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Validity of net primary productivity estimation by light and dark bottle oxygen technique in tropical inshore waters, with a note on primary productivity of the surf zone at Cochin

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ABSTRACT

Light and dark bottle oxygen technique is widely used to estimate the rate of gross and net photosynthetic production (G.P.P. and N.P.P.) in the marine environment, of which N.P.P. determines the fertility of the sea and is also used for the assessment of potential fishery resources. But in the shallow coastal waters of tropical seas, where organic load is considerably high, bacterial oxidation and reduction processes involving utilisation and release of oxygen tend to interfere significantly in the L - I values often leading to unreliable and unrealistic values of net photosynthetic production.

This paper deals with the validity of N.P.P. estimation in shallow coastal waters of the tropical sea based on 100 primary productivity experiments conducted in the water samples collected from the intertidal surf zone of the sea at Cochin during July 1996 - June 1999. Record of negative values of L - I with higher G.P.P. values and at times L - I values exceeding G.P.P. values in the water samples confirmed the interference of other biochemical (bacterial) consumption and production of oxygen in the estimation of N.P.P. in tropical inshore waters. In such cases, 0.8 (L-D) could be considered as valid estimation for N.P.P.

Introduction

Among the several methods available to assess photosynthetic production in water, Light and Dark bottle Oxygen Technique is the easiest and convenient method in use which provides the rate of gross and net production in the marine environment (Gaarder and Gran, 1927); of which net production is important for the assessment of potential fishery resources in the inshore waters of the sea. But it has been in practice that certain aspects in the methodology adopted in the open seas of higher latitudes to assess the photosynthetic production are directly applied in the tropical waters also, such as (a) using ten light hours for the day; (b) ignoring bacterial interference on the utilisation and release of oxygen; and (c) usage of same photosynthetic

quotient (PQ) to assess the primary production by photosynthesis and chemosynthesis together; which hamper the net photosynthetic production results and ultimately the assessment of potential fishery resources also in the tropical inshore waters. This paper deals with the validity of photosynthetic production estimates by Light and Dark bottle Oxygen Technique in the shallow tropical waters of the sea based on the results obtained from 100 incubation experiments conducted in the water samples collected from the surf zone at Cochin during July 1996 - June '99.

Material and Methods

Data used in the present study were obtained from the results of a total of 100 primary productivity experiments conducted by Light and Dark bottle oxygen method on water samples collected from three stations on monthly basis during July 1996 - June 1999 (Table 1) at a depth of one metre from the intertidal surf zone of the sea at Cochin, *viz.*, Fort Cochin (Station 1) in the north, Manaserry (Station 2) in the middle and Kannamaly (Station 3) in the south covering a distance of 10 km along the coast.

For productivity experiments, water samples were filtered through the zooplankton filter (0.4 mm mesh) fixed at the holed bottom of a plastic bucket (15 litres capacity). While collecting water samples, this bucket was placed tightly inside another plastic bucket of same capacity (which does not have holes at the bottom) and the water samples thus collected manually in the inner bucket was filtered gently by lifting the inner bucket out so that the filtered water (free from zooplankton and other suspended particles) could be collected in the outer bucket. The water sample thus collected in the outer bucket was kept undisturbed for few minutes for uniform distribution of phytoplankters before collecting in the 125 ml BOD glass bottles for incubation experiment.

The filtered water samples thus collected in three 125 ml bottles from each station (without entangling air bubbles) were set for incubation experiment atleast 30 minutes after sampling to bring the micro-organisms physiologically stabilized inside the bottles. Simulated *in situ* experiments were conducted giving uniformly 2.5 hours of incubation in each experiment for the 'L' bottle in light and 'D' bottle in darkness under normal temperature.

Dissolved oxygen values were determined for the Initial (I), Dark (D) and Light (L) bottle samples adopting Winkler's method (Strickland and Parsons, 1972). Gross Photosynthetic Production (G.P.P.) values were determined using L-D values. For the assessment of Net Photosynthetic Production (N.P.P.), oxygen values obtained from L-I and 0.8 (L-D) were compared and the latter was considered as valid.

