Hydrobiologia 246: 141–145, 1992.

The use of photosynthesis inhibitor (DCMU) for *in situ* metabolic and primary production studies on soft bottom benthos

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Received 9 October 1991; in revised from 11 March 1992; accepted 28 April 1992

Key words: DCMU, metabolic studies, primary production, photosynthesis inhibitor, benthos

Abstract

A selective chemical photosynthesis inhibitor, DCMU (Dichorophenyl-dimethylurea), dissolved in DMSO (Dimethyl sulfoxide) was substituted for the dark incubation method commonly used to measure the oxygen consumption in metabolic and primary production studies. We compared oxygen fluxes during light incubations with DCMU and dark incubations procedure, on soft bottom benthos. For this purpose, we studied the effects of different DCMU concentrations. A concentration of $5 \cdot 10^{-5}$ mol l^{-1} inside a clear incubation enclosure completely inhibits photosynthesis without affecting the metabolism of soft bottom benthos.

Introduction

A number of primary production studies have been based on oxygen concentrations differences between light and dark incubations (Charpy-Roubaud & Sournia, 1990). Dark consumption of oxygen is generally recognized as similar to night consumption and easier to process. Many authors consider that dark is sufficient to inhibit photosynthesis and stop oxygen production. Since Bishop (1958) demonstrated that DCMU (Dichorophenyl-dimethylurea) was an inhibitor of photosynthesis (photosystem II), some authors have used it *in vitro* for metabolic experiments, on isolated organisms such as microalgae (Pohl & Wagner, 1972; Legendre *et al.*, 1983), on symbiotic organisms (Vandermeulen *et al.*, 1972), and in phytoplankton incubation bottle experiments (Putt *et al.*, 1987). No *in situ* global primary production studies on soft bottoms have used DCMU although it is easier to use than to darken an incubation chamber. In this paper, we compare oxygen fluxes during light incubations with DCMU and dark incubation procedures on soft bottom benthos. We also studied the effects of different DCMU concentrations. One experiment was carried out at night to compare oxygen consumption measured with three incubation methods: night, DCMU + light and dark.

Material and methods

This study was conducted in August 1990, during a cruise on board the R.V. ALIS, in the South West lagoon of New Caledonia, near Seiche-Croissant reef (22°19'40S–166°21'30E). Depth varied from 10 m to 11 m according to the tide. During the experiments, the temperature ranged from 22 °C to 24 °C. Sediment was composed of well sorted fine sand with 3.9% silt content (Cla-

vier *et al.*, 1990). The bottom was covered by a mixture of seagrass meadow intermixed with seaweeds. Oxygen fluxes were measured *in situ*, inside enclosures, as described by Boucher & Boucher-Rodoni (1988) and Boucher & Clavier (1990). Three PVC tubes (0.2 m^2) were pushed by SCUBA diver into the sediment, to a minimum depth of 5 cm. The tubes were closed with clear acrylic hemispheres trapping a known volume of water (54 l to 63 l according to core insertion in the substrate). Submersible pumps, connected to waterproof 12 V batteries, maintained a 10 l min^{-1} closed circuit flow rate within each enclosure, allowing good mixing without noticeable resuspension of sediments. A calibrated polarographic electrode, connected to a dissolved oxygen meter (YSI, mod. 58 in a submersible container), was placed in each dome for continuous measurements. Incubations were conducted between 9 and 12 AM, while light intensity was increasing. First dark incubations were conducted and continued 1 h. Each enclosure was covered with black plastic sheet. To prevent radiation of light absorption by black cover which increases water temperature inside the enclosure, an aluminium cover reflecting radiations was put on top of it. Oxygen concentration in each chamber was recorded every 10 minutes by a SCUBA diver. Next the covers were lifted, and the clear hemispheres were removed for 1 h, then relocked on the bases for light incubations. 60 ml of a DCMU solution was injected in each enclosure. As the inhibitor must be able to reach its site of activity to be effective (D'Elia, 1978) it was dissolved in DMSO (Dimethyl sulfoxide) to facilitate its passage through plant cellular membranes and its penetration into the sediment. Dilutions were made to obtain 6 final concentrations tested in the enclosures (10^{-3}, 10^{-4}, $5 \cdot 10^{-5}$, 10^{-5}, 10^{-6} and 10^{-7} mol l^{-1}). DCMU incubations lasted 2 h. Oxygen concentration in each enclosure was checked every 10 minutes. During incubations, light energy at the sea-surface was recorded using a LICOR integrator. The amount of available light reaching the bottom was then calculated using an extinction coefficient of water obtained by underwater vertical profiles of light.

To check the relationships between night, DCMU and dark oxygen consumptions in enclosures, triplicated night incubations were carried out between 2 h and 4 h after sunset, followed next morning at the same place by dark incubations and DCMU incubations. Concentration of DCMU solution used for these experiments was $5 \cdot 10^{-5}$ mol l^{-1}.

