Vol. 52: 119–130, 2008
doi: 10.3354/ame01208

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Printed August 2008
Published online July 24, 2008

Oxygen production and carbon fixation in oligotrophic coastal bays and the relationship with gross and net primary production

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ABSTRACT: Planktonic primary production and respiration in 2 coastal oligotrophic sites of the Northwest Mediterranean Sea were examined. Primary production was quantified using 3 methods (light and dark changes in dissolved O_2, ^{18}O-labeling and ^{14}C uptake technique) using *in situ* bottle incubations. Gross primary production (GPP) based on the O_2 light-dark technique was not significantly different from that using the ^{18}O-labeling technique, indicating that the former technique provides accurate estimates of GPP in these environments. Respiration in the dark was not significantly different from respiration in the light. Total ^{14}C uptake (including the dissolved and particulate organic carbon fractions) during the whole duration of the light period approached GPP and consequently overestimated net primary production.

KEY WORDS: Primary production · Oligotrophic coastal bays · Light-dark O_2 changes · ^{18}O-labeling · ^{14}C uptake · Respiration in the light

INTRODUCTION

Oceanic primary production (PP) accounts for about 50% of total carbon fixation in the biosphere (Field et al. 1998). Accurate quantification of marine PP is therefore fundamental in understanding and characterizing global carbon cycling. To avoid confusion, it is important to distinguish between some of the terms related to PP. PP can be quantified at the initial stage of the photosynthetic process by following changes in dissolved oxygen or at a later stage by measuring carbon fixation. Gross primary production (GPP) is the photosynthetic carbon uptake by phytoplankton which can be estimated using the O_2 light-dark and the ^{18}O-labeling methods. Net community production (NCP) is GPP less the organic carbon lost through community respiration and can be measured using the O_2 light–dark method. Net primary production (NPP) is the GPP minus autotrophic respiration and can be measured using the ^{14}C method when the ^{14}C and ^{12}C carbon pools are in equilibrium.

Traditionally, PP is estimated from either oxygen changes during light/dark incubations or the transfer of ^{14}C from bicarbonate to the particulate organic matter pool produced, thus ignoring the dissolved organic matter fraction (see references in Barber & Hilting 2002). Subsequently, the ^{18}O-labeling technique for gross oxygen production, and bottle incubation independent techniques such as the triple oxygen isotope method ($^{18}O_2$, $^{17}O_2$, and $^{16}O_2$), fast repetition rate fluorometry (FRRF) and indirect methods (O_2 and CO_2 changes, net transports of allochthonous organic carbon) have been used (e.g. Grande et al. 1989, Luz & Barkan 2000, Juranek & Quay 2005, Sarma et al. 2005, Pringault et al. 2007). However, each technique has its strengths and weaknesses (e.g. Robinson & Williams 2005).

Unfortunately, most of these methods involve incubation over a period of time and this may induce a

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bottle effect, which can result in changes in biomass and community structure (e.g. Gattuso et al. 2002). For instance, incubations are often carried out on the deck of a ship or in an incubator in which temperature, and in particular light conditions, deviate from those *in situ*. Therefore, there is a critical need to develop alternative techniques with no incubation. A number of incubation-free approaches have been developed, e.g. FRRF and the measurement of the expression of the gene coding for RUBISCO (Corredor et al. 2004), but these do not yet have the required precision. Air-sea gas exchange of oxygen provides another direct method to estimate PP (e.g. Bates et al. 1998), but the uncertainty of the air-sea gas exchange estimates is rather high (Juranek & Quay 2005). Recent studies (e.g. Luz & Barkan 2000) consider the 3 oxygen isotopes method as one of the more accurate methods to estimate GPP. Nevertheless, it relies on uncertain parameterization of the air-sea gas exchange which may lead to relatively large errors of GPP (Juranek & Quay 2005).

Several studies have compared methods to derive PP in the open ocean (e.g. Williams et al. 1983, Bender et al. 1987, Grande et al. 1989, Juranek & Quay 2005, Sarma et al. 2005) and have identified a number of difficulties. First, a photosynthetic quotient (PQ = moles of O_2 released/moles of CO_2 fixed) is required to compare PP based on carbon fixation and oxygen release methods. This PQ can vary widely although it generally ranges between 1.2 and 1.8 (Laws 1991). Second, interpreting ^{14}C uptake is not as straightforward as the significance of recycling, i.e. the release of fixed C as CO_2, varies depending on the duration of the incubation and community structure. According to some authors (see references in Langdon et al. 1995), ^{14}C uptake should theoretically approximate NPP or GPP depending on how fast the pool of organic carbon reaches isotopic equilibrium with the dissolved inorganic carbon pool. Although there is no consensus, ^{14}C fixation is thought to approach NPP when 24 h incubations are used; when shorter incubations are used, it approximates GPP (e.g. Weger et al. 1989). Marra (2002) has argued that ^{14}C fixation approximates NPP otherwise it would require an unrealistic PQ. However, others (e.g. Langdon et al. 1995) consider that ^{14}C fixation gives a good estimation of GPP or something between GPP and NPP (e.g. Laws et al. 2000). Third, the ^{14}C uptake rates reported in the literature often concern only particulate ^{14}C fixation. If dissolved organic carbon ($DO^{14}C$) is produced but not measured, the ^{14}C fixation rate will consequently underestimate total PP (Karl et al. 1998). Fourth, light respiration may be higher than dark respiration as a result of photoenhancement of mitochondrial respiration, photorespiration and photoreduction of O_2 (e.g. Bender et al. 1987, Dickson & Orchardo 2001, Dickson et al. 2001, Pringault et al. 2007). The differences in respiration in the light and dark are another contentious issue of direct relevance to the accuracy of the light-dark technique which assumes that these rates are similar.

The current debate on the measurement of PP remains unresolved because of the lack of *in situ* assessments, artefacts associated with incubations, the difficult distinction between NPP and GPP, the rapid release and consumption of photosynthesis derived dissolved organic matter, the impact of metabolic processes not directly related to photosynthesis and the critical assumption of equal community respiration in light and darkness. In addition, the present debate is largely limited to open ocean communities (Grande et al. 1989, Laws et al. 2000, Juranek & Quay 2005) and fresh-water ecosystems (e.g. Carignan et al. 2000), while methods inter-comparisons in coastal ecosystems are rather limited (Sarma et al. 2005, Gazeau et al. 2007).

The main goal of the present work was to provide a critical appraisal of GPP measurements. Such an evaluation is essential to further our understanding of global carbon cycling. We present PP rates from Mediterranean oligotrophic coastal waters, based on 3 methods (light and dark changes in dissolved O_2, ^{18}O-labeling and ^{14}C-uptake technique) using *in situ* bottle incubations. These data will be used to revisit the controversial topics of whether respiration rates in the light and dark are equal and whether ^{14}C fixation approximates GPP or NPP.

