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Biogeosciences, 4, 105–114, 2007
www.biogeosciences.net/4/105/2007/

Biogeosciences

Consequences of respiration in the light on the determination of production in pelagic systems

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Received: 31 August 2006 – Published in Biogeosciences Discuss.: 14 September 2006
Revised: 11 December 2006 – Accepted: 24 January 2007 – Published: 26 January 2007

Abstract. Oxygen microprobes were used to estimate Community Respiration (R), Net Community Production (NCP) and Gross Primary Production (GPP) in coastal seawater samples. Using this highly stable and reproducible technique to measure oxygen change during alternating dark and light periods, we show that respiration in the light could account for up to 640% of respiration in the dark. The light enhanced dark respiration can remain elevated for several hours following a 12 h period of illumination. Not including R_{light} into calculations of production leads to an underestimation of GPP, which can reach up to 650% in net heterotrophic systems. The production: respiration (P:R) ratio is in turn affected by the higher respiration rates and by the underestimation of GPP. While the integration of R_{light} into the calculation of P:R ratio does not change the metabolic balance of the system, it decreases the observed tendency, thus net autotrophic systems become less autotrophic and net heterotrophic systems become less heterotrophic. As a consequence, we propose that efforts have to be focused on the estimation and the integration of R_{light} into the determination of GPP and R for a better understanding of the aquatic carbon cycle.

1 Introduction

Fundamental to an understanding of the global carbon cycle is the determination of whether the oceans are net autotrophic or net heterotrophic (del Giorgio et al., 1997; Williams, 1998; del Giorgio and Duarte, 2002). In order to do this, the ratio between photosynthesis (P) and biological respiration (R) is calculated, with P:R>1 indicating net autotrophy and P:R<1 net heterotrophy. Gross Primary Production (GPP) of organic carbon in aquatic systems is generally measured by the fixation of $H^{14}CO_3$ whereas R is determined from the change in oxygen concentration during incubations. However, this approach requires the application of conversion coefficients that vary as a function of several factors including, community composition, nutrient status and the chemical nature of the organic carbon molecules (del Giorgio and Cole, 1998). These problems can be circumvented by measuring GPP and R using the same technique, for example the Winkler technique, which measures changes in oxygen concentration during incubation in the light and dark. Thus, GPP is determined from the sum of net community production (NCP, measured in the light bottle) and R (measured in the dark bottle). This assumes that R in the light is equivalent to that in the dark, an assumption which has already being shown to be problematic (Grande et al., 1989b; Luz et al., 2002). Indeed, it is well known that production and respiration are tightly coupled in aquatic systems (Paerl and Pinckney, 1996) leading to a stimulation of respiration by photosynthesis (Epping and Jørgensen, 1996). The coupling between autotrophs and heterotrophs is generally a function of grazing and the utilization of the DOM excreted by the autotrophs which fuels the respiration activity of the heterotrophs. The composition of exudates and grazing rates can vary as a function of the phytoplankton composition, which is dependent on nutrient supply and other environmental factors. As a consequence, even though this coupling may vary, it should be considered.

The assumption R_{light}=R_{dark} is necessary, because the most commonly used technique (dark/light bottle technique combined with oxygen measurements by Winkler titration) does not allow the determination of respiration occurring in the light. Nevertheless, anecdotal evidence suggests that respiration in the light can be higher than that in the dark (Williams and del Giorgio, 2005), which would result in an underestimation of GPP and R. Light enhanced dark respiration (LEDR) occurs separately from the Mehler reaction, which is not involved in the organic carbon metabolism (Raven and Beardall, 2005). LEDR has been documented in

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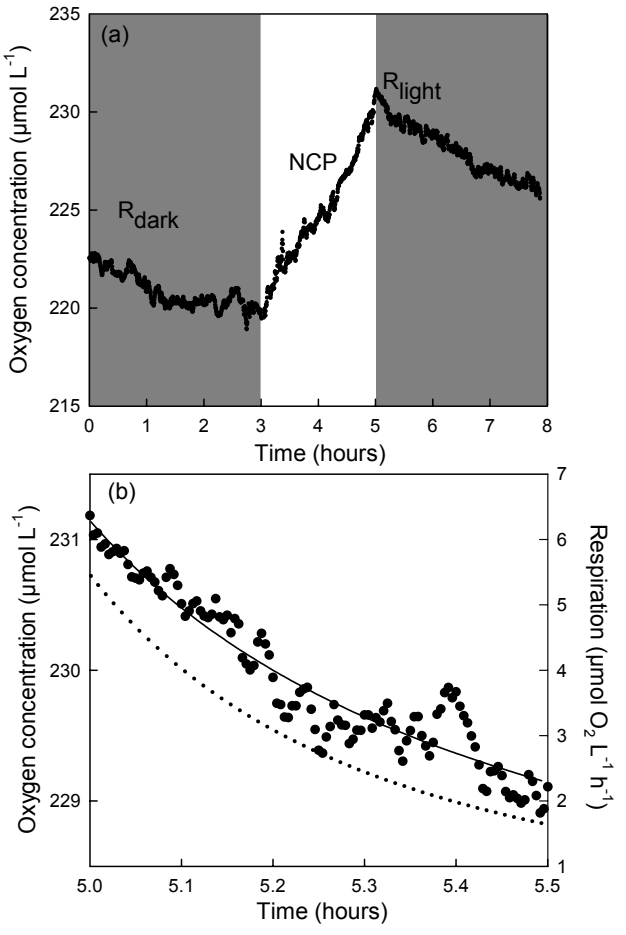

