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Planktonic primary production in estuaries: comparison of ^{14}C, O_2 and ^{18}O methods

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ABSTRACT: Rates of primary production were measured in 2 estuaries (Randers Fjord, Denmark, and the Scheldt estuary, Belgium/The Netherlands) using 3 different incubation methods: (1) the oxygen light-dark method (O_2-LD), (2) ^{14}C incorporation and (3) ^{18}O labeling. Estimates based on the ^{14}C incorporation technique were not significantly different from those obtained using the O_2-LD technique. The ^{18}O approach provided rates significantly lower than the 2 other techniques. Ratios of O_2-LD to ^{18}O-based rates (range: 0.99 to 3.54) were often statistically significantly higher than 1 and increased with decreasing salinities and/or lower oxygen concentrations. The underestimation of gross primary production by the ^{18}O method may be due to an intracellular recycling of labeled oxygen which increased in magnitude with decreasing external oxygen conditions. These results suggest that the ^{18}O method must be used with extreme care in nutrient-rich, low oxygen systems.

KEY WORDS: Primary production · Phytoplankton · Estuaries · Incubation method · Oxygen · Carbon-14 · Oxygen-18

INTRODUCTION

Concerns about rising atmospheric CO_2 have initiated research on the role of the ocean as a sink for anthropogenic CO_2. Sabine et al. (2004), based on a high-resolution inorganic-carbon database, estimated the ocean sink as accounting for nearly half of the total CO_2 emissions from fossil fuel burning and cement manufacturing during the period 1800 to 1994. The capacity of the oceans to store anthropogenic carbon is governed by chemical, physical and biological processes, the latter partly depending on the balance between gross primary production (GPP) and community respiration (CR), i.e. net ecosystem production. Primary production in the oceans represents about 50% of global primary production (Field et al. 1998) and it is therefore essential to understand its governing factors and to accurately quantify its magnitude.

Historically, this process was first investigated on regional scales using the oxygen light-dark (O_2-LD) method (Riley 1939). In the 1950s, Steemann-Nielsen (1952) introduced the ^{14}C incorporation method which, at the time, was much more precise than the O_2-LD method and allowed shorter incubation times. This method has been extensively used and has become the most common way for measuring primary production,

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although the sensitivity of the O_2-LD technique has since been considerably improved (Howarth & Michaels 2000). Moreover, several problems with the use of the ^{14}C method have been identified and it is advisable to be careful when interpreting data based on it (Peterson 1980).

There is still a strong debate as to whether the ^{14}C method measures GPP, net primary production (NPP = GPP – autotrophic respiration), net community production (GPP – CR) or something inbetween. One of the shortcomings of the ^{14}C technique is that the ^{14}C incorporated in the algal cell can be respired or excreted as dissolved organic carbon (DOC) and then incorporated and/or respired by bacteria. This fraction is generally not accounted for, but some of it can be taken into account by measuring $DO^{14}C$ release. These processes introduce a bias and can make the results difficult to interpret. Numerous studies have focused on the comparison between GPP estimates based on the O_2-LD technique (O_2-GPP) and primary production measured by the ^{14}C method (^{14}C-PP). Several studies suggested that the ^{14}C method with incubations lasting from 12 to 24 h provides a rate closer to NPP (e.g. Eppley 1980) while others found a reasonable correspondence between the 2 methods in oligotrophic and eutrophic environments (Williams et al. 1983, Davies & Williams 1984, Bender et al. 1987, Langdon et al. 1995).

The O_2-LD method also has its problems. In order to estimate the increase of O_2 due to GPP in the light, additional samples need to be incubated in the dark to quantify the O_2 uptake due to CR and other potential O_2-consuming processes such as nitrification. Therefore, this method makes the implicit assumption that these processes are constant over 24 h and are not influenced by light. Nevertheless, several studies have shown enhanced CR rates in the light (Bender et al. 1987, Grande et al. 1989b, 1991, Kana 1990, Martinez 1992), suggesting that GPP rates measured by the O_2-LD method are underestimated.

Grande et al. (1982) developed a method based on ^{18}O tracing *in vitro*. Photosynthesis includes the splitting of H_2O to form O_2. Hence, a water sample spiked with $H_2{}^{18}O$ and incubated in the light allows estimation of GPP (hereafter referred to ^{18}O-GPP) as the increase in $\delta^{18}O$ in dissolved O_2 assuming that the ambient O_2 pool is large compared to respiratory O_2 uptake during the incubation. This method is considered to give reasonable estimates of GPP and usually provides higher rates of GPP than the O_2-LD and the ^{14}C methods (Bender et al. 1987, 1992, 1999, Grande et al. 1989a, 1991, Kiddon et al. 1995).

The aim of the present study was to compare these 3 methods for the first time in nutrient-rich coastal environments. Rates of GPP were measured by the O_2-LD and ^{18}O methods in 2 estuarine systems: the Randers Fjord (Denmark) and the Scheldt estuary (Belgium/The Netherlands). In the latter system, additional measurements by the ^{14}C method were performed.

MATERIALS AND METHODS

Study sites. This study was conducted in the framework of the EUROTROPH project (www.ulg.ac.be/oceanbio/eurotroph/) which aimed to determine the metabolic status of 3 European coastal ecosystems using several techniques and to compare the estimates at several time scales.

The Randers Fjord is the longest Danish estuary on the east coast (Fig. 1). The river and fjord drain an area of 3260 km^2 and receive treated sewage water from 600 000 inhabitants. The estuary is 27 km long and covers an area of 23 km^2. The main freshwater input comes from the river Gudenå, which drains 80% of the catchment area and enters the innermost part of Randers Fjord (Nielsen et al. 2001). The tidal range is small (0.2 to 0.3 m) and highest in the inner estuary (Nielsen et al. 2001). The mean annual water residence time within the estuary is about 13 d (Nielsen et al. 2001). A pycnocline is present throughout the year in almost the entire estuary (Nielsen et al. 1993).

The Scheldt estuary (Fig. 1) is one of the most eutrophic estuaries in Europe as a result of urban wastewater drainage and runoff from agriculture (Wollast 1988). The river Scheldt, with a catchment area of 19 500 km^2 (Heip 1989), is the most important freshwater source for the estuary. The freshwater residence time is long in this macrotidal estuary, ranging from 70 d in the inner to 10–15 d in the outer estuary (Soetaert & Herman 1995). Due to strong tidal currents (up to 1.5 m s^{-1}) and low freshwater discharges, the water column is well mixed throughout the estuary (Wollast 1988).