MATERIALS AND METHODS

Study site and sampling. Experiments were carried out in the Bay of Palma (BP) and at Point B, Bay of Villefranche-sur-mer (BV) (43° 41′ N, 7° 19′ E) (Table 1). Both embayments are oligotrophic systems in the NW Mediterranean Sea with extensive seagrass (*Posidonia oceanica*) meadows and negligible freshwater inputs. The bottom depth of the stations varied between 13 m and 33 m in the BP and was 80 m in the BV. The study at the BP was conducted on board the RV 'Mytilus' during June 2002 and the experiments were performed at 4 stations: Posidonia (Pos), Enderrocat (End), Bahía (B) and Stn 4 (S4) (Table 1). The study in the BV was carried out using the Sagitta at the Point B time series station (Table 1) in October 2002, December 2002, March 2003 and July 2003. Further details about the BP and BV study sites are provided in Gazeau et al. (2005) and Gomez & Gorsky (2003), respectively.

Hydrography, irradiance and nutrients. Vertical CTD profiles were carried out at each station using a CTD SeaBird SBE19 (BP) or SBE25 (BV). Profiles of

Table 1. Summary of selected variables within photic layer at stations sampled at Bay of Villefranche (BV) and Bay of Palma (BP). Also shown are averages ± SE of each vertical depth, BV and BP. %cyano: % cyanobacteria; PER: percent of extracellular release TOC: total organic carbon; Pos: *Posidonia*; B: Bahía; S4: Stn 4; End: Enderrocat

Location	Depth (m)	Sunrise-sunset (h)	Irradiance ($\mu E\ m^{-2}\ s^{-1}$)	NO_3 ($\mu mol\ l^{-1}$)	Chl *a* ($\mu g l^{-1}$)	%cyano (%)	PER (%)	TOC ± SE ($\mu mol\ l^{-1}\ d^{-1}$)
Oct02 (BV)	0.5	11.43	1287	0.47	0.15	43.86	69.21	0.56 ± 0.07
	10	11.43	300	0.24	0.15	41.39	50.41	0.32 ± 0.07
	20	11.43	153	0.75	0.15	17.13	65.68	0.40 ± 0.03
	30	11.43	110	2.21	0.36	28.03	37.82	0.21 ± 0.11
Dec02 (BV)	0.5	9.09	739	1.14	0.31	13.8	5.01	0.25 ± 0.02
	10	9.09	219	0.69	0.29	6.66	19.46	0.20 ± 0.03
	20	9.09	69	0.44	0.29	5.69	19.37	0.13 ± 0.01
	30	9.09	20	0.68	0.17	4.45	37.74	0.05 ± 0.01
Mar03 (BV)	0.5	11.21	374	2.16	1.09	1.39	37.25	2.19 ± 0.20
	10	11.21	215	2.95	0.66	2.14	31.20	2.86 ± 0.09
	20	11.21	100	3.75	0.84	1.15	64.26	1.88 ± 0.07
	30	11.21	48	3.01	0.40	2.21	86.59	1.03 ± 0.31
Jul03 (BV)	0.5	15.21	1598	0.64	0.13	30.13	21.41	0.71 ± 0.04
	10	15.21	593	0.30	0.12	47.40	16.96	0.65 ± 0.10
	20	15.21	288	0.54	0.11	36.71	44.76	0.33 ± 0.04
	30	15.21	221	1.20	0.21	35.80	43.19	0.58 ± 0.03
Pos (BP)	0.5	15.00	998	0.30	0.42	4.86	16.44	1.92 ± 0.31
	3	15.00	677	0.32	0.44	0.14	45.09	3.74 ± 0.35
	6	15.00	424	0.55	0.44	3.48	47.86	2.57 ± 0.15
	10	15.00	228	0.32	0.39	6.76	36.82	2.27 ± 0.95
B (BP)	0.5	15.00	974	0.26	0.29	12.38	40.80	1.00 ± 0.38
	5	15.00	659	0.22	0.26	14.00	31.78	1.34 ± 0.30
	15	15.00	276	0.30	0.49	5.42	37.29	1.04 ± 0.42
	30	15.00	75	0.27	0.26	11.75	36.62	1.13 ± 0.00
S4 (BP)	0.5	15.00	921	0.32	0.14	7.93	61.57	1.13 ± 0.23
	5	15.00	635	0.32	0.08	13.24	55.13	0.60 ± 0.13
	16	15.00	277	0.40	0.07	28.70	67.54	0.41 ± 0.27
	30	15.00	80	0.51	0.12	32.36	60.69	0.24 ± 0.01
End (BP)	0.5	15.00	1060	0.40	0.21	7.64	68.86	0.30 ± 0.02
	5	15.00	699	0.30	0.18	11.09	80.36	0.29 ± 0.07
	10	15.00	441	0.30	0.13	14.18	80.63	0.20 ± 0.02
	15	15.00	278	0.36	0.12	19.14	83.28	0.35 ± 0.20
Average ± SE								
Depth 1				0.71 ± 0.23	0.34 ± 0.11	15.2 ± 5.1	40.0 ± 8.7	1.01 ± 0.25
Depth 2				0.67 ± 0.33	0.27 ± 0.07	17.0 ± 6.2	41.3 ± 7.4	1.25 ± 0.47
Depth 3				0.88 ± 0.41	0.32 ± 0.09	14.1 ± 4.5	53.4 ± 6.9	0.87 ± 0.32
Depth 4				1.07 ± 0.36	0.25 ± 0.04	17.6 ± 4.7	52.8 ± 7.5	0.73 ± 0.26
BV				1.32 ± 0.28	0.34 ± 0.07	19.9 ± 4.3	40.7 ± 5.5	0.77 ± 0.21
BP				0.34 ± 0.02	0.25 ± 0.04	12.1 ± 2.2	53.1 ± 4.9	1.16 ± 0.25

light penetration were performed with a spherical sensor (LI-193SA, LI-COR Biosciences) connected to a LI-1400 data-logger. Seawater was collected at 4 depths within the photic layer at each station (Table 1) using single 12 l Niskin bottles. Dissolved inorganic nutrients were measured using an automated colorimetric method and chlorophyll *a* (chl *a*) was measured by HPLC. In order to convert chl *a* to biomass (total autotrophic biomass), we used the ratio of carbon/chl *a* = 120 (Marañón 2005). The *Prochlorococcus* spp., and *Synnechococcus* spp. biomass were estimated following the methodology and conversion factors described in González et al. (2003). The percent of *Prochlorococcus* spp., and *Synnechococcus* spp. to total autotrophic biomass (% cyanobacteria) was estimated as the ratio of *Prochlorococcus* spp. and *Synnechococcus* spp. biomass to total autotrophic biomass. Samples for measurement of PP and respiration were incubated *in situ* on a vertical line from sunrise to sunset or for 24 h. The processes measured are summarized in Table 2.