Fig. 1. (a) Oxygen time course during incubation of water from the Southwest lagoon of New Caledonia. Sample was collected in Anse Vata on 30 June 2005. The concentration of chlorophyll *a* was $9\,\mu g\,L^{-1}$. Shaded boxes represent the dark periods and the unshaded box represents the illumination period. **(b)** Oxygen time course for the second period of darkness consecutive to light exposure and exponential decrease (solid line) fitted to the raw data. Respiration (dotted line) was calculated from the first derivative of the fitted exponential decrease curve.

phytoplankton cultures (Grande et al., 1989a; Ekelund 2000, Heraud and Beardall 2002), in lakes (Luz et al., 2002), and in seawater can be 300 to 800% of dark respiration (Grande et al., 1989b). The close coupling between GPP and R has also been extensively studied in phototrophic benthic environments. The use of oxygen microsensors for the determination of both processes permits a precise estimation of light respiration (Epping and Jørgensen, 1996; Epping and Kühl, 2000; Wieland and Kühl, 2000). In this type of environment, light respiration can represent up to 700% of dark respiration (Wieland and Kühl, 2000). Yet, despite the increasing evidence demonstrating the importance of quantifying light respiration, this phenomenon has been rarely examined in oceanic environments (Grande et al., 1989b) and as a consequence its ecological significance has largely been ignored. Therefore, the aims of this work were 1) to estimate light respiration in coastal waters, and 2) to determine the consequences of R_{light}=R_{dark} on the determination of P and P:R ratios.

2 Material and methods

In this study, we collected water in the South West lagoon of New Caledonia in the vicinity of the city of Nouméa. Map of the study area and sampling location can be found in Briand et al. (2004). Oxygen concentration was measured using oxygen microsensors. We used the same protocol as described by Briand et al. (2004). For the estimation of NP, the incubators were exposed to a photon flux density (PFD) of 1000 μmol photons $m^{-2}\,s^{-1}$, which represents the average PFD observed in the first few meters of the water column in the study area. The microprobes (Unisense, Denmark) are designed with an exterior guard cathode (Revsbech, 1989), which results in extremely low oxygen consumption by the electrodes themselves ($4.7–47\times10^{-7}\,\mu$mol $O_2\ h^{-1}$). Probes have a response time shorter than 1 second and a precision of 0.05%. The precision of the oxygen microprobe (0.05%) is equivalent to highly precise Winkler techniques described by Sherr and Sherr (2003). However, as described in Briand et al. (2004), this high precision is counterbalanced by the background noise, therefore we considered a difference of 0.5μM as significant to measure NCP or R rates. This highly precise and reproducible technique permits the continuous measurement of oxygen concentration during incubations (Briand et al., 2004). By exposing the sample to dark and light cycles (Fig. 1), it is possible to estimate within the same sample, dark respiration (R_{dark}), NP and the effect of light on R determined just after light exposure (R_{light}). After switching off the light, oxygen concentration showed an exponential decrease with time (Fig. 1). Therefore for the determination of R_{light}, we fitted an exponential decay to the raw data, and respiration was then calculated from the first derivative of the fitted equation. The value within the first few minutes consecutive to darkness was assumed to represent the best estimate of the respiration that occurs in the light as previously described by Falkowski et al. (1985).

Oxygen consumption estimates using the Winkler method are usually performed in replicates. In our study, the availability of the equipment to measure oxygen concentration prevented us from estimating O_2 consumption systematically in replicates. However, on several occasions, we checked reproducibility in duplicate water samples. We observed that the time course of oxygen concentration is very similar in two samples of the same station (Fig. 2) as described by Briand et al. (2004).

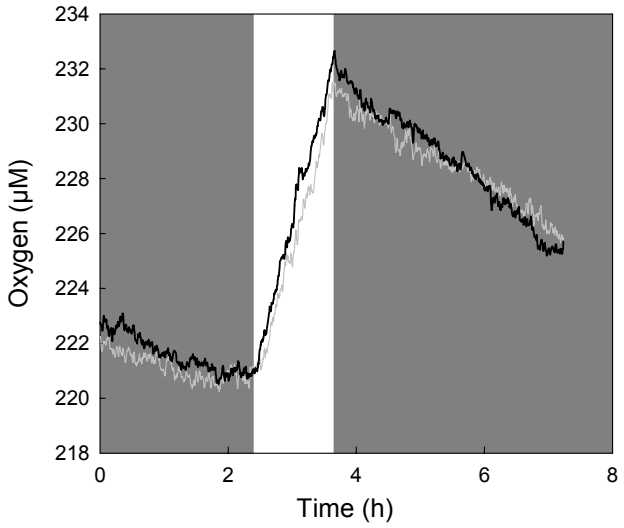

Fig. 2. Oxygen concentration during dark and light incubation in duplicate. Sample was collected in Anse Vata on 29 June 2005. Shaded boxes represent the dark periods and the unshaded box represents the illumination period.