Sampling. Planktonic primary production was measured using the O_2-LD and the ^{18}O methods during a campaign in Randers Fjord (21 to 28 August 2001) and using the O_2-LD, the ^{18}O and the ^{14}C methods during 2 cruises in the Scheldt estuary (6 to 12 November 2002 and 2 to 9 April 2003, referred to hereafter as Scheldt 1 and 2, respectively). In Randers Fjord, samples were taken and incubated *in situ* at 4 depths from sunrise to sunset (incubation time ~15 h). As the water column was well mixed, only surface samples were taken in the Scheldt estuary and incubated in a 5-compartment on-deck incubator from sunrise to sunset (incubation time ~9 h in November and ~13 h in April). Samples were kept at *in situ* temperature by flowing water, and irradiance was controlled in each compartment by means of filters (100, 19, 13, 8 and 0% of surface irradiance). In order to avoid sedimentation of particulate

material in the samples, the incubated bottles were fixed on a rotating device (1 rpm). Concomitantly, samples were taken for salinity and pigment analysis. For pigment analysis, 500 to 1500 ml samples were filtered through GF/F membranes, which were stored frozen pending extraction and analysis by high-performance liquid chromatography using the same technique as described in Barranguet et al. (1998). Irradiance in the water column was measured on 3 to 4 occasions during the course of the incubations using a LI-COR LI-193SA spherical quantum sensor connected to a LI-COR LI-1400 datalogger.

O_2-GPP method. In Randers Fjord, at each depth, samples (5 replicates) were incubated in both transparent and dark 60 ml biological oxygen demand (BOD) bottles; in the Scheldt estuary, 5 replicate samples were incubated in each of the 5 compartments of the incubator (4 light, 1 dark). Concentrations of dissolved O_2 were measured at the beginning and end of the incubations using an automated Winkler titration technique with potentiometric end-point detection. Analyses were performed with an Orion redox electrode (9778-SC) and a custom built titrator. Reagents and standardizations were similar to those described by Knap et al. (1996). In Randers Fjord, samples incubated in the dark were used to estimate CR. In the Scheldt estuary, the O_2 consumption due to CR and nitrification was estimated by incubating samples in the dark, with and without addition of nitrification inhibitors. The following inhibitors were used: N-serve (nitrapyrine, 5 mg l^{-1} in ethanol) and sodium chlorate (10 mmol l^{-1}). As the N-serve inhibitor was dissolved in ethanol, its addition might enhance bacterial respiration; therefore, control samples were also incubated in the dark with addition of ethanol only (~0.8 ml l^{-1}). Nitrification and CR rates were estimated as:

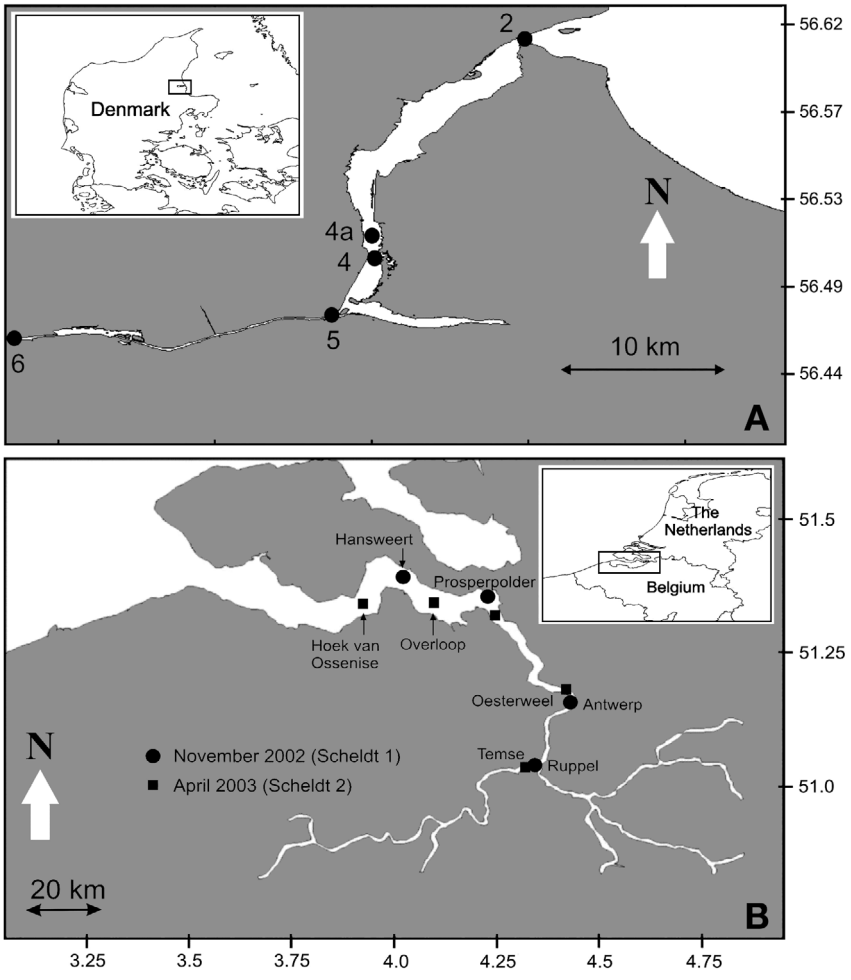

Fig. 1. (A) Randers Fjord (Denmark) and (B) Scheldt estuary (Belgium/The Netherlands), showing sampling stations of present study. Scheldt 1 and 2: 16 to 12 November 2002 and 2 to 9 April 2003 cruises, respectively

$$\text{Nitrification} = \frac{\Delta O_{2d\_eth} - \Delta O_{2d\_inh}}{\Delta O_{2d\_eth}} \times \Delta O_{2d} \quad (1)$$

$$CR = \Delta O_{2d} - \text{nitrification} \quad (2)$$

where ΔO_{2d}, ΔO_{2d_eth} and ΔO_{2d_inh} are O_2 variations during the incubations (sunrise to sunset) in the dark and with addition of ethanol (eth) and inhibitors (inh), respectively (mmol O_2 m^{-3} incubation $time^{-1}$). $\Delta O2_{d\_eth}$ was, on average, 3 and 1.3 times higher than ΔO_{2d}, during Scheldt 1 and 2, respectively, illustrating the need for control incubations. At each depth or irradiance level, net community production during the day (NCP_d in mmol O_2 m^{-3} incubation $time^{-1}$) was estimated as the variation in the O_2 concentration during the course of the incubations. Planktonic O_2-GPP rates (mmol O_2 m^{-3} d^{-1}) were then calculated as the difference between NCP_d and ΔO_{2d}. The combined error was calculated as:

$$SE_{x-y} = \sqrt{SE_x^2 + SE_y^2} \quad (3)$$

^{14}C-PP method. In the Scheldt estuary, samples were first filtered through a 63 µm sieve, and then immediately incubated in bottles identical to the ones used for the O_2-LD method, after spiking with 83.25 kBq of sodium [^{14}C] bicarbonate. Samples incubated in the dark were used to quantify dark ^{14}C uptake and for standardization of the initial ^{14}C activity. Light- and dark-incubated samples were filtered on glass fiber filters (Whatmann GF/F) under gentle vacuum. Wet filters

were then treated with 200 µl HCl (0.01 N) to remove excess $H^{14}CO_3^-$ and deep-frozen on board ship. ^{14}C activity remaining on the filters was measured in the shore laboratory using a liquid scintillation counter (Packard Tri-Carb 1600TR) after addition of 10 ml scintillation cocktail (Beckman Ready-Safe) to the unfrozen filters. pH and total alkalinity were determined in all samples and were used to compute dissolved inorganic carbon (DIC) concentrations. The relative ^{14}C uptake was determined by reference to the initial ^{14}C activity (5 replicates).

Total carbon uptake was computed as the product of relative ^{14}C uptake and an isotopic discrimination factor equal to 1.05 (IOC-SCOR 1994). Finally, these values were corrected for dark uptake for ^{14}C-PP estimation.

In April 2003, additional measurements of $DO^{14}C$ release were performed in the 100% light compartment. The $DO^{14}C$ excreted by the phytoplankton during the course of the incubation was evaluated by determination of the ^{14}C activity in the filtrated samples, after removal of DIC (including the remaining $DI^{14}C$). This was done by acidification of the filtrates with H_3PO_4 Suprapur® (0.87 M) to pH 3–4 and air bubbling for 30 min. $DO^{14}C$ activity was measured as described above for a 1 ml aliquot.

^{18}O-GPP method. Samples were incubated in 27 ml transparent glass bottles, spiked with 25 µl of 95% $H_2{}^{18}O$ which resulted in a final isotopic composition ($\delta^{18}O$-H_2O) of 300 to 500‰ that was substantially enriched relative to the natural isotopic composition in the 2 estuaries (–7.1 to –2.3 and –6.7 to 0.4‰ in Randers Fjord and the Scheldt estuary, respectively). The bottles were immediately closed after spiking to prevent air contamination. The isotopic composition is defined as:

$$\delta^{18}O = \left[\frac{{}^{18}O/{}^{16}O_{sample}}{{}^{18}O/{}^{16}O_{std}} - 1\right] \times 1000 \quad (4)$$

where std corresponds to V-SMOW (Vienna standard mean ocean water).

At each station, triplicate samples were taken to measure initial $\delta^{18}O$-O_2 values, immediately poisoned with $HgCl_2$ (~1 ml l^{-1}), and closed to limit air contamination. Incubations (in triplicate) took place at 4 depths in the Randers Fjord and in the 4 light compartments of the on-deck incubator in the Scheldt estuary. After incubation, samples were poisoned with $HgCl_2$ and stored upside down in the dark pending measurement. In the laboratory, 500 µl of headspace was created by extracting water with a helium flow and allowed to equilibrate at room temperature for 24 h (^{18}O-O_2 measurements). The extracted water was injected in helium-flushed vials (^{18}O-H_2O); 100 µl of pure CO_2 was then added and the samples were allowed to equilibrate at room temperature for 24 h. $\delta^{18}O$-H_2O was therefore measured as $\delta^{18}O$-CO_2. Determinations of $\delta^{18}O$-O_2 and $\delta^{18}O$-CO_2 were carried out using an elemental analyzer interfaced with a Finnigan Deltaplus isotope ratio mass spectrometer (IRMS). A gas volume of 500 µl was manually sampled from the vials with a syringe and directly injected while a helium overflow technique was used to limit air contamination of the needle. Gas chromatographic separation was achieved with a molecular sieve 5Å GC column (60 to 80 µm mesh, length 2 m, diameter 6.35 mm) and a Haysep-Q GC column (60 to 80 µm mesh, length 2 m, diameter 6.35 mm) for $\delta^{18}O$-O_2 and $\delta^{18}O$-CO_2, respectively. Before separation, at a temperature of 60ºC and under a helium flow of ~60 ml min^{-1}, residual water was removed using magnesium perchlorate, and residual CO_2 was removed by means of sodium hydroxide for $\delta^{18}O$-O_2 determination. For $\delta^{18}O$-O_2, calibration was done with external air and was checked regularly (every 10 samples). An analytical standard deviation of 0.16‰ was achieved during the calibration process. Based on initial triplicate $\delta^{18}O$-O_2 values obtained in the 2 estuaries, this technique allowed a standard deviation of 0.3 to 0.4‰. For $\delta^{18}O$-CO_2, calibration was done with V-SMOW and checked with IAEA-GISP (Greenland ice sheet precipitation) and -SLAP (standard light arctic precipitation); absolute differences between the certified and analyzed values were less than 0.5‰.

GPP rates (mmol O_2 m^{-3} d^{-1}) were calculated using the following (Kiddon et al. 1995):

$$\delta^{18}O - GPP = \left[\frac{\delta^{18}O{-}O_{2final} - \delta^{18}O{-}O_{2init}}{\delta^{18}O{-}H_2O - \delta^{18}O{-}O_{2init}} - 1\right] \times [O_2]_{init} \quad (5)$$

where $\delta^{18}O$-O_{2init} and $\delta^{18}O$-O_{2final} are measured $\delta^{18}O$-O_2 before and after incubation (‰), respectively, $\delta^{18}O$-H_2O is the final isotopic composition of the spiked water (‰), and $[O_2]_{init}$ is the oxygen concentration before incubations (mmol O_2 m^{-3}). The overall error was based on propagation of errors, as for O_2-GPP.

Statistical analysis. In order to compare the rates obtained by the 3 methods, and as each method is subject to measurement error, Model II regressions were used. Slopes and intercepts were tested using Student's *t*-tests.

RESULTS AND DISCUSSION

Rates obtained using the O_2-LD, the ^{18}O and ^{14}C (when applicable) methods in the Randers Fjord and in the Scheldt estuary are shown in Tables 1 & 2, respectively. Functional (Model II) regressions between rates based on the ^{14}C and the O_2-LD methods in the Scheldt estuary are presented in Fig. 2 and Table 3. Statistically significant relations were found in both seasons; the O_2-GPP to ^{14}C-PP ratio was 1.62 in November and 1.24 in April and the intercepts were not significantly different from zero (*t*-test, $p > 0.05$).