^{14}C technique. The incorporation of carbon into the dissolved and particulate fractions was measured using the ^{14}C technique (Steeman Nielsen 1952). Water samples (30 ml) were transferred to transparent glass bottles at each station and depth. The samples were incubated from sunrise to sunset in 1 dark

Table 2. Summary of different processes and how they were determined

Acronym	Definition	Comments
GPP-^{18}O	Photosynthetic release of ^{18}O from $H_2{}^{18}O$ during daytime	There is no ^{18}O release at night-time
NCP_{12h}	Change in O_2 during daytime	GPP less community respiration during daytime: GPP – CR_{light}
NCP	Change in O_2 over 24 h	GPP less community respiration over 24 h: GPP – CR
CR	O_2 decrease in the dark bottles during 24 hours	Community respiration over 24 h in the dark. It is assumed that light and dark respirations are equal
CR_{light}	GPP-$^{18}O_2$ – NCP_{12h}	Community respiration during daytime
CR_{dark}	NCP_{12h} – NCP_{24h}	Community respiration during night-time
GPP-O_2	CR + NCP	GPP, assuming that light and dark respiration are equal
GPP_{light}	CR_{light} + CR_{dark} + NCP	GPP estimates take into account light respiration
DO^{14}C	Photosynthetic production of dissolved organic ^{14}Carbon	From sunrise to sunset
PO^{14}C	Photosynthetic production of particulate organic ^{14}Carbon	From sunrise to sunset
TO^{14}C	Photosynthetic production of total organic ^{14}Carbon (DO^{14}C + PO^{14}C)	NCP ≤ TO^{14}C ≤ GPP

and 3 transparent biological oxygen demand bottles, after the addition of 30 µCi (1110 kBq) of sodium bicarbonate ($NaH^{14}CO_3$). Upon completion of the incubation, two 5 ml aliquots of each sample were placed into 20 ml scintillation vials for determination of total labeled organic carbon (TO^{14}C). Two more 5 ml aliquots of each sample were filtered over 0.22 µm of membrane filter for determination of total particulate organic carbon (PO^{14}C) and the filtrate collected for determination of total dissolved organic carbon (DO^{14}C). Filters were placed into vials and exposed to concentrated hydrochloric acid fumes for a minimum of 12 h before the addition of 3.5 ml of Ultima Gold liquid scintillation cocktail (Packard Instruments). Liquid samples (TO^{14}C and DO^{14}C) were acidified with 100 µl of hydrochloric acid (5 N) and bubbled with air for 2 h before adding 15 ml of Ultima Gold XR scintillation cocktail. Radioactivity (dpm) was measured in a Tri-Carb 4000 scintillation counter (Packard Instruments). Counts from the dark bottles were subtracted from counts measured in the light bottles to correct for non-photosynthetic ^{14}C-incorporation.

GPP-^{18}O technique. Gross oxygen production was determined by spiking seawater with $H_2{}^{18}O$ and measuring the quantity of ^{18}O produced during the incubation. At each water depth, 8 headspace vials of 27 ml were filled carefully to prevent trapping of air bubbles. Four vials were sealed immediately, fixed with saturated mercuric chloride (6.5%) and stored upside down in the dark pending the isotope analysis. The other 4 vials were spiked with $H_2{}^{18}O$ to a final isotopic composition (δ^{18}O-H_2O) of 300 to 500‰. This isotopic composition is defined as:

$$\delta^{18}O = \left[\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{std}} - 1 \right] \cdot 1000$$

where std corresponds to V-SMOW (Vienna Standard Mean Ocean Water).

Samples were deployed *in situ* from sunrise to sunset alongside the samples used for measurement of ^{14}C uptake and fixed with saturated mercuric chloride upon completion of the incubation. In the laboratory, 500 µl of headspace was created with helium and allowed to equilibrate for 24 h (^{18}O-O_2 measurements). The extracted water was injected into helium-flushed vials (^{18}O-H_2O). Pure CO_2 (100 µl) was then added and samples were allowed to equilibrate for 24 h. δ^{18}O-H_2O was therefore measured as δ^{18}O-CO_2. Determinations of δ^{18}O-O_2 and δ^{18}O-CO_2 were accomplished using an elemental analyzer interfaced Finnigan Deltaplus Isotope Ratio Mass Spectrometer. An overflow technique was used to limit air contamination of the needle.

Gross oxygen production (GPP-^{18}O) was calculated from the isotopic composition of the dissolved oxygen in the initial and incubated samples following the equation of Dickson et al. (2001). Standard deviation is based on propagation of error assuming that the errors of the measurements were independent.

GPP-O_2 technique. For estimating production and consumption of dissolved oxygen, seawater samples (20 replicates) were transferred into dark and transparent BOD bottles (60 ml) and incubated at sunrise *in situ* alongside the samples used with the GPP-^{18}O and ^{14}C techniques. At each station and depth, 5 bottles were immediately fixed (time 0) with Winkler reagents (Knap et al. 1996), 5 dark bottles were fixed after 24 h, 5 transparent bottles were fixed at sunset (about 12 h incuba-

tion) and the remaining 5 bottles at the following sunrise (24 h incubation). In the BP, 10 extra dark bottles at each depth and station were incubated and fixed after 12 and 48 h, to compare the effect of the incubation time on the respiration rates. The dissolved oxygen concentration was determined by the Winkler titration technique with potentiometric end-point detection. Analyses were performed with a redox electrode (9778-SC, Orion) and a custom built titrator and standardizations were similar to those described by (Knap et al. 1996). The planktonic community respiration (CR) and net community production during the light period (NCP_{12h}) and 24 h (NCP) were respectively estimated by regressing O_2 in the dark and transparent bottles against time. Daily GPP-O_2 was estimated as the difference between NCP and CR (Table 2). We obtained a pooled variation coefficient of 0.19 ± 0.03% (mean ± SE, n = 176) for NCP and CR. Dark community respiration during night time (CR_{dark}) was estimated as the difference between NCP_{12h} and NCP (Table 2). Community respiration in the light (CR_{light}) was estimated as the difference between GPP-^{18}O and $NCP_{12h.}$ This avoids the use of CR estimated in bottles incubated for 24 h in the dark, which assumes that dark respiration is equivalent to light respiration. Consequently, we could distinguish between GPP-O_2 and GPP_{light}, which takes into account light respiration (Table 2). We filtered the data for occasions when CR_{dark} and CR_{light} values were anomalously negative. These values are considered impossible when the data are less than twice its standard error (Williams 2000). However, we do not have standard errors for CR_{light} and CR_{dark}. Therefore, we compared NCP_{12h} and NCP and GPP-^{18}O and $NCP_{12h,}$ respectively, using paired *t*-tests. A few pairs that were significantly different and gave negative values of CR_{dark} and CR_{light}, were then excluded from the analyses. In order to compare PP in oxygen and carbon units, molar flux ratios ($\Delta O_2/\Delta CO_2$, apparent PQ) were estimated (Williams et al. 1983) as the ratio between GPP-O_2 or GPP-^{18}O (µmol O_2 l^{-1} h^{-1}) and $TO^{14}C$ (µmol C l^{-1} h^{-1}).