Dissolved oxygen values thus obtained from the incubation experiments were extrapolated for 12 light hours of the day. Microalgal respiratory loss of oxygen (ml/l) for 24 hours of the day was considered as double the value obtained for 12 light hours, i.e., 2[0.2 (L-D) 12 hrs] = 0.4 (L-D) 12 hrs, while G.P.P. and N.P.P. values obtained for 12 light hours were same for 24 hours of the day (including 12 night hours). Oxygen values obtained from L-D and 0.8 (L-D) per 12 light hours were converted into mg.C using the average photosynthetic quotient (PQ) of 1.25 (1 ml $O_2 = \frac{0.536}{1.25} = 0.429$ mg. C.) to assess the gross and net photosynthetic production respectively in mg. C/l/d = g.C/m^3/d.

Other biochemical (bacterial) release and consumption of oxygen in the samples were assessed from the positive and negative values obtained respectively from [(L-I)-0.8(L-D)] for the 12 light hours of the day.

Results and Discussion

Monthly average values of photosynthetic and bacterial production and consumption of oxygen are depicted in Figure 1. Frequencies of water samples showing bacterial production and consumption of oxygen during photosynthesis are given in Table 2. Stationwise and seasonwise average values of photosynthetic production and bacterial production and consumption rates of oxygen are given in Tables 3 and 4 respectively.

The results revealed that bacterial population interfere significantly in the surf waters at Cochin on the consumption and release of oxygen during photosynthetic experiments by Light and Dark bottle oxygen method leading to unreliable and unrealistic values of L-I in the determination of net photosynthetic production. While the oxygen value obtained in the 'L' bottle (after incubation) includes initial oxygen value ('I' value), photosynthetic production of oxygen, respiratory loss of oxygen by photosynthetic community, consumption and release of oxygen by other biochemical (bacterial) oxidation and reduction processes, that in the 'D' bottle is the resultant of factors cited in 'L' bottle excluding the gross photosynthetic production of oxygen. As a result, L-D value, which is the abbreviation of [(L-I) - (D-I)], would give only the gross photosynthetic production of oxygen assuming that 'D' value serves as the control for the other factors cited above; whereas L-I value excludes the initial oxygen value ('I' value) leaving behind the net oxygen production / consumption value resulting from photosynthetic production, bacterial release, microalgal respiration and bacterial consumption of oxygen during incubation. As a result, L-I values often tend to show unreliable results such as negative values when L-D shows positive values as observed at Station 1 (Fort Cochin) and Station 3 (Kannamaly) during southwest monsoon season (Table 3). Out of 100 data analysed, L-I showed negative values in 17 samples distributed as 5, 10 and 2 numbers for the premonsoon, southwest monsoon and postmonsoon seasons respectively when L-D (G.P.P.) showed positive values (Table 2). Highest negative value of L-I of -0.95 ml 0_2/ 1/12 light hours was recorded in August when the G.P.P. value was 1.23 ml 0_2/1 (Fig. 1 a). Record of negative values of L-I with positive values of G.P.P. in the water samples confirmed the interference of bacterial consumption of oxygen during incubation in primary productivity experiment.

At times, L-I values were found to exceed L-D values indicating release of oxygen by other biochemical (bacterial) reduction processes in the water samples during incubation. Out of 100 data examined, 33 showed bacterial production of oxygen, of which 20 were recorded during premonsoon season (Table 2). In such cases, usage of Phtosynthetic Quotient (PQ) of 1.25 or 1.3 for the tropical inshore waters (Raghuprasad and Nair, 1962) in L-I values which include photosynthetic and bacterial production of oxygen might lead to erroneous estimation of net photosynthetic production.