Following the equation of carbohydrate production using nitrate as a nitrogen source:

Table 1. Date, geographical location and characteristics of incubation stations in Randers Fjord. S: salinity; *E*: mean irradiance (µmol photons m^{-2} s^{-1}); Chl *a*: concentration of chl *a* (mg m^{-3}); O_2-GPP, ^{18}O-GPP: gross primary production (mmol O_2 m^{-3} d^{-1}) measured by the O_2 light-dark (O_2-LD) and ^{18}O methods, respectively, where data are means ± SD (N = 5 and 3 for O_2-LD and ^{18}O methods, respectively)

Date (2001)	Stn	Lat. (°N)	Long. (°E)	Depth (m)	S	*E*	Chl *a*	O_2-GPP	^{18}O-GPP
24 Aug	2	56.61	10.30	0.5	16.3	400.1	2.0	13.7 ± 1.7	14.8 ± 1.9
				1.5	18.3	228.5	2.2	9.7 ± 0.6	8.4 ± 0.5
				3	19.8	98.7	2.4	5.6 ± 0.4	3.1 ± 1.0
				7	21.8	10.5	1.5	0.2 ± 0.4	1.2 ± 0.2
28 Aug	4	56.52	10.23	0.5	11.3	294.9	3.1	47.8 ± 1.9	34.5 ± 2.5
				1.5	11.6	101.5	3.6	14.3 ± 0.8	14.9 ± 1.8
				3	13.6	20.5	3.0	2.0 ± 0.8	2.0 ± 0.2
				5	19.8	2.4	2.2	−0.8 ± 0.3	0.4 ± 0.0
30 Aug	4a	56.52	10.23	0.5	6.9	324.5	4.2	80.0 ± 3.3	50.3 ± 1.3
				1.5	8.0	131.9	5.7	35.5 ± 3.5	26.3 ± 1.9
				3	13.2	34.2	4.7	6.1 ± 0.8	5.9 ± 0.5
				6	20.2	2.3	2.7	−0.1 ± 0.3	0.4 ± 0.1
26 Aug	5	56.47	10.21	0.5	2.7	113.2	3.0	17.3 ± 1.3	8.8 ± 1.2
				1.5	8.7	43.1	2.9	4.0 ± 0.9	3.3 ± 0.6
				3	13.5	10.1	2.7	0.4 ± 0.4	1.2 ± 0.2
				6	16.1	0.6	2.9	−0.6 ± 0.4	0.2 ± 0.1
21 Aug	6	56.46	10.04	0.5	0.2	375.2	4.7	27.1 ± 2.3	13.4 ± 0.1
				1	0.2	239.2	4.7	20.6 ± 1.1	11.4 ± 0.5
				1.5	0.3	152.5	3.8	11.7 ± 1.5	6.4 ± 0.4
				3.5	11.4	25.2	1.8	−0.5 ± 1.7	0.5 ± 0.1

$$106HCO_3^- + 16NO_3^- + HPO_4^{2-} + 16H_2O + 124H^+ \rightarrow (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \quad (6)$$

one can compute a photosynthetic quotient (PQ, defined as the amount of O_2 produced per CO_2 consumed [in molar units]) of 1.3. If the nitrogen source is ammonium, the amount of O_2 produced is less (the nitrogen source is already in a more reduced form) and a theoretical PQ of 1 is expected:

$$106HCO_3^- + 16NH_4^+ + HPO_4^{2-} + 16H_2O + 124H^+ \rightarrow (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 106O_2 \quad (7)$$

Therefore, in the case of carbohydrate production, the theoretical PQ can vary between these 2 values and will be higher when nitrate is the primary nitrogen source (Williams et al. 1979). Dissolved organic nitrogen can be an important nitrogen source for phytoplankton (Antia et al. 1991, Veuger et al. 2004); this implies a further reduction in the amount of O_2 produced. The PQ is also dependent on the nature of the terminal organic carbon product (Rabinovitch 1945), with more reduced compounds such as lipids inducing a higher PQ and more oxidized products (organic acids) implying a lower PQ.

Therefore, when comparing primary production by the O_2-LD and the ^{14}C methods, one can expect a ratio close to PQ (between ~1 and 1.6: Williams et al. 1979) if both techniques measure rates of GPP. Using the whole data set during the 2 cruises, these ratios did not statistically differ from this (Table 3). Considering each station separately, again there was no reason to believe that the ^{14}C method underestimated GPP in our system. Although the slopes obtained at the different stations were not statistically significantly different from each other during each cruise, there is nevertheless a trend of increasing O_2-GPP : ^{14}C-PP ratios as a function of decreasing salinity. This suggests (1) a higher recycling of labeled organic matter in the brackish part of the estuary, with high respiration rates (Gazeau et al. 2005) and high bacterial production rates (Boschker et al. 2005), and/or (2) a higher rate of DO^{14}C release by primary producers which was not

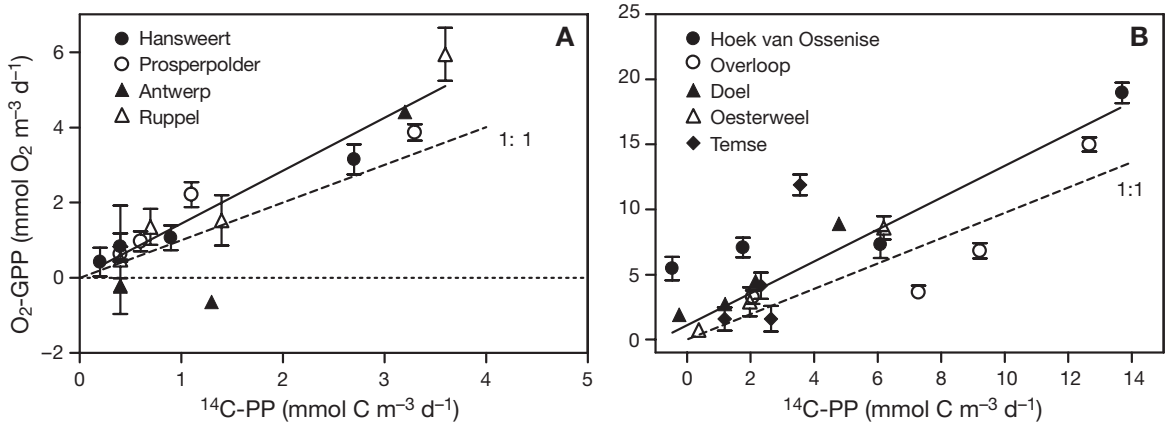

Fig. 2. Relationships between gross primary production measured by O_2 light-dark method (O_2-GPP) and by ^{14}C method (^{14}C-PP) in (A) Scheldt estuary in November 2002 (Scheldt 1) and (B) April 2003 (Scheldt 2). Continuous line: functional regression fit using whole data set; dashed line: 1:1 line