Usually oxygen production (GPP) determined with the light and dark bottle technique is calculated from the following equation:

$$\text{GPP=NCP} + |\text{R}_{\text{dark}}| \quad (1)$$

with $|\text{R}_{\text{dark}}|$ representing the absolute value of R, also known as community respiration, measured in the dark and NCP, the net community production. With this approach it is assumed that R measured in the dark is equivalent to that in the light.

In this study, GPP was computed from NCP and R_{light} using the following equation:

$$\text{GPP=NCP} + \left|\text{R}_{\text{light}}\right| \quad (2)$$

with NCP and $\left|\text{R}_{\text{light}}\right|$ being measured as described above (Fig. 1). Hereafter, GPP_{dark} represents the production when R_{dark} is used in the calculation, as in the light and dark bottle method, and $\text{GPP}_{\text{light}}$ when R_{light} is used. Therefore for the same water sample, we distinguish between GPP estimates of the traditional method (GPP_{dark}) that assumes that R_{light}=R_{dark}, from GPP estimates that take into account light respiration ($\text{GPP}_{\text{light}}$). Consequently, we estimated the effects of R_{light} on the determination of P by comparing GPP_{dark} and $\text{GPP}_{\text{light}}$ using the following equation:

$$\text{Underestimation of GPP}\,(\%) = \frac{(\text{GPP}_{\text{light}} - \text{GPP}_{\text{dark}})}{\text{GPP}_{\text{dark}}} \times 100 \quad (3)$$

The P:R ratio which describes the trophic status of the system is calculated from daily rates of GPP and R. Daily rates taking into account R_{light} were calculated from hourly rates using the sum of R_{dark}+R_{light} considering 12 h darkness and 12 h light, and $\text{GPP}_{\text{light}}$ by considering 12 h light. In order to estimate the effects of the assumption R_{light}=R_{dark} on the estimation of P:R ratios, we also calculated daily rates from the hourly rates of GPP_{dark} and R_{dark} considering 12 h of light and 24 h of darkness, respectively. This latter calculation is commonly used for the light and dark bottle method. For the same water sample, we therefore distinguished between P:R ratios calculated from Eq. (1), that do not take into account R_{light} and those calculated from Eq. (2) that do take into account R_{light}. For all the experiments, the determination of R_{dark}, NCP and R_{light} was achieved within a maximum incubation time of 8 h in order to decrease bottle effects, which can result in changes in biomass and community structure as described by Gattuso et al. (2002).

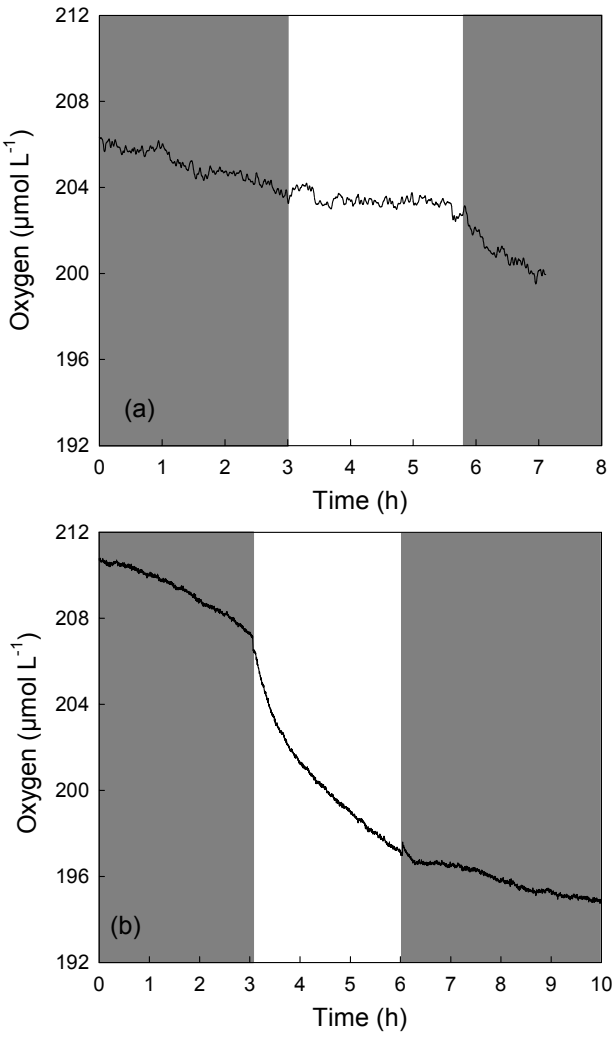

Fig. 3. Oxygen time course during incubation of water from Southwest lagoon of New Caledonia. Shaded boxes represent the dark periods and unshaded box represents the illumination period. Samples were collected in N12 on 16 March 2005 **(a)** and in M41 on 11 May 2006 **(b)**, concentration of chlorophyll *a* was 0.75 μg L^{-1} (a) and 0.27 μg L^{-1} (b). See Table 1 for rate values.

Table 1. Respiration and production for different natural water samples. The light regime was as follows for all experiments: 2–3 h dark/2–3 h light/2–4 h dark. Processes are expressed in μmol O_2 L^{-1} h^{-1}, Chlorophyll *a* in μg L^{-1}. To avoid a negative GPP_{dark} (see Eq. 1), we assume that R_{light} has to be at least equal to NCP. In this case, a precise estimation of R_{light} is not possible, therefore GPP_{light} was not calculated.

