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

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Abstract

Oxygen microprobes were used to estimate Respiration (R), Net Production (NP) and Production (P) in coastal sea water 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 represent up to 640% of respiration in the dark. This light enhanced dark respiration can remain elevated for several hours following a 12 h period of illumination. The non inclusion of R_{light} into calculations of production leads to an underestimation of P, which can reach up to 650% in net heterotrophic systems. The P:R ratio is in turn affected by the higher respiration rates and by the underestimation of P. While the integration of R_{light} in to 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 P 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. Production (P) 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). By measuring P and R using

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the same technique, for example the Winkler technique, which measures changes in oxygen concentration during incubation in the light and dark, these problems can be circumvented. Thus, P is determined from the difference between net production (NP, 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 subject to caution (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). Although, the strength of this coupling between autotrophic and heterotrophic compartments will vary as a function of the organic matter and nutrient concentration, it cannot be considered as negligible.

The assumption that $R_{light}=R_{dark}$, is made 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 is higher than that in the dark (Williams and del Giorgio, 2005), which would result in an underestimation of P and R. Light enhanced dark respiration, separate from the Mehler reaction, which is not involved in the organic carbon metabolism (Raven and Beardall, 2005), has been largely documented in phytoplankton cultures (Grande et al., 1989a; Ekelund, 2000; Heraud and Beardall, 2002), in lakes (Luz et al., 2002), and in seawater (Bender et al., 1987; Grande et al., 1989b) and, can be 300 to 800% of dark respiration (Grande et al., 1989b). The close coupling between P and R has also been extensively studied in phototrophic benthic environments where the methodology (oxygen microsensors) used for the determination of both process 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 mounting 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, firstly to estimate light respiration in coastal waters and secondly to determine the consequences on the determination of P and P:R ratios of the assumption that R in the light is equivalent to that in the dark.

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 ($4.7–47\times10^{-7}$ μmol O_2 h^{-1}). Probes have a response time shorter than 1 s and a precision of 0.05%. The precision of 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 0.5 μM as significant to measure NP 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}. 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 1s) means that we can precisely measure the respiration rate immediately consecutive to the onset of darkness as previously described by Falkowski

et al. (1985). 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. In this water sample, R_{light} was 5.41 μmol O_2 L^{-1} h^{-1} (Fig. 1B), corresponding to more than 630% of R_{dark}.

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

$$P = NP + |R_{dark}| \quad (1)$$

where $|R_{dark}|$ represents the absolute value of R, also known as community respiration, measured in the dark and NP the net production. With this approach it is assumed that R measured in the dark is equivalent to that in the light.

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

$$P = NP + \left|R_{light}\right| \quad (2)$$

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

$$\text{Underestimation of P (\%)} = \frac{(P_{light} - P_{dark})}{P_{dark}} \times 100 \quad (3)$$

The P:R ratio which describes the trophic status of the system is calculated from daily rates. Daily rates taking into account R_{light} were calculated from the hourly rates using

the sum R_{dark} + R_{light} considering 12 h darkness and 12 h light and P_{light} considering 12 h light. In order to estimate the effects of the assumption that R in the light is equivalent to that in the dark on the estimation of P:R ratios, we also calculated daily rates from the hourly rates of P_{dark} and R_{dark} considering 12 h of light and 24 h of darkness, respectively. This latter calculation is that which is 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}, NP 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).

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

We applied this procedure in different water samples covering a range of chlorophyll *a* (Chl *a*) concentrations from 0.27 $\mu g\,L^{-1}$ to 45 $\mu g\,L^{-1}$ (Fig. 2). R_{dark}, R_{light}, NP were estimated from the oxygen changes as a function of time in light and dark conditions, and P_{dark} and P_{light} were calculated according to Eq. (1) and Eq. (2), respectively (Table 1). Thus, we used R_{light} to determine P_{light} (Eq. 2) and then compared these values with P_{dark} obtained when R_{dark} was used (Eq. 1) instead of R_{light}. When NP (in absolute values) was greater than R_{dark}, resulting in physiologically impossible negative values of P, we consider that R_{light} needs to be greater than NP in order to get a positive value for P. 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 P when R_{light} was not taken into account reached up to 649% in net heterotrophic conditions (i.e., NP<0), whereas in net autotrophic conditions (NP>0) underestimation was less important despite R_{light} values of 636% (Table 1).