Table 2. Date, geographical location and characteristics of incubation stations in the Scheldt estuary during 2 cruises. S: salinity; $\%E_0$: percent of ambient irradiance; E: mean irradiance (µmol photons m^{-2} s^{-1}); Chl *a*: concentrations of chl *a* (mg m^{-3}); ^{14}C-PP: primary production measured by ^{14}C method (mmol C m^{-3} d^{-1}); O_2-GPP, ^{18}O-GPP: gross primary production measured by the O_2 light-dark method (mmol O_2 m^{-3} d^{-1}) and by ^{18}O method (mmol O_2 m^{-3} d^{-1}), respectively. For O_2-GPP and ^{18}O-GPP, data are means ± SD (N = 5 and 3 for O_2-LD and ^{18}O methods, respectively)

Date	Stn	Lat. (° N)	Long. (° E)	S	$\%E_0$	E	Chl *a*	^{14}C-PP	O_2-GPP	^{18}O-GPP
Scheldt 1										
10 Nov 2002	Hansweert	51.41	4.04	19.7	100	72.9	1.7	2.7	3.1 ± 0.4	2.7 ± 0.4
					19	13.8		0.9	1.1 ± 0.3	1.3 ± 0.3
					13	9.5		0.4	0.8 ± 0.3	0.6 ± 0.3
					8	5.8		0.2	0.4 ± 0.4	0.7 ± 0.3
12 Nov 2002	Prosperpolder	51.39	4.21	6.0	100	98.0	2.3	3.3	3.9 ± 0.2	2.9 ± 0.7
					19	18.6		1.1	2.2 ± 0.3	1.5 ± 0.3
					13	12.7		0.6	1.0 ± 0.4	1.0 ± 0.3
					8	7.8		0.4	0.6 ± 0.2	0.8 ± 0.5
08 Nov 2002	Antwerpen	51.23	4.40	3.0	100	47.5	6.1	3.2	4.4 ± 0.2	3.5 ± 0.9
					19	9.0		1.3	−0.7 ± 0.2	1.5 ± 0.4
					13	6.2		0.4	−0.2 ± 0.2	0.4 ± 0.8
					8	3.8		0.4	−0.2 ± 0.2	0.6 ± 0.7
06 Nov 2002	Ruppel	51.13	4.31	0.6	100	75.8	17.4	3.6	5.9 ± 0.7	2.8 ± 0.4
					19	14.4		1.4	1.5 ± 0.7	1.8 ± 0.3
					13	9.9		0.7	1.4 ± 0.5	1.1 ± 0.3
					8	6.1		0.4	0.5 ± 1.4	1.0 ± 0.3
Scheldt 2										
08 Apr 2003	Hoek van Ossenise	51.38	3.93	20.9	100	601.6	3.6	13.7	19.0 ± 0.8	17.7 ± 2.0
					19	114.3		6.1	7.3 ± 1.0	9.2 ± 1.5
					13	78.2		1.8	7.1 ± 0.7	6.7 ± 0.7
					8	48.1		−0.5	5.5 ± 0.9	4.3 ± 0.4
09 Apr 2003	Overloop	51.37	4.09	12.9	100	369.9	3.2	12.7	15.0 ± 0.5	12.2 ± 1.2
					19	70.3		9.2	6.8 ± 0.6	6.0 ± 1.4
					13	48.1		7.3	3.6 ± 0.5	4.5 ± 0.9
					8	29.6		2.1	3.3 ± 0.5	3.2 ± 0.6
04 Apr 2003	Doel	51.35	4.25	7.6	100	160.4	5.2	4.8	8.9 ± 0.4	4.1 ± 2.3
					19	30.5		2.2	4.5 ± 0.3	2.4 ± 0.7
					13	20.9		1.2	2.7 ± 0.3	1.3 ± 0.4
					8	12.8		−0.3	1.9 ± 0.3	1.5 ± 0.9
06 Apr 2003	Oesterweel	51.24	4.37	2.9	100	270.3	3.8	6.2	8.6 ± 0.9	3.3 ± 0.9
					19	51.4		–	3.6 ± 1.0	1.7 ± 0.6
					13	35.1		2.0	2.9 ± 1.0	1.5 ± 0.0
					8	21.6		0.4	0.7 ± 1.1	1.0 ± 0.8
02 Apr 2003	Temse	51.12	4.30	1.2	100	199.8	4.7	3.6	11.9 ± 0.8	4.1 ± 0.9
					19	38.0		2.3	4.1 ± 1.0	1.9 ± 0.8
					13	26.0		2.6	1.6 ± 1.0	1.6 ± 0.5
					8	16.0		1.2	1.6 ± 0.9	0.7 ± 0.8

Table 3. Relationships between primary production measured by the ^{14}C method (^{14}C-PP, mmol C m^{-3} d^{-1}; *x* variable) and gross primary production measured by the O_2 light-dark method (O_2-GPP, mmol O_2 m^{-3} d^{-1}; *y* variable) in the Scheldt estuary, calculated according to functional regressions, showing 95% confidences limits (CL) of slopes and *y*-intercepts (95% CL), number of data points (N), and significance levels (p). ns: slope of regression not significantly different from 0 ($p > 0.05$)

Stn	Slope	95% CL	Intercept	95% CL	N	p
Scheldt 1						
Entire data set	**1.62**	**1.19, 2.06**	**−0.52**	**−1.28, 0.24**	**16**	**<0.0001**
Hansweert	1.07	0.73, 1.40	0.24	−0.24, 0.72	4	0.0053
Prosperpolder	1.10	0.21, 2.00	0.43	−1.17, 2.02	4	0.0340
Antwerpen	1.90	−0.63, 4.44	−1.70	−6.13, 2.75	4	0.0841^{ns}
Ruppel	1.71	0.80, 2.62	−0.29	−2.09, 1.51	4	0.0150
Scheldt 2						
Entire data set	**1.23**	**0.77, 1.69**	**1.10**	**−1.57, 3.76**	**19**	**<0.0001**
Hoek van Ossenise	1.00	−0.10, 2.10	4.45	−3.86, 12.8	4	0.0601^{ns}
Overloop	1.27	−1.10, 3.64	−2.76	−23.36, 17.84	4	0.1469^{ns}
Doel	1.48	0.64, 2.32	1.60	−0.67, 3.87	4	0.0171
Oesterweel	1.34	1.33, 1.35	0.25	0.22, 0.29	3	0.0004
Temse	6.14	−8.09, 20.37	−10.09	−46.65, 26.47	4	0.2044^{ns}