At the end of the incubations, triplicate sediment syringe-cores (5.31 cm^2, 1 cm depth) were taken inside each enclosure for functional chlorophyll *a* and phaeopigments contents. Sediment were deep-freezed and freeze-dried. The pigments were extracted using 90% acetone in a refrigerator (4 °C) for 18 to 24 h (Garrigue & Di Matteo, 1991). Pigments were measured using the spectrophotometric method of Lorenzen (1967). Macrophytobenthos was collected in each enclosure by scuba diver and fixed in 10% formalin. In the laboratory, species were identified and biomass, expressed as g m^{-2} AFDW, were calculated after dessication at 60 °C and ash content determination at 550 °C.

Oxygen fluxes were calculated by linear regression 7 or 15 measures of oxygen content according to sampling frequency and incubation duration. Oxygen consumption, corrected for water volume trapped in the enclosure and for bottom surface area, was expressed as mg O_2 m^2 h^{-1}. DCMU efficiency (*EF* = oxygen consumption in DCMU incubation/oxygen consumption in dark incubation $\times 100$) represents the percentage of respiration in presence of DCMU compared to the dark respiration. Simple linear regressions were calculated between DCMU efficiency and other parameters such as light, functional chlorophyll *a* and macrophytobenthic biomass. Night, DCMU and dark incubations were compared using a Friedman non-parametric test (Siegel, 1956).

Results

The light available near the enclosures during the DCMU incubations varied from 63.5 to 242.6 μmol m^{-2} s^{-1}. The list of macrophytes on

the study site and their abundance are presented in Table 1. The macrophytobenthic biomass collected in the enclosures varied from 32 to 98 g m^{-2} AFWD and the microphytobenthic chlorophyll *a* varied from 20 to 100 mg chlorophyll *a* m^{-2}.

Oxygen concentration evolution in an enclosure during an incubation is presented Fig. 1. In darkness oxygen consumption is linear; in the light primary production occurs consequently oxygen concentration in increase; then after DCMU injection, oxygen concentration decreases regularly (Fig. 1).

DCMU efficiency (Fig. 2) is not complete for 10^{-7} to 10^{-5} mol l^{-1} concentrations. With a 10^{-7} mol l^{-1} solution, photosynthesis inhibition was so slight that oxygen production was measured in two of the incubations ($EF<0$). The efficiency of the inhibitor increases gradually from 10^{-7} to 10^{-5} mol l^{-1}. Total photosynthesis inhibition occurs at DCMU concentrations ranging from $5\cdot10^{-5}$ to 10^{-4} mol l^{-1}. For those concentrations, the DCMU respiration represents respectively 111% and 108% of the dark respiration. The percentage decreases to 94% for a 10^{-3} mol l^{-1} concentration. However, at the end of the 10^{-3} mol l^{-1} incubation, fauna, such as ophiurids and fishes, were observed to be in a poor condition. This suggests that *EF* decrease could be related to inhibitor deleterious effects on animals. The linear regression between light and DCMU efficiency is not significant if the smallest DCMU concentration (10^{-7} mol l^{-1}) incubations values are omitted ($r=-0.33$, $F=2.28$, $p>0.05$, $n=20$). This supports a close relationship between light and oxygen fluxes during 10^{-7} mol l^{-1} incubations ($r=-0.98$, $F=54.90$, $p<0.01$, $n=4$) which is explain by the primary production observed with this small DCMU concentration insufficient to completely stop photosynthesis. Such dependence seems to be restricted to low concentrations, no other linear regression being significant greater than 10^{-7} mol l^{-1}. The linear regressions between DCMU efficiency and functional chlorophyll *a* or macrophytobenthic biomass, are not significant (respectively $r=-0.18$, $F=0.71$, $p>0.05$, $n=24$ and $r=-0.37, F=3.30, p>0.05, n=24$). Thus results

Table 1. Abundance of macrophytobenthic species on the study site (1: rare, 2: scattered, 3: abundant, 4: very abundant).

Macrophytes species	Abundance
Cymodocea serrulata	4
Halodule uninervis	4
Halophila ovalis	2
Avrainvillea erecta	1
Caulerpa sertularioides	1
Caulerpa taxifolia	1
Halimeda cylindracea	3
Halimeda discoidea	2
Halimeda incrassata	3
Halimeda macroloba	2
Amansia glomerata	1
Galaxaura sp.	1
Tolypiocladia sp.	3
Padina australis	1

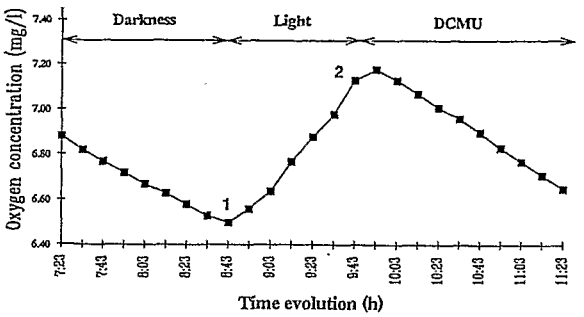

Fig. 1. Example of oxygen concentration evolution (mg l^{-1}) in an enclosure during an incubation. Black covers were removed at 1 and $5\cdot10^{-5}$ mole l^{-1} DCMU were injected at 2.