Sample	Chl *a*	T °C	NCP	R_{dark}	GPP^{a}_{dark}	R_{light}	GPP^{b}_{light}	R_{light} (% of R_{dark})	Underestimation[c] of GPP (%)
M41 11/05/2006	0.27	24°C	−2.12	1.14	−0.98	2.12	n.d.	186	n.d.
M33 11/05/2006	0.67	24°C	−1.12	0.41	−0.71	1.12	n.d.	273	n.d.
N12 16/03/2005	0.75	26°C	−0.19	0.76	0.57	4.46	4.27	587	649
N12 30/03/2005	0.98	26°C	−0.20	0.82	0.62	1.85	1.65	225	165
N12 09/06/2005	2	22°C	−2.40	0.99	−1.41	2.40	n.d.	242	n.d.
Anse Vata 10/05/2006	1.8	24°C	−0.10	0.46	0.36	1.43	1.33	310	268
Anse Vata 27/03/2006	3	26°C	0.28	0.50	0.78	1.39	1.67	279	114
Anse Vata 09/05/2006	3.3	24°C	0.85	0.65	1.50	2.10	2.95	323	97
Anse Vata 30/06/2005	9	22°C	4.77	0.85	5.62	5.41	10.18	636	81
Anse Vata 14/03/2006	10	26°C	18	1.80	19.80	3.06	21.06	170	6
Anse Vata 29/06/2005	13	22°C	9.49	0.42	9.91	2.08	11.57	495	17
Anse Vata 17/06/2005	45	22°C	23.13	1.14	24.27	5.96	29.09	523	20

[a] GPP_{dark} represents the Production when R_{dark} is used in the calculation (see Eq. 1).
[b] GPP_{light} represents the Production when R_{light} is used in the calculation (see Eq. 2).
[c] Underestimation of P when R_{dark} is used to determine P instead of R_{light} (see Eq. 3).
n.d.: Not determined.

Chlorophyll a was measured on samples collected on GF/F filters using the method of Jeffrey and Humphrey (1975). The filters were frozen (−20°C) until measurement which was always within 72 h and generally within 24 h.

3 Results

3.1 Determination of R_{light}

It is important to note that the continuous measurement of oxygen concentration does not allow a direct determination of respiration in the light itself. However, the fast response of the oxygen microelectrode (less than 1 s) means that we can precisely measure the respiration rate immediately consecutive to the onset of darkness as previously described by Falkowski et al. (1985). We applied this procedure in different water samples covering a range of chlorophyll *a* (Chl *a*) concentrations from 0.27 μg L^{-1} to 45 μg L^{-1} (Fig. 3). Metabolic processes (hourly rates) determined for the different water samples are listed in Table 1. On some occasions, we observed that the value of NCP was more negative than R_{dark}, as it is illustrated in Fig. 3b. According to Eq. (1), the derived GPP value is negative, which is theoretically impossible (Table 1). For example, for the water sample M41 (11/05/2006), NCP and R_{dark} values were −2.12 and 1.14 μmol O_2 L^{-1} h^{-1}, respectively. According to Eq. (1), GGP_{dark} would equal −0.98 μmol O_2 L^{-1} h^{-1}. For these particular cases, R_{light} was not estimated from the O_2 changes consecutive to darkness. We consider that R_{light} needs to be greater than NCP in order to get a positive value for GPP. Therefore, for this water sample, R_{light} was assumed to be at least equal to 2.12 μmol O_2 L^{-1} h^{-1}. In all samples, respiration was stimulated by light and R_{light} represented up to 636% of R_{dark}. Taking into account the in situ hourly rates (Table 1), we calculated that on average R_{light} represented 354% of R_{dark}. It is also interesting to note that the percentage of stimulation was not dependent upon Chl *a* concentration. The underestimation of GPP when R_{light} was not taken into account reached up to 649% in net heterotrophic conditions (i.e., NCP<0), whereas in net autotrophic conditions (NCP>0) underestimation was less important despite R_{light} values of 636% relative to R_{dark} (Table 1).

3.2 Respiration in the light and P:R ratios

From the hourly rates we calculated daily rates (assuming 12 h dark and 12 h light) of GPP and R in order to determine the P:R ratio. Results are presented in Table 2. The daily rates of R when R_{light} is taken into account are on average more than twice the daily rates of R when it is assumed that R_{light}=R_{dark}. All the experiments were performed under saturating light conditions and previous measurements conducted at the sampling sites have shown that phytoplankton photosynthesis is subject to saturating irradiances during 80–90% of the day. As a consequence we assume that the hourly rates are representative of the prevailing conditions occurring in the 12 h of light. From measurements of R_{light} in a phytoplankton culture under different light conditions (data not shown), we have estimated the error introduced by using a

Table 2. Respiration and production ratios for different natural water samples. Processes are expressed in μmol O_2 L^{-1} d^{-1}. Hourly rates are from Table 1. When NCP (hourly rates, Table 1) was more negative than R_{dark} resulting to a negative value for GPP_{dark} (see Eq. 1), we assumed that R_{light} has to be at least equal to NCP in order to get a positive value for GPP. In this case a precise estimation of R_{light} is not possible, therefore GPP_{light} was not calculated.