From the hourly rates we calculated daily rates (assuming 12 h dark and 12 h light) of P 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 as much as the daily rates of R when it is assumed that respiration in the dark is equivalent to that in the light. All the experiments were performed under saturating light conditions; as a consequence we assume that the hourly rates are representative of the prevailing conditions occurring in the 12 h of light. We have estimated the error introduced by using a fixed PFD instead of a variable PFD for the calculation of daily rates of R_{light} and P and 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 P and the determination of the daily rates of R. Under net heterotrophic conditions (i.e. NP<0), P:R ratios were underestimated. For example for the station N12 (16 March 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 in the light is equivalent to that in the dark ((0.38–0.82)/0.038×100). On the other hand, under net autotrophic conditions (NP>0), P:R ratios are overestimated when we assume that R in the light and R in the dark are equivalent. For example, for the station Anse Vata (30 June 2005), we calculated a P:R ratio of 1.63 (Table 2), however, if we make the assumption that R in the light is equivalent to that in the dark, P:R ratio would be equal to 3.31, which represents an overestimation of 51% ((3.31–1.63)/3.31×100).

It has been shown (Falkowski et al., 1985) that light respiration can be still measured a few minutes after the onset of darkness, and we used the same approach to estimate R_{light}. However, 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 3a shows the variations

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of R_{dark} as a function of time after two hours of light exposure. In this sample, R_{light} measured immediately (within 5 min) after the onset of darkness was three fold greater than R_{dark} and then respiration exponentially decreased to reach, after four hours of darkness, R_{dark} value (Fig. 3c). Figure 3c shows the oxygen concentration variations 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. 3d). 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 within the shortest period allowable in order to have a significant change in oxygen concentration (i.e. 0.5 μM) after the onset of darkness.

Obviously, the incubation procedure doesn't 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 summing this value with 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 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 in the light is equivalent to that in the dark. Similarly, we can calculate P and then determine the P:R ratio, taking into account R_{light} and the daily rates of P and R. When R_{light} was taken into account, P 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 P value of 290 μmol O_2 $L^{-1}d^{-1}$and a P:R ratio of 5.47, when it is assumed that R in the light is equivalent to that in the dark. This represents an underestimation for P

of 56% and an overestimation for P:R ratio of 68%.

It is clear that the long tailing off of R_{light} means that we can not ignore this phenomenon 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

In our study, the range of hourly rates of R_{light} is of the same order of magnitude as those measured for phytoplankton cultures (Ekelund, 2000; Heraud and Beardall, 2002). In these studies, respiration in the light was measured from light-dark shift using an oxygen microsensor with the same procedure as the one used in our study. Respiration in the light has also been estimated in lakes or in phytoplankton cultures using a mass spectrometry technique, which is based on the metabolic isotopic fractionation of ^{18}O (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, respiration in the light can be up to 5 fold more than the value measured in the dark (Luz et al., 2002), values that are similar to those measured in our study. Using the same isotopic fractionation technique, Grande et al. (1989b) have shown that in the North Pacific Gyre, respiration in the light can be up to 8 times greater than the rate in the dark. This strong stimulation of respiration in the light leads to an underestimation of P of more than 135% when it is assumed that respiration in the light is equivalent to that in the dark (Grande et al., 1989b). Consequently, despite mounting evidence that respiration in the dark can not be equal to that in the light, the ecological consequences of the assumption that respiration in the light is equivalent to that in the dark continue to be ignored. This is maybe 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 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 incubation allowed to show that oxygen concentration is not always linear with time (Briand et al., 2004), especially during the change of light conditions (Figs. 1 and 2). 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) and 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 P 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, P is the dominant process. Thus, the error introduced in the calculation of P 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, we observed during net heterotrophic conditions, NP rates (in absolute values) greater than R rates (Table 1, Fig. 2b) resulting in physiologically impossible negative values of P (see Eq. 1). Similar phenomenon have been observed in the ALOHA station in the Central Pacific by Williams et al. (2004), where negative values of P were reported in deep waters under net heterotrophic conditions. In our study (Table 1), we consider that R_{light} needs to be greater than NP in order to get a positive value for P. For example for the sample collected in M41 on 11 May 2006, this results in a calculated R_{light} that is 186%

higher than R_{dark}. This value should be considered as conservative as the percentage of stimulation is probably higher (P>0). Indeed, the strong decrease observed just after light exposure (Fig. 2b) clearly indicates that respiration was strongly stimulated by the onset of illumination suggesting thus a tight coupling between respiration and production. Obviously, respiration measured in this study represents the community respiration, i.e. sum of phytoplankton respiration and bacterial respiration. Therefore, both components of this community respiration might be stimulated by light, including the Melher 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 compound for photosynthetic production resulted in a 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 P. 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 an interesting exercise to use it to estimate the potential error in the calculations of P (Eqs. 1 and 2), the calculations of daily R, and hence the P:R ratio, when it is assumed that respiration in the dark is equivalent to that in the

light. We perform this exercise with literature data collected in costal waters exhibiting similar levels of chlorophyll a concentration as those measured in this study. From our field experiments we calculated that on average hourly rates of R_{light} represent 354% of R_{dark}. We used this average value to estimate the underestimation of P from literature data from coastal ecosystems according to Eq. (3).