Statistical analysis. A series of random complete block designs (RCB; Quinn & Keough 2002) were performed to evaluate differences among depths and between bays, taking the different stations within the BP and BV as blocks. When the sphericity was not met we used the Greenhouse-Geisser adjustment (Quinn & Keough 2002). Variance homogeneity was tested with Cochran's analysis.

We used reduced major axis regressions (RMA, Model II regression) because it is more appropriate than standard ordinary least squares regression (OLS, Model I regression) when both variables are subject to measurement error (Sokal & Rohlf 1995). However, results obtained with both analyses are reported, in order to enable comparison with previous studies. The regression lines were compared using Student's *t*-tests (Zar 1999) because the analysis of covariance assumes a Model I regression. Data are reported as mean ± standard error of the mean (SE).

RESULTS

Irradiance, nutrients and chlorophyll

The profiles of irradiance were measured at approximately mid-day at each station (Table 1). Obviously, the highest irradiances were measured at surface and during summer. The highest nitrate (NO_3) values were observed during the most productive time, in March 2003 (see Table 1). NO_3 concentration did not show any significant vertical variability but tended to be higher at the BV (Table 3) because all stations in the BP were measured during summer (June 2002). Concentrations of chl *a* were below 1.1 µg l^{-1}, and did not exhibit any vertical variability or difference across the study sites (Table 3).

Table 3. Random complete block design (RCB). Bay of Villefranche (BV) and Bay of Palma (BP)

Parameter	Source	df	*F*-statistic	p-value	*A posteriori* comparison (Simple)
Nitrate (log)	Bay	1	5.01	0.06	
	Depth	3	1.98	0.15	
	Bay × Depth	3	0.43	0.73	
Chl *a*	Bay	1	0.30	0.59	
	Depth	3	0.77	0.52	
	Bay × Depth	3	0.24	0.86	
$TO^{14}C$	Bay	1	0.33	0.58	
	Depth	3	2.3	0.10	
	Bay × Depth	3	0.3	0.80	
NCP[a]	Bay	1	0.64	0.45	
	Depth	1.2	1.04	0.35	
	Bay × Depth	1.2	0.33	0.61	
CR	Bay	1	39.3	0.001	BV<BP
	Depth	3	0.32	0.80	
	Bay × Depth	3	0.43	0.73	
GPP-O_2	Bay	1	2.32	0.17	
	Depth	3	7.69	0.002	1=2>3=4[b]
	Bay × Depth	3	0.22	0.88	
GPP-^{18}O	Bay	1	5.05	0.06	
	Depth	3	7.70	0.002	1>4[b]
	Bay × Depth	3	1.7	0.18	

[a]Sphericity was not met so the Greenhouse-Geisser adjustment was used
[b]The numbers correspond to each depth

Carbon fixation, oxygen production and consumption

$TO^{14}C$ production ranged from 0.05 to 3.74 µmol l^{-1} d^{-1} (Table 1). The highest $TO^{14}C$ values were observed in March at Point B station whereas the *Posidonia* station in summer was the most productive of the stations studied in the BP. $TO^{14}C$ did not show a difference across study sites (Table 3) but showed a marginally vertical variability (i.e. a decrease) as a function of depth (Table 1). We observed a wide range of variability in the relative contribution of DOC production to total PP (% DOC) with an average of 40 and 54% (Table 1) for the BV and BP, respectively.

Estimates of NCP and NCP_{12h} ranged from –6.01 to 5.25 and –3.99 to 6.39 µmol l^{-1} d^{-1}, respectively. Estimates of NCP (µmol $l^{-1}h^{-1}$) were significantly higher than NCP_{12h} (*t*-test, t_{31} = 4.5, n = 32, $p < 0.0001$). NCP showed a tendency to decrease with depth (Table 4) but this was not significant (Table 3). Differences between areas in NCP were not significant (Table 3). Estimates of CR ranged from 0.25 to 7.0 µmol l^{-1} d^{-1} and did not exhibit significant vertical variability but were significantly higher in the BP than in the BV (Table 3).

Table 4. Summary of selected biological variables within the photic layer (4 depths, see Table 1) at the stations sampled in BV and BP. dt = daytime (from sunrise to sunset) nt = night-time; nd = no data. Also shown are averages (± SE) of each vertical depth, BV and BP. See Table 1 for location abbreviations