Sample	NCP	R_{dark}	GPP^{a}_{dark}	R_{light}	GPP^{b}_{light}	P_{dark}:R_{dark}	P_{light}:R_{light}	Over- or Underestimation of P:R (%)[c]
M41 11/05/2006	−39.1	27.4	−11.8	39.1	n.d.	−0.43	n.d.	n.d.
M33 11/05/2006	−18.4	9.8	−8.5	18.4	n.d.	−0.87	n.d.	n.d.
N12 16/03/2005	−11.4	18.2	6.8	62.6	51.2	0.38	0.82	−118
N12 30/03/2005	−12.2	19.7	7.4	32.0	19.8	0.38	0.62	−63
N12 09/06/2005	−40.7	23.8	−16.9	40.7	n.d.	n.d.	n.d.	n.d.
Anse Vata 10/05/2006	−6.7	11.0	4.3	22.6	15.9	0.39	0.70	−80
Anse Vata 27/03/2006	−2.6	12.0	9.4	22.7	20.1	0.78	0.88	−13
Anse Vata 09/05/2006	2.4	15.6	18.0	33.0	35.4	1.15	1.07	7
Anse Vata 30/06/2005	47.0	20.4	67.4	75.1	122.1	3.31	1.63	51
Anse Vata 14/03/2006	194.4	43.2	237.6	58.3	252.7	5.50	4.33	21
Anse Vata 29/06/2005	108.8	10.1	118.9	30.0	138.8	11.80	4.63	61
Anse Vata 17/06/2005	263.9	27.4	291.2	85.2	349.1	10.64	4.10	62

[a] GPP_{dark} represents the Production when R_{dark} is used in the calculation (see Eq. 1).
[b] GPP_{light} represents the Production when R_{light} is used in the calculation (see Eq. 2).
[c] Over or underestimation of P:R ratio when R_{light} is not taken into account.
Negative values indicate an underestimation whereas positive values indicate an overestimation.
n.d.: Not determined

fixed PFD instead of a variable PFD for the calculation of daily rates of R_{light} and GPP. The resultant error is of the order of 10%. Since our method takes into account R_{light}, we can calculate the error introduced in the P:R ratio estimation when R_{light} is not taken into account in the estimation of GPP and in the determination of the daily rates of R. Under net heterotrophic conditions (i.e. NCP<0), P:R ratios were underestimated. For example for the station N12 (16/03/2005), we calculated a P:R ratio of 0.82, indicating that the system is net heterotrophic. With the assumption that R in the light is equivalent to that in the dark, the P:R ratio would be equal to 0.38, which represents an underestimation of 116% relative to the value estimated when it is assumed that R_{light}=R_{dark} ((0.38–0.82)/0.38×100). On the other hand, under net autotrophic conditions (NCP>0), P:R ratios are overestimated when we assume that R_{light}=R_{dark}. For example, for the station Anse Vata (30/06/2005), we calculated a P:R ratio of 1.63 (Table 2), however, if we assume that R_{light}=R_{dark}, P:R ratio would be equal to 3.31, which represents an overestimation of 51% ((3.31–1.63)/3.31×100).

3.3 Time of light exposure and R_{light}

Depending on the time exposed to light, R_{light} can remain higher than R_{dark} for up to several hours after the onset of darkness. Figure 4a shows the variations of R_{dark} as a function of time after two hours of light exposure. In this sample, R_{light} measured immediately (within 5 minutes) after the onset of darkness was three fold greater than $R_{dark.}$ After four hours of darkness, respiration decreased exponentially to reach R_{dark} (Fig. 4c). Figure 4b shows the variations of oxygen concentration as a function of time for a seawater sample exposed for 12 h in the light and 12 h in the dark. In order to estimate dark respiration, the sample was initially exposed to darkness for two hours. After 12 h of light, oxygen decreased exponentially in the dark. Respiration calculated from the first derivative of the exponential decay fitted to the oxygen concentration also decreased exponentially (Fig. 4d). Just after darkness, R_{light} represented 800% of R_{dark}, and the initial R_{dark} value was reached after 10 h of darkness. Thus, while the stimulation of R by light can be observable for several hours after the onset of darkness, we propose that the determination of R_{light} should be done immediately after the onset of darkness in order to have a significant change in oxygen concentration (i.e. 0.5 μM).

Obviously, the incubation procedure does not accurately mimic in situ conditions as changes in biomass and community structure are likely to occur during this 26 h incubation (Gattuso et al., 2002; Briand et al., 2004). However, we can calculate daily rates of R by integrating the exponential decrease of R during the dark period consecutive to light exposure, and adding this value to R_{light}, assuming that R_{light} is constant during the illumination period. This leads to an R_{dark} value of 77 μmol O_2 L^{-1} for 12 h and an R_{light} value of 180 μmol O_2 L^{-1} for 12 h. Consequently, daily respiration is equal to 257 μmol O_2 l^{-1} d^{-1}. This value