It is clear that the assumption that R in the light is equivalent to that in the dark can have non-negligible consequences on the determination of the P:R ratios of pelagic systems. This will be particularly important in regions characterized by permanent net autotrophy or net heterotrophy where the stimulation of respiration by light may lead to an erroneous estimation of the percentage of heterotrophy or autotrophy. 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 used our average R_{light} of 354% R_{dark}, we estimate for the same periods P:R ratios medians of 2.05 (spring), 1.42 (summer), and 1.82 (fall), which represents an overestimation of P:R ratios 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, representing a 106% underestimation.

The use of P:R ratio to estimate whether a system is net autotrophic or net heterotrophic can be subject to caution since uncertainties remain regarding the estimation of P and R. Therefore, it can be preferable to use NP as it represents the real balance between P and R and its estimation is less inexact due to the fact that it takes into account respiration in the light. However, although NP 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 P and R estimations, numerous studies on the carbon cycle have compared P 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 P, which separates net heterotrophic from net autotrophic communities in Southern Ocean. Assuming that R in the light is equivalent to that in the dark, they calculated a threshold value for P of 2.2 mmol O_2 m^{-3} d^{-1} (Fig. 4). If R_{light} is integrated into the calculation (R_{light}=3.54×R_{dark}), we estimate that the threshold for P is 6.5 mmol O_2 m^{-3} d^{-1}. 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 of the different metabolic pathways involved in the carbon cycle on a global scale (e.g. Del Giorgio et al., 1997). Therefore, the uncertainties regarding the estimation of P and R alter 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 that respiration in the light is equivalent to that in the 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 for the last two decades alternative methods are available for the measurement of oxygen concentration under conditions that allow for the determination of respiration in the light. This undoubtedly explains why the ecological significance of the assumption that respiration in the light is equivalent to that in the dark has generally been considered as inconse-

quential. In our study, we show that respiration in the light should not be ignored for the determination of P nor for the estimation of the daily rate of R, at least in coastal environments. This emphasizes the fact that, 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.

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Table 1. Respiration and production for different natural water samples. The light regime was as follow 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}.

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

[a] P_{dark} represents the Production when R_{dark} is used in the calculation (see Eq. 1).
[b] P_{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.

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.

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

[a] P_{dark} represents the Production when R_{dark} is used in the calculation (see Eq. 1).
[b] P_{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.

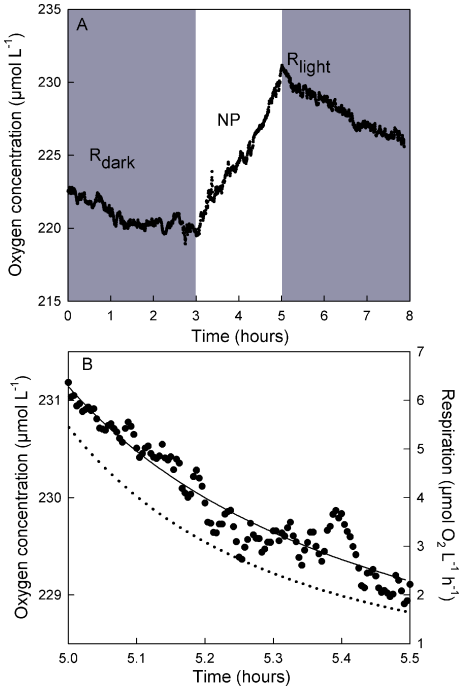

Fig. 1. (a) Oxygen time course during incubation of water from the Southwest lagoon of New Caledonia. Sample was collected in Anse Vata on 30 June 2005. The concentration of chlorophyll *a* was 9 μg L^{-1}. Shaded boxes represent the dark periods and the unshaded box represents the illumination period. **(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.

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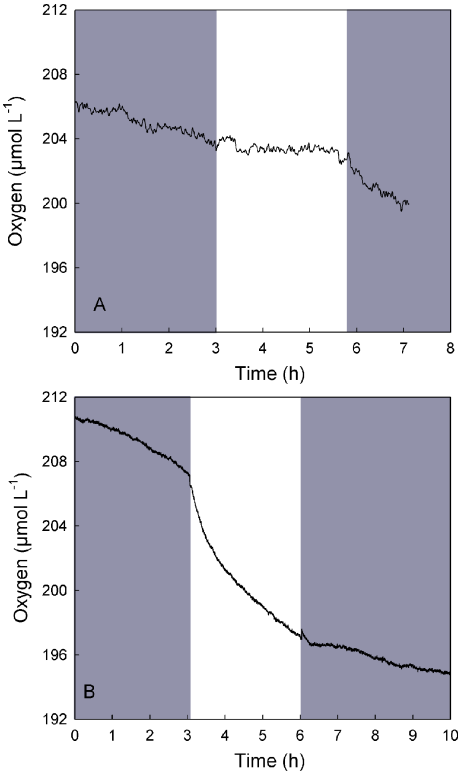