Location	NCP_{12h} ± SE (µmol O_2 l^{-1} d^{-1})	NCP ± SE (µmol O_2 l^{-1} d^{-1})	R ± SE (µmol O_2 l^{-1} d^{-1})	GPP-O_2 (µmol O_2 l^{-1} d^{-1})	GPP-^{18}O ± SE (µmol O_2 l^{-1} d^{-1})	CR_{light} (µmol O_2 l^{-1} dt^{-1})	CR_{dark} (µmol O_2 l^{-1} nt^{-1})
Oct02	–0.24 ± 0.12	–0.83 ± 0.15	2.64 ± 0.21	1.82	0.76 ± 0.12	1.00	0.58
	0.00 ± 0.06	–0.47 ± 0.16	1.84 ± 0.15	1.37	1.20 ± 0.33	1.19	0.47
	0.43 ± 0.14	–0.34 ± 0.25	0.89 ± 0.21	0.55	0.76 ± 0.21	0.33	0.77
	0.51 ± 0.21	–0.16 ± 0.26	0.49 ± 0.25	0.32	0.43 ± 0.17	–0.08	0.67
Dec02	1.02 ± 0.38	1.38 ± 0.26	0.76 ± 0.26	2.14	0.50 ± 0.06	–0.52[a]	–0.36
	1.04 ± 0.39	0.30 ± 0.35	0.25 ± 0.26	0.55	0.33 ± 0.10	–0.71[a]	0.74
	–0.27 ± 0.26	–0.29 ± 0.31	1.03 ± 0.30	0.74	0.12 ± 0.07	0.39	0.02
	nd nd	1.01 ± 0.24	0.40 ± 0.28	1.41	0.47 ± 0.09	nd	5.38
Mar03	5.07 ± 0.99	5.25 ± 0.77	0.61 ± 1.69	5.86	3.45 ± 0.38	–1.62[a]	–0.18
	4.55 ± 0.40	2.66 ± 0.55	0.73 ± 0.30	3.39	4.39 ± 0.40	–0.16	1.89
	1.77 ± 0.92	2.02 ± 0.77	0.96 ± 0.81	2.99	1.57 ± 0.19	–0.19[a]	–0.26
	–0.47 ± 0.41	0.73 ± 0.45	0.64 ± 0.25	1.37	0.37 ± 0.18	0.84	–1.20[a]
Jul03	0.62 ± 0.48	–0.31 ± 0.11	1.38 ± 0.04	1.07	0.52 ± 0.73	–0.10	0.92
	0.35 ± 0.24	–0.57 ± 0.18	1.99 ± 0.22	1.42	1.50 ± 0.45	1.15	0.92
	–0.07 ± 0.24	–0.26 ± 0.24	0.28 ± 0.23	0.02	0.50 ± 0.13	0.57	0.19
	0.12 ± 0.26	0.19 ± 0.30	0.27 ± 0.27	0.46	0.45 ± 0.12	0.32	–0.07
Pos	3.82 ± 0.90	0.38 ± 0.64	4.44 ± 0.98	4.83	6.33 ± 0.69	2.51	3.44
	4.14 ± 0.67	0.44 ± 1.08	5.34 ± 0.51	5.77	5.55 ± 1.65	1.41	3.71
	3.35 ± 0.31	0.84 ± 0.50	4.97 ± 0.45	5.81	5.87 ± 0.62	2.52	2.51
	6.18 ± 0.48	4.41 ± 0.77	1.01 ± 0.77	5.42	5.03 ± 1.07	–1.15[a]	1.77
B	2.15 ± 0.43	0.83 ± 0.68	3.63 ± 0.41	4.45	5.43 ± 1.03	3.29	1.32
	2.60 ± 0.56	1.10 ± 0.89	2.02 ± 0.48	3.12	4.88 ± 0.95	2.28	1.50
	1.49 ± 0.67	–0.28 ± 1.08	2.60 ± 0.73	2.31	3.47 ± 0.22	1.98	1.77
	0.45 ± 0.24	0.41 ± 0.38	1.68 ± 0.30	2.08	2.48 ± 0.31	2.03	0.04
S4	2.03 ± 0.49	0.68 ± 0.79	nd 0.98	nd	1.87 ± 0.57	–0.16[a]	1.35
	1.32 ± 0.32	0.90 ± 0.51	1.65 ± 0.81	2.55	1.40 ± 0.33	0.08	0.42
	0.30 ± 0.41	–0.94 ± 0.66	1.50 ± 0.48	0.55	1.11 ± 0.27	0.81	1.24
	–1.67 ± 0.64	–4.49 ± 1.02	5.79 ± 0.30	1.30	0.48 ± 0.24	2.14	2.82
End	2.30 ± 0.27	0.84 ± 0.16	2.48 ± 0.41	3.31	4.67 ± 0.19	2.37	1.46
	0.72 ± 0.16	–1.12 ± 0.25	4.03 ± 0.22	2.91	2.52 ± 0.58	1.80	1.84
	1.56 ± 0.24	–0.77 ± 0.38	1.22 ± 0.25	0.45	2.25 ± 0.30	0.69	2.33
	–3.99 ± 0.28	–6.01 ± 0.44	7.10 ± 0.08	1.09	1.81 ± 0.24	5.80	2.02
Average ± SE							
Depth 1	2.10 ± 0.61	1.03 ± 0.65	2.28 ± 1.45	3.35 ± 1.77	2.94 ± 0.83	1.81 ± 0.60	1.07 ± 0.42
Depth 2	1.84 ± 0.61	0.40 ± 0.42	2.23 ± 1.68	2.64 ± 1.61	2.72 ± 0.69	1.11± 0.33	1.44 ± 0.38
Depth 3	1.07 ± 0.43	0.00 ± 0.34	1.68 ± 1.49	1.68 ± 1.96	1.96 ± 0.67	1.04 ± 0.32	1.07 ± 0.38
Depth 4	0.94 ± 1.28	–0.49 ± 1.16	2.17 ± 2.70	1.68 ± 1.61	1.44 ± 0.58	1.84 ± 0.87	1.80 ± 0.72
BV	1.30 ± 0.53	0.64 ± 0.39	0.95 ± 0.17	1.59 ± 0.37	1.08 ± 0.30	0.50 ± 0.15	0.77 ± 0.36
BP	1.67 ± 0.59	–0.17 ± 0.59	3.30 ± 0.49	3.06 ± 0.48	3.45 ± 0.48	2.12 ± 0.36	1.84 ± 0.24

[a]Values anomalously negative; these are not included in the analyses

GPP-O_2 estimates were below 5.8 µmol l^{-1} d^{-1} and showed significantly higher values at the surface decreasing with depth (Table 3) but did not differ between the 2 bays. The highest values of PP (GPP-O_2, NCP_{12h}, NCP) were found at the Posidonia station in the BP and in March 2003 in the BV. More than half (55%) of the GPP/CR ratios were higher than 1 (net autotrophy) with an average of 1.71 ± 0.35. However, the average respiration rate was not significantly different from that of average GPP (*t*-test, t_{30} = 0.60, n = 31, p = 0.55).

Estimates of CR based on incubations lasting 12 h (0.08 ± 0.02 µmol O_2 l^{-1} h^{-1}), 24 h (0.10 ± 0.02 µmol O_2 l^{-1} h^{-1}) or 48 h (0.12 ± 0.02 µmol O_2 l^{-1} h^{-1}) were not significantly different ($F_{1,21}$ = 1.09, p = 0.35).

GPP-^{18}O was below 6.3 µmol l^{-1} d^{-1} and similar to GPP-O_2 (Table 4). The highest values of GPP-^{18}O coincided with the highest concentrations of chl *a* and the highest values of the other metabolic rates (GPP-O_2, NCP_{12h}, NCP). GPP-^{18}O showed significant depth variability with the highest values in the surface waters (Table 3) but no differences between areas were evident.

Estimates of CR_{light} were not significantly different from those of CR_{dark} (*t*-test t_{22} = 0.45, n = 23, p = 0.65). However, incubation times for CR_{light} and CR_{dark} were different and for that reason we normalized these estimates by time. We observed that CR_{dark} h^{-1} was not significantly different from CR_{light} (*t*-test, t_{22} = 1.89, n = 23, p = 0.07). Consequently, we observed no differences between $CR_{dark\,+}$ CR_{light} and CR (paired *t*-test, t_{25} = 0.36, n = 26, p = 0.71), but they were strongly correlated (r^2 = 0.82, p < 0.001, Fig. 1) with a $slope_{RMA}$ not significantly different from 1 (Table 5). In addition, GPP-O_2 was not significantly different from GPP_{light} (paired *t*-test, t_{23} = 1.70, n = 24, p = 0.10). In order to precisely estimate the effect of light on the rates of oxygen consumption, we examined the relation between irradiance (µE m^{-2} s^{-1}) and CR_{light}/CR_{dark} but the correlation was not significant (r_s = 0.22, n = 23, p = 0.32). Also, there was no significant correlation between percentage of cyanobacteria and CR_{light}/CR_{dark} (r_s = 0.1, n = 23, p = 0.65). Finally, we compared GPP-O_2 vs. GPP_{light} as a function of the water metabolic conditions. We found that GPP-O_2 was not significantly different from GPP_{light} in autotrophic (*t*-test, t_9 = 1.97, n = 10, p = 0.07) or in heterotrophic (*t*-test, t_{13} = 0.49, n = 14, p = 0.63) waters.