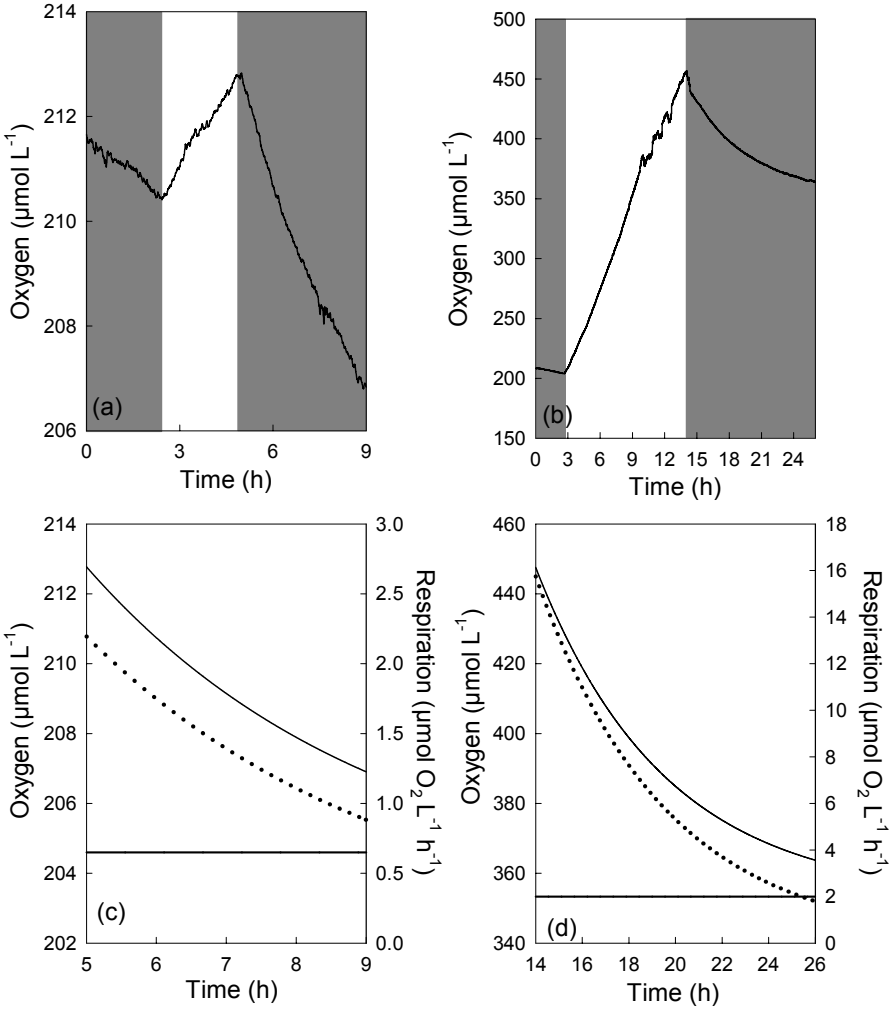

Fig. 4. (a) and **(b)** Oxygen time course during incubation of water from Southwest lagoon of New Caledonia. Shaded boxes represent the dark periods and unshaded box represents the illumination period. **(c)** and **(d)** Exponential decrease (solid lines) fitted to the raw data of oxygen concentration in the dark period consecutive to light exposure. Respiration (dotted lines) was calculated from the first derivative of the fitted exponential decrease curve. The horizontal line represents the initial R_{dark}. For (a) and (c), sample was collected in Anse Vata on 9 May 2006. The concentration of chlorophyll *a* was 3.3 μg L^{-1}. For (b) and (d) sample was collected in Anse Vata on 30 March 2006. The concentration of chlorophyll *a* was 20 μg L^{-1}.

is much greater than the daily R of 53 μmol O_2 L^{-1} d^{-1} calculated from the initial dark value assuming that R_{light}=R_{dark}. Similarly, we can calculate GPP and then determine the P:R ratio, taking into account R_{light} and the daily rates of GPP and R. When R_{light} was taken into account, GPP was equal to 453 μmol O_2 L^{-1} d^{-1}, leading to a P:R ratio of 1.76. This has to be compared with a GPP value of 290 μmol O_2 L^{-1} d^{-1} and a P:R ratio of 5.47, when R_{light}=R_{dark} is assumed. This represents an underestimation for GPP of 56% and an overestimation for P:R ratio of 68%.

The long tailing off of R_{light} cannot be ignored in respiration measurements. It is obvious that initial sampling time is extremely important as respiration measurements conducted on samples previously exposed to sunlight would have a higher R than those collected at sunrise.

4 Discussion

From oxygen monitoring in phytoplankton cultures with an oxygen macroprobe, Falkowski et al. (1985) have shown that light respiration can be still measured a few minutes after the onset of darkness, and this was confirmed by Weger et al. (1989), using the technique of ^{18}O isotopic fractionation. In our study we used the same approach as described by Falkowski et al. (1985) to estimate R_{light} in natural field water samples. The range of hourly rates of R_{light} is of the same order of magnitude as those measured in natural field water samples using the stable ^{18}O technique (Grande et al., 1989a; Luz et al., 2002). With this latter technique, respiration in the light can be directly measured, and it has been shown that in natural lake communities, R_{light} can be up to 5 fold R_{dark}

(Luz et al., 2002). These results are similar to those of our study where on average R_{light} represented more than 350% of R_{dark}. Using the same isotopic fractionation technique, Grande et al. (1989b) have shown that in the North Pacific Gyre, R_{light} can be up to 8 times greater than R_{dark}. This strong stimulation of respiration in the light leads to an underestimation of GPP of more than 135% when it is assumed that R_{light}=R_{dark} (Grande et al., 1989b). A similar range for the underestimation of GPP was obtained in our study (see Table 1). Respiration in the light has also been estimated in phytoplankton cultures using both techniques (Grande et al., 1989a; Ekelund, 2000; Heraud and Beardall, 2002). Studying several naturally abundant marine phytoplankton species, Grande et al. (1989a) have shown that R_{light} was often greater than R_{dark} with values up to 10 fold more than R_{dark} in some cases.