Thus, the interference of bacterial consumption and production tends to mask the real net photosynthetic production values while using L-I value to assess N.P.P. (Fig. 1 a & c). In such cases, separation of bacterial consumption and production of oxygen from net photosynthetic production of oxygen has become highly essential. Since the microalgal respiratory rate of oxygen during photosynthesis is directly related to photosynthetic production of oxygen (Selvaraj, 1999) and since the microalgal respiratory loss of oxygen is same for the day and night hours (Steemann Nielsen, 1955), determination of average respiratory loss of oxygen during photosynthesis and deduction of the same from gross

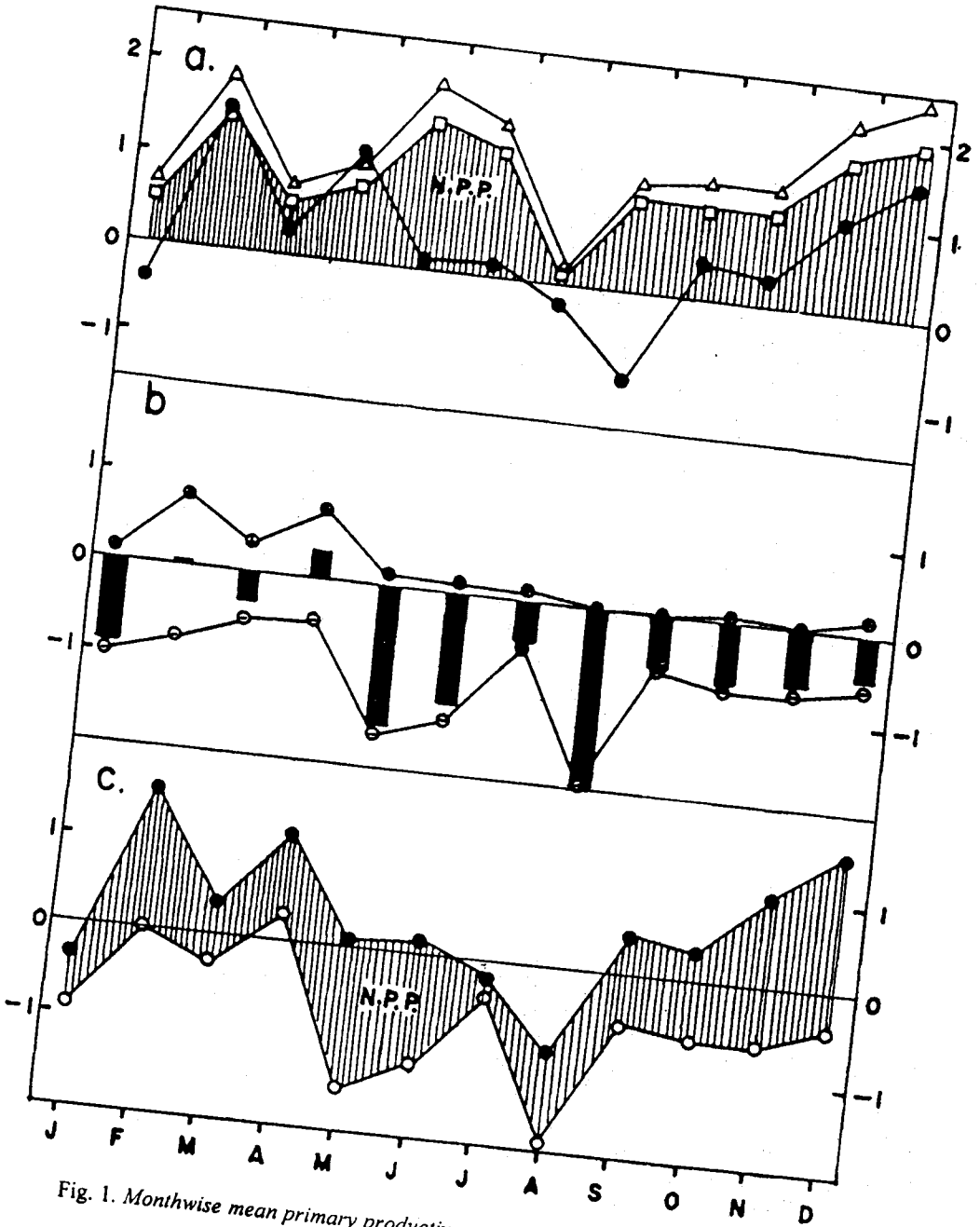

Fig. 1. *Monthwise mean primary productive potential of the surf zone at Cochin.*