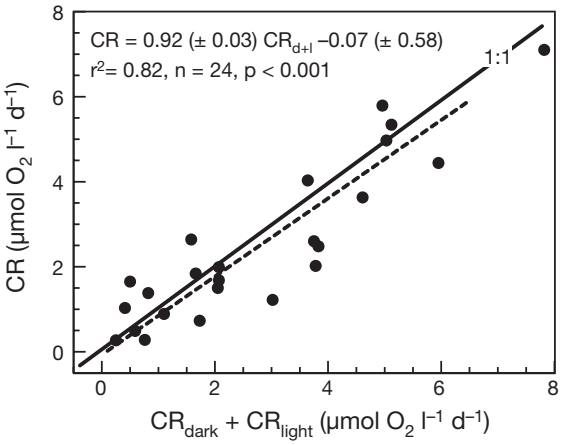


Fig. 1. Relationship between community respiration (CR_{dark} + CR_{light} [CR_{d+l}]) estimated as the sum of dark and light respiration rates and community respiration (CR) estimated from the light–dark O_2 method. Dashed line = reduced major axis (RMA) regression

Comparison of oxygen production and carbon fixation rates

GPP-O_2 closely correlated with GPP-^{18}O (r^2 = 0.74, n= 31, p < 0.001, Fig. 2). The $slope_{RMA}$ (0.89 ± 0.03) was not significantly different from 1 and the intercept

Table 5. Parameters of the regressions (RMA: reduced major axis; OLS: ordinary least squares) and Student's *t*-tests for the slope and intercept of regression lines

	r^2	n	df	RMA	OLS
CR_{light} + CR_{dark} vs. CR	0.82	24	22	t_{slope} = 0.94, p > 0.05 $t_{intercept}$ = 0.27, p > 0.05	
GPP-O_2 vs. GPP-^{18}O	0.74	31	29	t_{slope} = 1.27, p > 0.05 $t_{intercept}$ = 1.07, p > 0.05	t_{slope} = 2.74, p < 0.01 $t_{intercept}$ = 2.18, p < 0.05
GPP-O_2 vs. TO^{14}C (PQ = 1)	0.66	31	29	t_{slope} = 4.41, p < 0.001 $t_{intercept}$ = 1.74, p > 0.05	t_{slope} = 2.69, p < 0.01 $t_{intercept}$ = 2.97, p < 0.01
GPP-O_2 vs. TO^{14}C (PQ = 1.5)	0.66	31	29	t_{slope} = 1.97, p > 0.05 $t_{intercept}$ = 1.73, p > 0.05	t_{slope} = 0.26, p > 0.05 $t_{intercept}$ = 2.97, p < 0.01
NCP vs. TO^{14}C (PQ = 1)	0.47	29	27	t_{slope} = 3.25, p < 0.001 $t_{intercept}$ = 3.46, p < 0.001	t_{slope} = 1.01, p > 0.05 $t_{intercept}$ = 1.77, p > 0.05
NCP vs. TO^{14}C (PQ = 1.5)	0.47	29	27	t_{slope} = 1.29, p > 0.05 $t_{intercept}$ = 3.46, p < 0.001	t_{slope} = 0.93; p > 0.05 $t_{intercept}$ = 1.77, p > 0.05

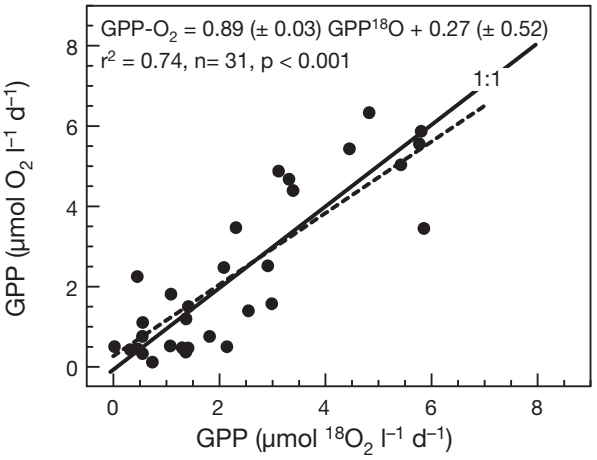


Fig. 2. Relationship between gross primary production measured by the light-dark O_2 method (GPP-O_2) and gross primary production measured by the ^{18}O technique (GPP-^{18}O). Dashed line = reduced major axis (RMA) regression

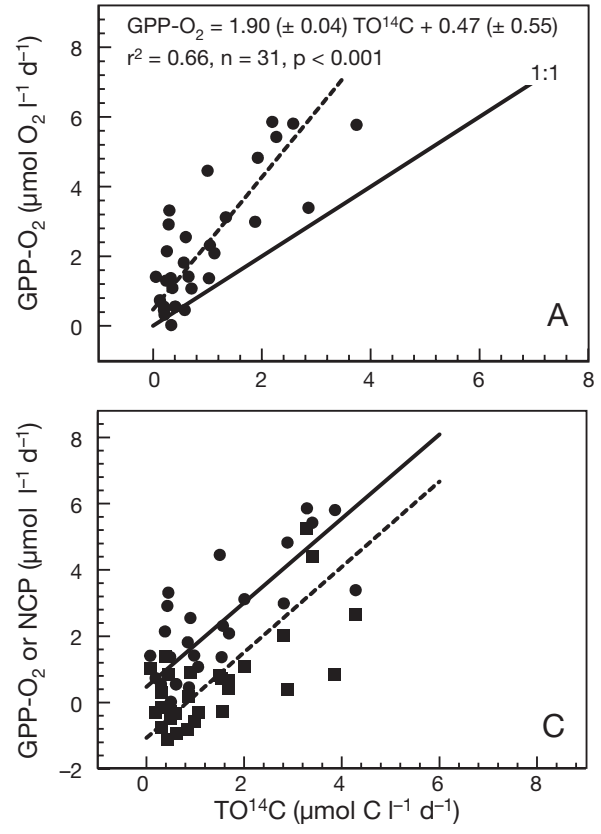
(0.27 ± 0.52) was not significantly different from 0 (Table 5). Note that the $slope_{OLS}$ was significantly different from 1 and the intercept was significantly different from 0 (Table 5).

GPP-O_2 was correlated with TO^{14}C (Fig. 3A) with a $slope_{RMA}$ (1.90 ± 0.04) significantly higher than 1 (Table 5). TO^{14}C and NCP were poorly correlated (r^2 = 0.26. $p < 0.05$). However, 3 outliers were identified and removed from the analysis (Zar 1999), improving the correlation (r^2 = 0.47, $p < 0.001$, Fig. 3B). The $slope_{RMA}$ (1.83 ± 0.08) was also significantly higher than 1 (Table 4). The average of the molar flux ratio was 1.5 ± 0.17 (GPP-O_2/^{14}C) and 2.1 ± 0.23 (GPP^{18}O/^{14}C), if we did not consider the outlier data with molar flux ratios > 6. The latter average was considered high and out of the general range (1.2 to 1.8). Therefore, the ^{14}C uptake rates were adjusted for a factor of 1.5 to compare ^{14}C uptake vs. GPP-O_2 and NCP. The new regression line between TO^{14}C and GPP-O_2 (Fig. 3C) had a $slope_{RMA}$ (1.19 ± 0.04) and an intercept (0.47 ± 0.55) which were not significantly different from 1 and 0, respectively (Table 5). The new comparison between TO^{14}C and NCP (Fig. 3C) showed a $slope_{RMA}$ (1.14 ± 0.08) not significantly different from 1, but the intercept (–1.07 ± 0.64) was significantly different from 0 (Table 5).