Consequently, despite increasing evidence that R_{dark} is not equal to R_{light}, the ecological consequences of the assumption R_{light}=R_{dark} continue to be ignored, probably because light respiration in natural field samples has been observed only using isotopic fractionation (e.g. Luz et al., 2002). The main drawback of this method is that it is technically demanding and requires measuring oxygen isotopes and estimating fractionation factors for a number of processes related to biological oxygen consumption and production as well as abiotic exchanges of oxygen (Luz et al., 2002). In this study, we showed that respiration in the light can be quantified in natural field seawater samples using a simple, reproducible and accurate methodology that allows the precise estimation of respiration in coastal waters under a wide range of Chl *a* concentrations from oligotrophic to eutrophic conditions (Briand et al., 2004). The continuous measurement of oxygen during incubations shows that changes in oxygen concentration are not always linear with time (Briand et al., 2004), especially during the change of light conditions (Figs. 1 and 3). In order to precisely estimate activities during this transient state characterizing the change from light to dark, we used a modeling approach, by fitting an exponential decay to the raw data. In benthic phototrophic environments, continuous measurements of oxygen from light to dark or dark to light allow the precise determination of production or respiration (Revsbech and Jørgensen, 1983, 1986). The modeling of these transient states has been developed to better estimate the dynamics of respiration or production rates (Lassen et al., 1998; Epping et al., 1999).

The degree of underestimation of GPP is highest under net heterotrophic conditions, with values reaching up to 650% (Table 1). The underestimation is less pronounced under net autotrophic conditions with values of 6 to 20%. This is intuitive as under net heterotrophy, R is the dominant process, whereas during periods of net autotrophy, GPP is the dominant process. Thus, the error introduced in the calculation of GPP when R_{light} is not taken into account has a greater impact during periods of net heterotrophy than it has during periods of net autotrophy. In our field study, on some occasions we observed NCP rates more negative than R_{dark} rates during net heterotrophic conditions (Table 1, Fig. 3b). According to Eq. (1), this results in negative values for GPP, which is theoretically impossible. Similar phenomena have been observed in the ALOHA station in the Central Pacific by Williams et al. (2004), where negative values of GPP were reported in deep waters under net heterotrophic conditions. In our study (Table 1), we consider that R_{light} needs to be at least equal to NCP in order to get a positive value for GPP. For example, for the sample collected in M41 on 11 May 2006 (Fig. 3b) this results in R_{light} that is 186% higher than R_{dark}. This value should be considered as conservative as the percentage of stimulation is probably higher (GPP$>$0). Even if a precise estimation of R_{light} cannot be achieved in this case, the strong decrease observed just after light exposure (Fig. 3b) clearly indicates that respiration was strongly stimulated by the onset of illumination thus suggesting a tight coupling between respiration and production. The oxygen changes presented in Fig. 3b result in a physiologically impossible negative value for GPP when assuming R_{light}=R_{dark}. This has been observed under several occasions for net heterotrophic waters (see Table 1). Obviously, respiration measured in this study represents the community respiration, i.e. sum of phytoplankton respiration and heterotrophic respiration. Therefore, both components of this community respiration might be stimulated by light, including the Mehler reaction for photosynthetic phytoplankton as well as the stimulation of bacterial respiration by freshly produced photosynthetic products. Tight coupling between both microbial compartments has been largely documented (Epping and Jørgensen, 1996; Paerl and Pinckney, 1996) and the stimulation of bacterial production under light conditions has been observed in pelagic systems (Church et al., 2004). In benthic environments the addition of limiting compounds for photosynthetic production resulted in a concomitant stimulation of respiration, indicating that heterotrophs are strongly dependent upon phototrophs for carbon supply (Ludwig et al., 2006).

The stimulation of R in the light affects the determination of the P:R ratio. As we have shown, an error in the determination of R leads to an error in the calculation of GPP. During net heterotrophy, we find P:R ratios that are higher than those estimated when R_{light} is not taken into account (Table 2). In contrast, under net autotrophic conditions, P:R ratios are lower than those estimated using only R_{dark}. Moreover, as the system becomes increasingly autotrophic or heterotrophic, the difference between both P:R estimations becomes more marked. Of course, the integration of R_{light} into the calculation of P:R ratio would not change the metabolic balance of the system (as indicated by NP), however it will decrease the observed tendency, in other words, net autotrophic systems become less autotrophic and net heterotrophic systems become less heterotophic.