a. △ G.P.P. [(L-D) ml O_2/1/12 light hrs.]; □ N.P.P. [0.8 (L-D) ml O_2/1/12 hrs.]; ● (L-I) ml O_2 / 1/12 light hrs.;
b. ⊕ Bact. prod. of O_2 (ml/1/12h); ⊖ Bact. cons. of O_2 (ml/1/12h); ■ Net bact. prod. /cons. (ml O_2/1/12h);
c. O Net bact. prod./cons. (ml O_2/1/12h); ● Net Biochem. prod./cons. (ml O_2/1/12h); ▨ Net photosynth. prod. (ml O_2/1/12h)

Table 1. *Monthwise and seasonwise number of data used for the analysis*

Month	No. of Expts.	Seasons	No. of data	Stations (No. of data) Fort Cochin	Manaserry	Kannamaly
February	10	Premonsoon	37	12	12	13
March	9					
April	9					
May	9	S.W.monsoon	31	11	10	10
June	7					
July	9					
August	6					
September	9	Post monsoon	32	11	11	10
October	9					
November	8					
December	6					
Janauary			100	34	33	33
Total	100					

Table 2. *Seasonwise distribution of data to indicate bacterial production and consumption of oxygen (3 stations pooled together)*

	Premonsoon	S.W.monsoon	Postmonsoon
Water samples with net bact. prod. of oxygen	20	6	7
Water samples with net bact. cons. of oxygen	17	25	25
Water samples with L-I value +ve	26	14	23
Water samples with L-I value -ve	11	17	9
Water samples with L-D (G.P.P.) value nil	6	7	7

photosynthetic production of oxygen, (i.e. L-D) would give the net photosynthetic production of oxygen in the water sample closer to the reality. Selvaraj (1999) has determined the average microalgal respiratory loss of oxygen in bacteria free seawater as 20% of L-D value for the 12 light hours of the day (photosynthetic period) which is very much applicable to the tropical inshore waters of the sea. Accordingly, the validity of average net photosynthetic production of oxygen (N.P.P.) would be 0.8 (L-D) per 12 light hours of the day (80% of G.P.P.). This value was found to be more realistic rather than the L-I value obtained for 12 light hours of the day (Table 3).

Stationwise analysis of data (Table 3) revealed that Station 2 (Manaserry) indicated the highest photosynthetic production trend, followed by Station 1 (Fort Cochin). Seasonwise analysis indicated higher photo-synthetic production during postmonsoon

Table 3. *Stationwise photosynthetic productivity values for the three seasons at Cochin*

Parameters	Fort Cochin (Stn. 1)			Manaserry (Stn. 2)			Kannamaly (Stn. 3)		
	Pre-monsoon	S.W. monsoon	Post-monsoon	Pre-monsoon	S.W. monsoon	Post-monsoon	Pre-monsoon	S.W. monsoon	Post-monsoon
G.P.P. = (L-D) 12 hrs									
a) (ml O_2/l/12 light hrs)	2.399	0.659	1.723	1.366	2.204	1.769	0.681	0.649	1.456
b) (g.C./m^3/d)	1.029	0.283	0.739	0.586	0.946	0.759	0.292	0.278	0.625
N.P.P. = 0.8 (L-D) 12 hrs									
a) (ml O_2/l/12 light hrs)	1.919	0.527	1.378	1.093	1.763	1.415	0.545	0.519	1.165
b) (g.C./m^3/d)	0.823	0.226	0.591	0.469	0.756	0.607	0.234	0.223	0.500
(L-I) 12 hrs									
(ml O_2/l/12 light hrs)	1.533	-0.943	0.286	0.500	0.902	1.117	0.417	-0.477	0.615

(October - January) followed by premonsoon (February - May) season (Table 4). Photosynthetic productivity was relatively low during southwest monsoon (June - September) season.