DISCUSSION

Oxygen production and consumption

The rates of GPP-O_2 (2.43 to 0.34 µmol l^{-1} d^{-1}) measured in the bays of BV and BP are similar to those results measured in other oligotrophic coastal systems



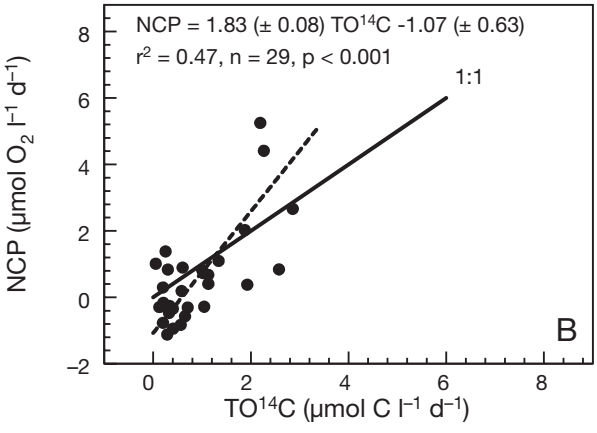


Fig. 3. (A) Relationship between gross primary production measured by the light-dark O_2 method (GPP-O_2) and daily ^{14}C uptake. (B) Relationship between net community production (NCP) measured by the light-dark O_2 method and daily ^{14}C uptake. Dashed lines = reduced major axis (RMA) regressions. (C) Relationship between TO^{14}C and NCP (dashed line and circles; 1.14 ± 0.08 × TO^{14}C – 1.07 ± 0.64), and GPP-O_2 (solid line and squares; 1.19 ± 0.04 × TO^{14}C + 0.47 ± 0.55), assuming a photosynthetic quotient of 1.5

(0.1 to 16.9 µmol l^{-1} d^{-1}; Duarte & Agustí 2004, Gazeau et al. 2005, Hashimoto et al. 2005). The rates of GPP^{18}O measured during the present study (2.27 ± 0.34 µmol l^{-1} d^{-1}) are higher than the rates reported for open ocean samples incubated *in situ* (0.71 to 0.09 µmol l^{-1} d^{-1}; Grande et al. 1989) and *in vitro* (0.96 µmol l^{-1} d^{-1}; Juranek & Quay 2005), similar to those reported for eutrophic estuaries (3.1 ± 0.6 µmol l^{-1} d^{-1}; Gazeau et al. 2007), but lower than those measured in laboratory incubations of samples from coastal mesocosms (43.5 to 1.2 µmol l^{-1} d^{-1}; Bender et al. 1987).

Dickson & Orchardo (2001) suggested that the $H_2{}^{18}O$ spike method should be preferred because it does not require the assumption that respiration in the light equals respiration in the dark, as does the oxygen light–dark method. However, in agreement with Bender et al. (1987) our results showed that GPP based on ^{18}O-labeling and the light-dark method, were not significantly different (Fig. 2). The $H_2{}^{18}O$ spike and O_2 light-dark method both involve bottle incubation. Sarma et al. (2005) observed that estimates of GPP based on *in situ* FRRF, which does not involve incubations, were similar to those obtained with the GPP-O_2 technique in the Bay of Sagami (Japan). Obviously, the bottle incubation procedure does not accurately mimic all aspects of *in situ* conditions. The light climate and hydrodynamics within the bottle may be different to that outside, and communities and biomass may change during incubation. However, our results revealed no significant differences in dark respiration rates when incubated for short (12 h) and long hours (24 and 48 h). Consequently, although GPP rates may be influenced by bottle incubations, we believe this artefact to be minor and our estimations robust.

Respiration in the light and dark

Most studies lack simultaneous determination of light and dark respiration, because of methodological difficulties, complicating comparison of our results with previous studies. The mean values of dark and light respiration in the BP and BV were higher than the values reported by Grande et al. (1989) for offshore oligotrophic waters (<0.5 µmol l^{-1} per 12 h). Oxygen consumption rates under light conditions were not significantly different from those in the dark, in agreement with Marra & Barber (2004). These results confirm that (daily) CR assuming CR_{light} = CR_{dark} is not significantly different from CR_{light} + CR_{dark} (Table 5). Consequently, GPP-O_2 based on dark respiration was not significantly different from GPP_{light} that takes CR_{light} into account. However, we did not observe a higher degree of underestimation of GPP-O_2 in net heterotrophic conditions, as observed in some other studies (e.g. Pringault et al. 2007). In an attempt to explain this difference, we note that most CR_{light} estimates from previous studies (e.g. Pringault et al. 2007) were obtained from *in vitro* conditions, under constant and saturating light (1000 µmol photons m^{-2} s^{-1}), while 90% of our samples received *in situ* irradiances much lower than saturating light, falling to values as low as 20 µmol photons m^{-2} s^{-1} at bottom depths in winter. Our data did not reveal evidence for respiration photoenhancement within our range of light intensities (20 to approximately 2000 µE m^{-2} s^{-1}). This is consistent with the findings of Lewitus & Kana (1995) showing limited differences between light and dark respiration under balanced growth conditions (i.e. respiration at the growth irradiance and not saturating light). Moreover, the lack of relationship between respiration in the light (CR_{light} or CR_{light}/CR_{dark}) and percentage of cyanobacteria is also consistent with literature reports (see references in Lewitus & Kana 1995), which showed important light stimulation of oxygen uptake by different phytoplankton species (e.g. *Synnechococcus* spp., diatoms) but only at high irradiances (light energy in excess). Our values for CR_{light} and CR_{dark} were estimated by difference and some negative values (see 'Materials and methods') were obtained. These negative values were retained and are presented because Williams (2000) argued that only the negative values derived from experimental anomalies (see 'Materials and methods') should be removed, otherwise it would bias the data set. However, this is not a common procedure (e.g. Grande et al. 1989, Pringault et al. 2007).