Fig. 2. Oxygen time course during incubation of water from Southwest lagoon of New Caledonia. Shaded boxes represent the dark periods and unshaded box represents the illumination period. Samples were collected in N12 on 16 March 2005 **(a)** and in M41 on 11 May 2006 **(b)**, concentration of chlorophyll *a* was 0.75 $\mu g\,L^{-1}$ (a) and 0.27 $\mu g\,L^{-1}$ (b). See Table 1 for rates values.

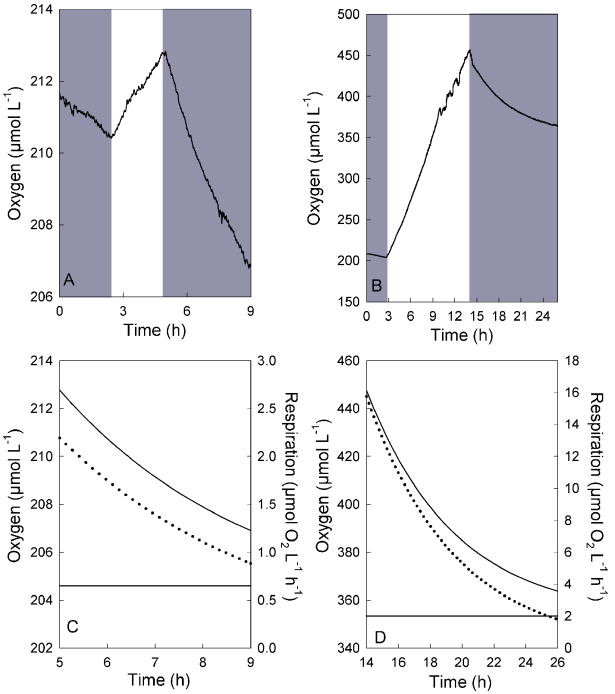

Fig. 3. **(a)** and **(b)** Oxygen time course during incubation of water from Southwest lagoon of New Caledonia. Shaded boxes represent the dark periods and unshaded box represents the illumination period. **(c)** and **(d)** Exponential decrease (solid lines) fitted to the raw data of oxygen concentration 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 $\mu g\,L^{-1}$. For (b) and (d) sample was collected in Anse Vata on 30 March 2006. The concentration of chlorophyll *a* was 20 $\mu g\,L^{-1}$.

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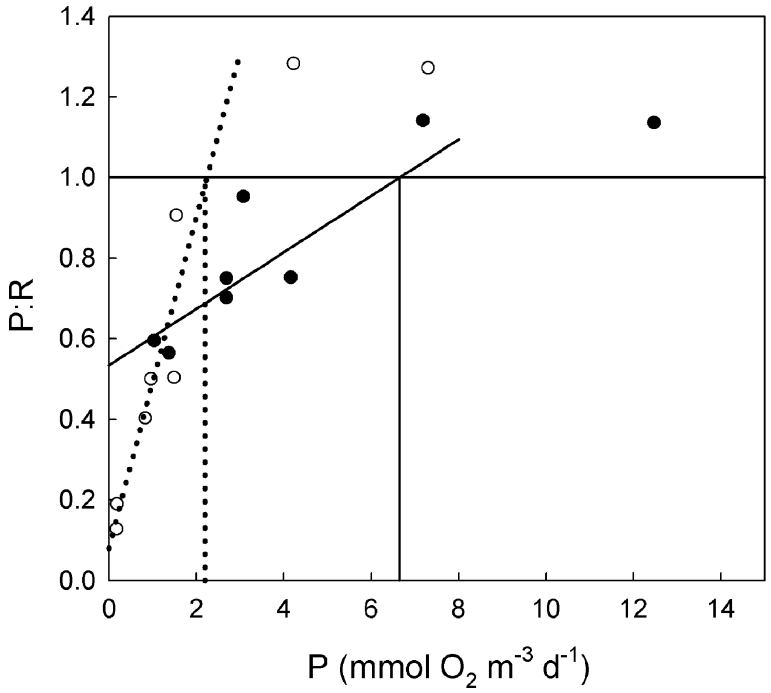

Fig. 4. Relationship between P:R ratio and Production for the determination of the threshold value of P separating net heterotophic from net autotrophic communities. Open symbols and dotted line data from Duarte and Agusti (2005). Closed symbols data from Duarte and Agusti corrected with $R_{light}=3.54\times R_{dark}$. 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.