Based on the percentages of microalgal respiration and N.P.P., other biochemical (bacterial) consumption and production of oxygen could be assessed using [(L-I) - 0.8 (L-D)] value of oxygen per 12 light hours which would be equal to the value obtained from [(D-I) + 0.2 (L-D)] representing 12 dark (night) hours of the day (+ = prod.; — = cons.).

The results revealed that both consumption and production of oxygen by bacterial action are going on simultaneously in the shallow coastal waters and in general, the net bacterial consumption of oxygen was higher than the net bacterial production of oxygen in the intertidal surf zone at Cochin (Fig. 1b; Table 4). According to Steemann Nielsen (1960), in areas where water is polluted, dark fixation by bacteria generally would exceed even the rate of photosynthesis by microalgae. In this context, the higher rate of bacterial consumption of oxygen indicated by negative values of L-I recorded at Station 1 and 3 during southwest monsoon season might be correlated with the low photosynthetic production recorded simultaneously in the surf zone (Tables 3 & 4).

It is well known that huge growth of bacteria starts as soon as seawater sample is enclosed in bottles. According to Steemann Nielsen (1960), the number of bacteria increases by a factor of about two during the first four hours of incubation in temperate waters. But, in the tropical environment, bacterial growth is likely to get doubled inside BOD bottles within 2-3 hours of sampling which influences the biochemical processes also proportionately in the water sample. Hence, for the assessment of bacterial

Table 4. *Seasonwise productivity potential of surf waters at Cochin (average of three stations values)*

Sl. No.	Parameters	Pre-monsoon	S. W. monsoon	Post monsoon	Annual average
1.	Diss. oxygen (ml / l)	3.912	4.035	3.721	3.889
2.	G.P.P. = (L-D) 12 hrs a) (ml 0_2/1/12 light hrs)	1.449	1.113	1.594	1.385
	b) (g.C./m^3/d)	0.622	0.477	0.684	0.594
3.	N.P.P. = 0.8 (L-D) 12 hrs a) (ml 0_2/1/12 light hrs)	1.159	0.890	1.275	1.108
	b) (g.C./m^3/d)	0.497	0.382	0.547	0.475
4.	(L-I) 12 hrs (ml 0_2/1/12 light hrs) (+ = prod. — = cons.)	+0.785	-0.148	+0.610	+0.416
5.	Bact. prod. / cons. of oxygen (ml/1/12 light hrs) = [(L-I) 12 hrs — 0.8 (L-D) 12 hrs] (+ = prod.; — = cons.) a) Bact. prod. of 0_2	0.485	0.070	0.099	0.218
	b) Bact. cons. of 0_2	(-) 0.859	(-) 1.108	(-) 0.764	(-) 0.910
	c) Net bact. prod. / cons. of 0_2 per 12 light hrs.	-0.374	-1.038	-0.665	-0.692

production / consumption of oxygen in the natural environment, 50% of the values obtained from [(L-I) - 0.8 (L-D)] per 12 hours in the incubation experiments could be considered as normal for 12 light hours of the day (during photosynthesis) and the entire value of [(L-I) - 0.8 (L-D)] obtained for 12 light hours could be considered as for 24 hours of the day. Since the present study is focussed on separating net photosynthetic production of oxygen from other biochemical (bacterial) production / consumption of oxygen, actual values of bacterial production / consumption of oxygen obtained in the incubation experiments were taken into account.

It is to be concluded that when L-D indicates zero or negative values in the experiments, it is to be presumed that there is no photosynthetic organisms present in the water sample and hence, the negative values of oxygen usually obtained from L-D and the values (+ or -) of L-I could be due to other biochemical (bacterial) processes going on in the `L' and `D' bottles. The difference in the oxygen values observed between 0.8 (L-D) and L-I could serve as an index to indicate the intensity of organic pollution and bacterial interference in the coastal water samples. If the L-I value falls wthin the range of ± 5% of 0.8 (L-D) value in any water sample, it might

indicate that bacterial interference in that sample would be nil or negligible. In such cases, the water samples are not polluted where L-I values would indicate the net photosynthetic production.

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