continuous light. Species in the littoral zone are exposed to high solar radiation and atmospheric changes in temperature during periods of low tide. In brown algae, adaptive mechanisms like phlorotannin production has been found to respond plastically to changes in light, depth, salinity and grazing [18]. Other adaptive roles include activation of oxidative response, increased activity of repair and recovery mechanisms or by the formation of UV-screening compounds [13].

5. Conclusions

Photosynthesis is probably the most intensively studied process in plant biology due to its central role in plant metabolism. Future studies regarding the light-dark cycles of other calcareous algae in the less studied tropics should be pursued. Unlike other algae, calcareous algae hold potential not just as models for photosynthetic production research, but also as a carbon sink and an indicator of climate change. This study is an initial step in that direction.

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