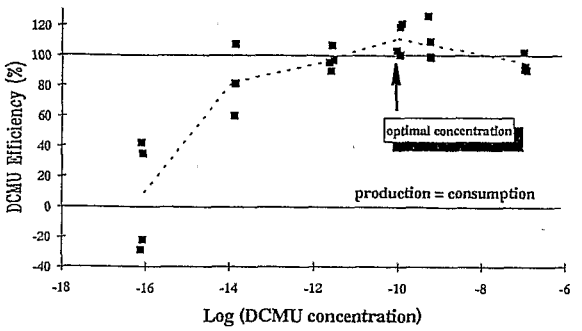

Fig. 2. DCMU efficiency in terms of DCMU concentration used.

Table 2. Friedman test for the comparison of night, dark and DCMU incubations. (Oxygen consumption is expressed in $mg\ m^2\ h^{-1}$).

	DCMU	(rank)	Night	(rank)	Dark	(rank)
Enclosure 1	125	(3)	105	(1)	121	(2)
Enclosure 2	86	(2)	95	(3)	69	(1)
Enclosure 3	102	(3)	77	(1)	84	(2)
S ranks		8		5		5

$X^2 = 2$; $P = 0.528$

H_0: DCMU = Night = Dark cannot be rejected

shown in Fig. 2 are not related to the heterogeneity of phytobenthos. Statistical tests of oxygen flux measurements obtained during DCMU, dark and night incubations (Table 2) are not significantly different and do not reject the hypothesis that the three procedures are equally valid.

Discussion

The present study confirms that inhibition of photosynthesis by DCMU is efficient for *in situ* soft bottom experiments. Legendre *et al.* (1983) were dissatisfied with dark C-14 uptake measurements because this measurement seems to contain an artifactual increase related to prior light exposure. Therefore incubations in the dark would yield different results than incubations in the light plus DCMU. That way they recommend DCMU measurements for correcting C-14 uptake in the light as a solution to the problem based in artifact. The present study, based on oxygen exchange, shows that DCMU treatment is interchangeable with dark or night incubations. That way we think that light enclosure with DCMU is a good method for field metabolism and primary production measurements. Indeed, a simple injection of the inhibitor is more convenient and easier to use than an underwater manipulation of black cover. Moreover, the inhibition seems to be reversible and the chemical has no lasting effects on the ecosystem (Vandermeulen *et al.*, 1972).

Our results suggest that the optimal DCMU concentration to inhibit soft bottom benthos photosynthesis is $5 \cdot 10^{-5}$ mol l^{-1}, even with a dense macrophytic cover. Such a concentration is lower than these found by Vandermeulen *et al.* (1972) in their study on the inhibition of photosynthesis in symbiotic algae. They consider that total inhibition occurred approximately at $5 \cdot 10^{-4}$ mol l^{-1} both *in vivo* on the coral *Pocillopora damicornis* and *in vitro* on isolated zooxanthellae. Compared to their control sample which consists in incubation without DCMU, the metabolic activity in a $5 \cdot 10^{-5}$ mol l^{-1} incubation still represented 17% for *P. damicornis* and 32% for the *in vitro* zooxanthellae. Vandermeulen *et al.* (1972) only dissolved DCMU in seawater, DCMU passage across the cellular membranes being not facilitated. This can explain the 17% of metabolic activity of the coral but it is not sufficient to explain the metabolic activity of the *in vitro* zooxanthellae incubation. In the present study DMSO was used in order to facilitate efficiency of the chemical.

We observed a latent time of about 10 minutes (Fig. 1) before any noticeable effect of the inhibitor. This observation differs from the results of Pohl & Wagner (1972) who noted, using a $3 \cdot 10^{-3}$ mol l^{-1} concentration, that chemical blocks photosynthesis from the moment of addition in an *in vitro* microalgae culture. Latent time must be necessary for the chemical to reach its site of activity on soft bottom benthos.

We can then conclude that the easiest way to measure oxygen consumption as a correction factor for primary productivity studies on soft bottom benthos is a light incubation plus DCMU. We suggest using a $5 \cdot 10^{-5}$ mol l^{-1} DCMU solution as a tool in sediment incubations to suppress rapidly photosynthesis and oxygen production without interfering with animal respiration rates.

If we are to partition metabolic activity of the different benthic compartments, the use of such photosynthesis inhibitor coupled with sediment poisoning should help to understand the benthic food web structure.

Acknowledgements

This research was supported by a French grant INSU/ORSTOM AIP: Récifs coralliens 90N50/0314. We thank the crew of R. V. Alis for their help at sea, S. Bonnet for chemical analyses and P. Laboute for diving assistance. We thank Dr J. Cullen and anonymous reviewer for critical reading of the manuscript.

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