Clearly, our average hourly value of R_{light} cannot be considered representative of all pelagic systems, however it is

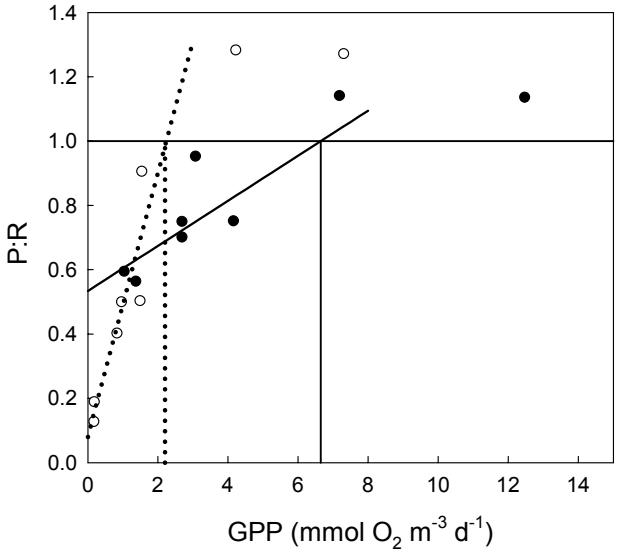

Fig. 5. Relationship between P:R ratio and Production for the determination of the threshold value of P separating net heterotophic from net autotrophic communities. Open symbols and dotted line data from Duarte and Agusti (2005). Closed symbols data from Duarte and Agusti corrected with $R_{light}=3.54\times R_{dark}$. The lines represent the fitted initial linear slope of the relationship. Dotted line: Duarte and Agusti (2005): $P:R=0.41\times P+0.08$, $R^2=0.75$, $p<0.05$. Solid line: Duarte and Agusti (2005) corrected: $P:R=0.09\times P+0.53$, $R^2=0.77$, $p<0.05$. The vertical lines represent the threshold value of P for a P:R=1.

an interesting exercise to use it to estimate the potential error in the calculations of GPP (Eqs. 1 and 2), the calculations of daily R, and hence the P:R ratio, when $R_{light}=R_{dark}$ is assumed. We performed this exercise with literature data collected in coastal waters exhibiting similar levels of Chl *a* concentration as those measured in this study. From our field experiments we calculated that hourly rates of R_{light} represent on average 354% of R_{dark}. We used this average value to test the underestimation of GPP of coastal ecosystems in the literature according to Eq. (3).

For example, Smith and Kemp (2001) have estimated that Chesapeake Bay is net autotrophic in spring, summer and fall with median P:R ratios of 3.33, 1.94, and 2.82, respectively. If we use our average R_{light} of 354% R_{dark}, we estimate for the same periods P:R ratios of 2.05 (spring), 1.42 (summer), and 1.82 (fall), which represents an overestimation of P:R ratios in the literature of 38%, 27%, and 35%, respectively. Furthermore, Caffrey (2004) has reported that most US estuaries are net heterotrophic, for example in Rookery Bay, the annual average P:R ratio is equal to 0.34. However, when we take into account R_{light}, calculated as 354% R_{dark}, we find a P:R ratio of 0.70, pointing to an underestimation of 106 %.

The use of P:R ratios to estimate whether a system is net autotrophic or net heterotrophic is problematic since the estimation of GPP and R is uncertain. NCP represents a more realistic balance between GPP and R and its estimation is less inexact due to the fact that it takes into account R_{light}. However, although NCP is a good estimate of whether or not a system is in metabolic balance, it provides no information about the degree of trophy of the system. Therefore, despite the uncertainties regarding GPP and R estimations, numerous studies on the carbon cycle have compared GPP and R rates on a global scale to define the trophic status of pelagic systems (see the review of Duarte and Agusti, 1998). Here, P:R ratios are calculated to estimate the percentage of net heterotrophy or net autotrophy (sensu chapter of the mass balance calculation in Robinson and Williams, 2005). For example, using the light and dark bottle technique applied in experimental mesocosms containing Chl *a* concentrations that are close to those we observed in our field experiments, Duarte and Agusti (2005) have estimated the threshold value for GPP, which separates net heterotrophic from net autotrophic communities in Southern Ocean. Assuming $R_{light}=R_{dark}$, they calculated a threshold value for P of 2.2 mmol O_2 m^{-3} d^{-1} (Fig. 5). If R_{light} is integrated into the calculation ($R_{light}=3.54\times R_{dark}$), we estimate that the threshold for GPP is 6.5 mmol O_2 m^{-3} d^{-1}. Community respiration and production can also be compared to other estimates of carbon production or utilization such as bacterial production or ^{14}C primary production to determine the interrelations between the different metabolic pathways involved in the carbon cycle on a global scale (e.g. Del Giorgio et al., 1997). In such cases, the uncertainties regarding the estimation of P and R will also affect the comparison of production and/or respiration with other estimates of carbon production and utilization.

5 Conclusions

Respiration represents a major area of ignorance in our understanding of the global carbon cycle (see the preface of Del Giorgio and Williams, 2005). The majority of studies of respiration in aquatic ecosystems have employed the Winkler technique (Williams and del Giorgio, 2005) despite the fact that this method cannot measure respiration in the light. As a consequence, this methodological problem has been circumvented by assuming $R_{light}=R_{dark}$. Since its first application in seawater by Gran in 1917 to measure oxygen flux, the Winkler technique has become the reference technique for oxygen measurements even though alternative methods are now available that allow for the determination of R_{light}. In our study, we show that R_{light} should not be ignored for the determination of GPP nor for the estimation of the daily rate of R, at least in coastal environments. In order to better estimate the contribution of pelagic systems to the global carbon cycle efforts have to be made to take into account the tight coupling between production and respiration and its consequences in the estimation of both processes.

Acknowledgements. We thank E. Smith, D. Ponton, and C. Payri for their invaluable comments on an earlier version of the manuscript. This study was supported by grants from the French "Programme National Environnement Côtier (PNEC)" and the French "Institut de Recherche pour le Développement (IRD)".

Edited by: A. Boetius

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