taken into account in this comparison between O_2-LD and particulate ^{14}C fixation. Irrespective, it appears that during the course of our incubations, the recycling of incorporated ^{14}C and/or exudation of DOC did not induce statistically significant underestimation of GPP. It should be stressed that, although not significantly different from 0, a high ratio (~6) was observed at the upstream station in April. During this cruise, production of $DO^{14}C$ in the 100% light compartment of the incubator was also measured, and is shown in Fig. 3. While $DO^{14}C$ production was low (<10% of total production) at most stations, a high value (65% of total ^{14}C uptake) was observed in the most upstream station. Muylaert et al. (2000) showed that, in the oligohaline Scheldt estuary, phytoplanktonic species originating from the river suffer from a strong saline stress and that freshwater taxa are unable to adapt to high salinities. Moreover, Boschker et al. (2005) presented isotopic evidence for lack of growth of riverine algae in the upper estuary. This could explain the high $DO^{14}C$ production rate found at the oligohaline station, although Muylaert et al. (2005) showed that phytoplanktonic blooms in the same area may not produce large amounts of DOC. As we are not aware of similar results for other estuaries, the reason for the observed large DOC production at this station remains unclear.

Functional regressions between rates based on the ^{18}O and the O_2-LD methods in the Randers Fjord and the Scheldt estuary are shown in Fig. 4 and Table 4. In Randers Fjord and in the Scheldt estuary in November, GPP estimated using the 2 methods was statistically

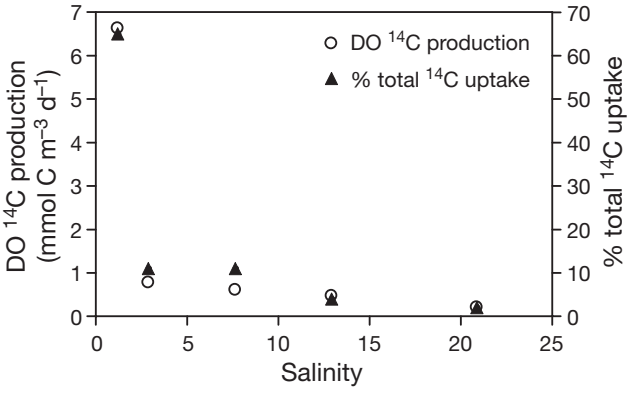

Fig. 3. Variation in rates of ^{14}C-dissolved organic carbon ($DO^{14}C$) production and percentage of $DO^{14}C$ production with regard to total ^{14}C uptake as a function of salinity in the Scheldt estuary, April 2003 (Scheldt 2)

significantly different when using the entire data set, with higher rates obtained using the O_2-LD technique. In the Scheldt estuary in April, using the whole data set, no statistically significant difference was found between rates obtained by the 2 methods. Moreover, a trend of increasing O_2-GPP:^{18}O-GPP ratio was observed with decreasing salinity in both estuaries (see below). These results were quite surprising and counterintuitive, since we would have expected higher rates using the ^{18}O method as reported in most studies (Bender et al. 1987, Grande et al. 1989a) and as demonstrated in a companion study carried out in 2 oligotrophic coastal bays (Bay of Palma, Spain, and

Table 4. Relationships between gross primary production measured by the ^{18}O method (^{18}O-GPP, mmol O_2 m^{-3} d^{-1}; *x* variable) and gross primary production measured by the O_2 light-dark method (O_2-GPP, mmol O_2 m^{-3} d^{-1}; *y* variable) calculated according to functional regressions, showing 95% confidences limits (CL) of slopes and *y*-intercepts, number of points (N) and significance levels (p). ns: slope of regression not significantly different from 0 ($p > 0.05$)

Stn	Slope	95% CL	Intercept	95% CL	N	p
Randers						
Entire data set	**1.95**	**1.68, 2.23**	**−2.00**	**−5.50, 1.50**	**24**	**<0.0001**
2	0.99	0.09, 1.89	0.53	−7.15, 8.22	4	0.0423
4	1.64	0.64, 2.64	−3.04	−19.59, 13.51	4	0.0195
4a	2.17	1.10, 3.25	−6.22	−30.43, 17.99	4	0.0132
5	2.39	0.67, 4.12	−2.94	−10.63, 4.76	4	0.0269
6	2.33	1.52, 3.13	−1.76	−8.55, 5.02	4	0.0065
Scheldt 1						
Entire data set	**2.04**	**1.40, 2.70**	**−1.49**	**−2.65, 0.32**	**16**	**<0.0001**
Hansweert	1.22	0.39, 2.04	−0.23	−1.53, 1.07	4	0.0248
Prosperpolder	1.54	0.83, 2.25	−0.51	−1.77, 0.76	4	0.0145
Antwerpen	1.75	−0.74, 4.23	−1.82	−6.70, 3.07	4	0.0948^{ns}
Ruppel	3.01	0.04, 5.98	−2.70	−8.11, 2.72	4	0.0492
Scheldt 2						
Entire data set	**1.13**	**0.83, 1.43**	**1.02**	**−0.80, 2.84**	**20**	**<0.0001**
Hoek van Ossenise	1.07	0.28, 1.86	−0.43	−8.93, 8.08	4	0.0284
Overloop	1.36	0.89, 1.83	−1.63	−5.06, 1.80	4	0.0063
Doel	2.50	1.29, 3.70	−1.23	−4.30, 1.83	4	0.0124
Oesterweel	3.40	2.65, 4.16	−2.47	−4.04, −0.91	4	0.0027
Temse	3.54	0.62, 6.46	−2.60	−9.68, 4.49	4	0.0349