The similarity of rates of light and dark respiration that we found requires discussion in the context of existing knowledge (Williams & Lefèvre 2007). The enhanced O_2 consumption during the light period reported by some authors (e.g. Bender et al. 1987, Dickson & Orchardo 2001, Dickson et al. 2001) has been attributed to photorespiration, the Mehler reaction, and mitochondrial respiration or its prokaryotic equivalent. However, photorespiration in phytoplankton is usually low due to the existence of a CO_2 concentrating mechanism (CCM) (Weger et al. 1989). Additionally, although the Mehler reaction can decrease real gross photosynthesis in cultures by 10% (Laws et al. 2000), its contribution to O_2 consumption in the light is minor (Weger et al. 1988). The alternative explanation for enhanced respiration in the light is increased mitochondrial respiration. However, although it can be inhibited (e.g. Bate et al. 1988) or stimulated (e.g. Weger et al. 1989), the current consensus is that it is similar in the dark and light (Geider 1992). Besides these physiological mechanisms, there is also a community level mechanism for enhanced respiration in the light: immediate release of fixed carbon by auto-

trophs and uptake and respiration by heterotrophic consumers. Although a major fraction (~47%) of the fixed carbon was excreted in our study, the rate of respiration was rather constant; there was not a difference among incubations lasting 12, 24 or 48 h. Hence, bacteria do not directly depend on current algal production of labile carbon. In oligotrophic ecosystems where PP is low and in coastal sites, bacteria carbon demand tends to be higher than algal DOC release (Morán et al. 2002). This implies the existence of other autochthonous (e.g. cellular lysis, grazer activity) or allochthonous dissolved organic sources in addition to algal release, making it possible that phytoplankton release did not significantly enhance respiration in the light.

The present work shows robust evidence that under natural conditions respiration in the light is not significantly different to respiration in the dark. Consequently, the light-dark method is an accurate and unambiguous method to measure marine PP.

O_2 vs. ^{14}C rates

^{14}C fixation data including DOC fraction are scarce, especially in oligotrophic coastal systems. The rates (from 0.96 to 0.16 µmol l^{-1} d^{-1}) were higher than those obtained in the open ocean with or without accounting for the DO-^{14}C fraction (< 1.5 µmol l^{-1} d^{-1}, Grande et al. 1989, Juranek & Quay 2005), and within (0.1 to 9.4 µmol l^{-1} d^{-1}, Hashimoto et al. 2005) or lower than the range of coastal environments (e.g. 4 to 60 µmol l^{-1} d^{-1}, Morán et al. 2002; >60 µmol l^{-1} d^{-1}, Marañón et al. 2004). ^{14}C uptake is lower than GPP if part of the ^{14}C pool in the dissolved organic form is not collected and measured or if some photosynthetically fixed ^{14}C is remineralized during the incubation. It is therefore important to consider the incubation time. Weger et al. (1989) suggested that the ^{14}C technique estimates GPP when short incubations (1 to 3 h) are used because respiratory losses can be ignored. In agreement with earlier works (e.g. Raine 1983, Langdon et al. 1995) our results showed that ^{14}C uptake in incubations of up to 6 to 12 h, overestimate NPP and are closer to GPP. There are also uncertainties regarding the magnitude of extracellular release of recently fixed carbon. Most previous papers that have concluded that ^{14}C uptake is closer to NPP, did not account for the DOC fraction (e.g. Marra 2002, Juranek & Quay 2005). However, the contribution of dissolved PP can be important, especially in oligotrophic systems where productivity tends to be low (Moran et al. 2002). Therefore, PP can be significantly underestimated if the DO-^{14}C fraction is not taken into account (e.g. Marañón et al. 2004). Dissolved organic carbon release accounted for about 47% of total carbon fixation in the present study. We have no evidence that the high DOC production rates were an artefact of improper handling of samples. For instance, care was taken to ensure that the phytoplankton assemblage was not light shocked during sampling (samples were collected before sunrise and were incubated *in situ*). However, incubation times were rather long (9 to 15 h) depending on the day light length and some ^{14}C may have been recycled. Dickson et al.(2001) and Hansell & Carlson (1998) employed shorter incubations (4 to 6 h) and nevertheless also obtained high percentages of DOC production (e.g. 79 ± 5% and 59 to 70%, respectively). Our results clearly highlight the need to include the dissolved organic fraction in oligotrophic coastal waters such as those found in the Mediterranean.

When the oxygen and ^{14}C fluxes are compared, careful attention needs to be paid to the PQ. It can vary widely (from 0.5 to 5) but generally ranges between 1.2 and 1.8 (Laws 1991). We obtained an apparent PQ of 1.5 (molar flux ratio $O_2/^{14}C$), which is within the PQ range, and was derived from natural assemblages and not from non-specific phytoplankton blooms or cultures. According to Rabinowitch (1945), PQ is different from the conventional value of 1.0, mainly due to the synthesis of lipids (PQ > 1) or organic acids (PQ < 1) rather than the carbohydrates. In contrast, Burris (1981) observed that PQs higher than 1 were more likely as a consequence of the nitrogen substrate used by phytoplankton. When nitrate as a replacement for ammonia is assimilated by phytoplankton PQ tends to increase. PQ rises as a result of O_2 release during the reduction of nitrate to ammonium, in the range of 1.5 to 1.8 (Williams et al. 1979) or up to 2 (Raine 1983). Laws (1991) used alternative methods to estimate PQ based on balanced chemical equations and reported PQ estimates of 1.4 for new production (nitrate as the main source of nitrogen) and 1.1 for recycled production (ammonium as the main source of nitrogen). In our study area (Northwest Mediterranean), we observed abundances of cyanobacteria of about 2×10^5 cells ml^{-1} (data not shown). This abundance is even higher than that reported by Duarte & Agustí (2004) in the Bay of Blanes (10^4 cells ml^{-1}), considering that cyanobacteria largely dominate the autotrophic biomass in summer. According to Bergman et al. (1997), aerobic N_2 fixation by cyanobacteria entails O_2 consumption as part of the nitrogenase autoprotection mechanism. Therefore, our data confirm that PQ values higher than the commonly used value of 1.25 are not unusual (e.g. Williams et al. 1979). In any case, and using more conservative PQs (<1.5), our results confirmed that PP based on ^{14}C method is closer to gross than NPP (Table 5).

Finally, it should be noted that these conclusions were obtained from comparing TO-^{14}C vs. GPP-O_2 and NCP rates using model II reduced major axis regres-

sion (RMA) instead of the ordinary least squares regression (OLS) model I (see Table 5). According to McArdle (1987), when the coefficient of determination is sufficiently large ($r^2 > 0.9$) it does not matter which model is used. However, when the coefficient of determination is low and the X variable is subject to significant error, it is recommended to carry out both regressions (model I and II), selecting always the RMA if there is any difference. In this respect, our paper differs from others that rely on model I regression (e.g. Marra 2002, Marra & Barber 2004, Juranek & Quay 2005).

Acknowledgements. Many individuals contributed to the successful completion of the field work, in particular J. Nieuwenhuize, M.-D. Pizay and the skippers of the research boats. The CTD data were kindly provided by C. Heyndrickx and the SOMLIT program. We thank an anonymous reviewer and Prof. Williams for constructive feedback and editor P. del Giorgio for his patience. This research was supported by the EU in the framework of the EUROTROPH project (contract # EVK3-CT-2000-00040) and by an M.C. individual fellowship (MCFI-2001-01425). Additional support was provided by NWO (Dutch-French co-operation on sea research program).

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Editorial responsibility: Paul del Giorgio, Montréal, Québec, Canada

Submitted: January 15, 2007; Accepted: May 13, 2008
Proofs received from author(s): July 11, 2008