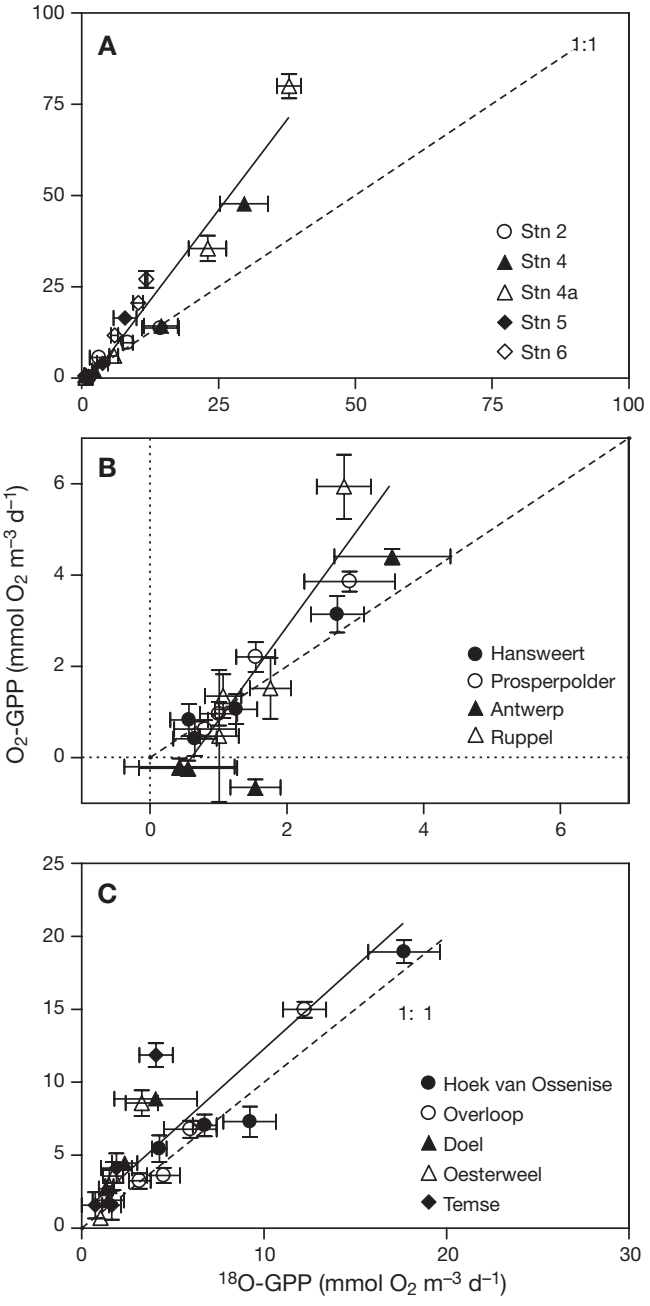

Fig. 4. Relationships between gross primary production measured by the O_2 light-dark method (O_2-GPP) and the ^{18}O method (^{18}O-GPP) in (A) Randers Fjord in August 2001, and (B) the Scheldt estuary in November 2002 (Scheldt 1), and (C) the Scheldt estuary in April 2003 (Scheldt 2). Continuous line: functional regression fit using whole data set; dashed line: 1:1 line

Bay of Villefranche-sur-Mer, France; N. González et al. unpubl.) using the same techniques. As already discussed, the O_2-LD method is based on the assumption that CR is the same in the light and in the dark. It is generally accepted that CR is higher in the light than in the dark because of enhanced mitochondrial respiration and/or photorespiration in the former (Bender et al. 1987). It seems therefore very unlikely that the GPP rates based on O_2-LD were overestimated because of an overestimation of CR measured in the dark. In the Scheldt estuary, we measured nitrification rates in the dark based on the use of inhibitors (nitrapyrine and chlorate). The results showed that nitrification represented up to ~70% of oxygen consumption (Fig. 5A), and therefore is an important process in O_2 dynamics. In contrast to CR, nitrification is inhibited by a factor of 40 to 50% in the light (Horrigan & Springer 1990, Ward 2005, Andersson et al. 2006). Therefore, as nitrification rates increased upstream (as also shown by Gazeau et al. 2005 and Andersson et al. 2006), inhibition in the light might have induced an overestimation of GPP using the O_2-LD method and thus explain the discrepancies between the 2 methods. However, even after correction assuming a 50% inhibition of nitrification in the 100% light compartment, the O_2-LD method nevertheless usually estimates still higher rates than the ^{18}O technique (Fig. 5B).

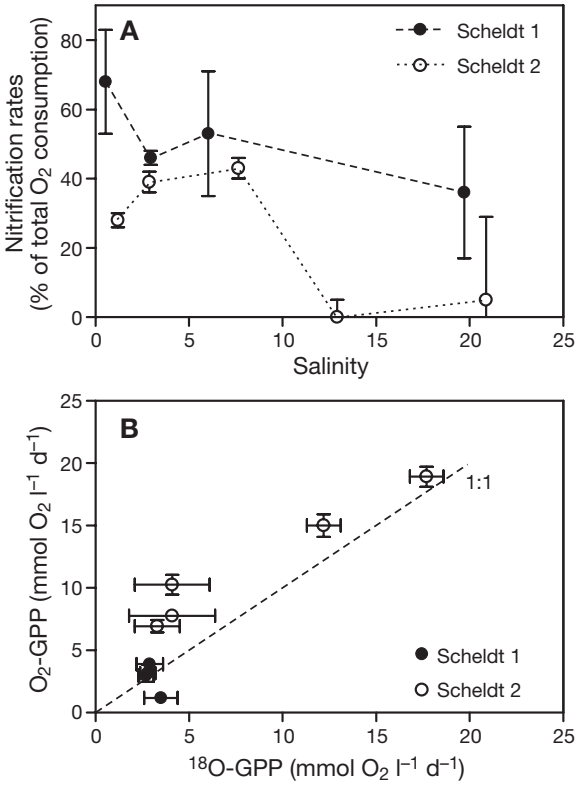

Fig. 5. (A) Contribution of nitrification to total O_2 consumption along the salinity gradient during the 2 Scheldt cruises. (B) Relationships between gross primary production measured in 100% light compartment by the O_2 light-dark method (O_2-GPP) and by the ^{18}O method (^{18}O-GPP) in the Scheldt estuary in November 2002 (Scheldt 1) and April 2003 (Scheldt 2). O_2-GPP rates corrected assuming that nitrification rates were underestimated by a factor of 50% in the light, see 'Results and discussion' for details

The relationships between the ^{14}C and the ^{18}O methods are shown in Fig. 6 and Table 5. Surprisingly, the ^{14}C-PP rates were higher than rates of GPP estimated using the ^{18}O technique. On theoretical grounds, ^{14}C-PP rates are expected to be lower than ^{18}O-GPP rates because of photorespiration and the Mehler reaction, which do not affect the O_2 and carbon fluxes to the same extent. When oxygen levels (Burris 1981) and light intensities (Beardall & Raven 1990) are high, photorespiration (oxygenase activity of RuBisCO) allows the oxidation of ribulose 1,5-biphosphate to produce glycolate and glycerate (Falkowski & Raven 1997). If the glycolate is excreted, then photorespiration is linked to $DO^{14}C$ production; otherwise, the glycolate is further metabolized and used for biosynthesis or (mainly) respired (Falkowski & Raven 1997). Therefore, this process consumes O_2 and does not involve CO_2 fixation (Bender et al. 1999). Similarly, the Mehler reaction (pseudocyclic electron transport) is the direct photoreduction of O_2 by Photosystem I (PSI). It involves an electron transport sequence from the donor side of Photosystem II (PSII) to the reducing side of PSI, where the O_2 generated by the oxidation of water is reduced, ultimately leading to the production of H_2O (Falkowski & Raven 1997). The Mehler reaction does not involve any net O_2 exchange, but it increases the $\delta^{18}O\text{-}O_2$ in the surrounding water because a molecule of labeled O_2 is produced (PSII) and a molecule of unlabeled O_2 is consumed (Laws et al. 2000). Therefore, the Mehler reaction should lead to an overestimation of GPP as measured by the ^{18}O method. It should also provide higher rates than the O_2-LD technique as the latter assumes that light respiration equals dark respira-

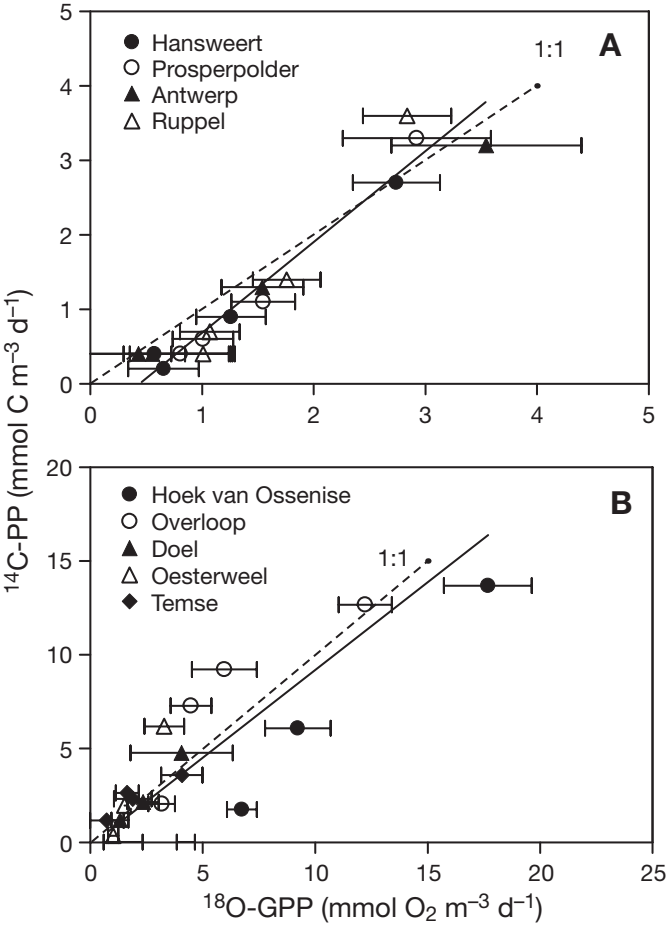

Fig. 6. Relationships between primary production measured by the ^{14}C method (^{14}C-PP) and gross primary production measured by the ^{18}O method (^{18}O-GPP) in the Scheldt estuary in (A) November 2002 (Scheldt 1) and (B) April 2003 (Scheldt 2). Continuous line: functional regression fit using whole data set; dashed line: 1:1 line

Table 5. Relationships between gross primary production measured by the ^{18}O method (^{18}O-GPP, mmol O_2 m^{-3} d^{-1}; *x* variable) and primary production measured by the ^{14}C method (^{14}C-PP, mmol C m^{-3} d^{-1}; *y* variable) in the Scheldt estuary, calculated according to functional regressions, showing 95% confidences limits (CL) of slopes and *y*-intercepts, number of points (N) and the significance levels (p). ns: slope of regression not significantly different from 0 ($p > 0.05$)

Stn	Slope	95% CL	Intercept	95% CL	N	p
Scheldt 1						
Entire data set	**1.22**	**1.04, 1.40**	**−0.54**	**−0.87, −0.21**	**16**	**<0.0001**
Hansweert	1.14	0.72, 1.55	−0.44	−1.09, 0.22	4	0.0072
Prosperpolder	1.40	0.92, 1.88	−0.85	−1.70, 0.00	4	0.0062
Antwerpen	0.92	0.80, 1.04	−0.07	−0.30, 0.16	4	0.0009
Ruppel	1.71	0.98, 2.44	0.78	−2.67, 0.01	4	0.0098
Scheldt 2						
Entire data set	**0.94**	**0.63, 1.25**	**−0.17**	**−2.09, 1.75**	**19**	**<0.0001**
Hoek van Ossenise	1.07	0.68, 1.46	−4.88	−9.10, −0.66	4	0.0072
Overloop	1.12	−0.66, 2.91	0.56	−12.52, 13.65	4	0.1139^{ns}
Doel	1.73	−0.07, 3.53	−2.01	−6.58, 2.56	4	0.0537^{ns}
Oesterweel	2.54	0.55, 4.53	−2.09	−6.43, 2.24	3	0.0393
Temse	0.69	−0.13, 1.51	1.00	−0.99, 2.99	4	0.0692^{ns}

tion although mitochondrial respiration can be enhanced in the light and photorespiration (only in the light) can be an important O_2 pathway. In summary, both the ^{18}O and O_2-LD techniques are expected to give higher rates than the ^{14}C method mainly because of (1) possible respiration of ^{14}C labeled organic matter in the cell to an extent that depends on incubation time, (2) possible excretion of $DO^{14}C$ (which is rarely taken into account), (3) stoichiometric relationships between O_2 and CO_2 (PQ) (which depends on the nature of the nitrogen substrate and of the organic carbon product), and finally (4) photorespiration and the Mehler reaction (with regard to ^{18}O-GPP only) which involve ^{18}O fluxes but no carbon fluxes.

While numerous studies have reported higher ^{18}O-GPP rates in various types of environments (Bender et al. 1987, Grande et al. 1989b, 1991, Kiddon et al. 1995, Bender et al. 1999, Laws et al. 2000, Luz et al. 2002, Juranek & Quay 2005), only 3 previous studies, to the best of our knowledge, reported discrepancies similar to those in our study (Grande et al. 1989a, Ostrom et al. 2005, Yacobi et al. in press). Grande et al. (1989a) suggested that the ^{18}O technique may underestimate GPP due to consumption of labeled O_2 by algal cells. Moreover, they suggested that glycolate produced by photorespiration and excreted may lead to a PQ <1, which could explain the higher values obtained with the ^{14}C method. Ostrom et al. (2005) proposed that the higher rates they obtained by the ^{14}C method than by the ^{18}O method could be due to the consumption of labeled $^{18}O_2$ within the cells and/or by the release of O_2 from supersaturated phytoplankton cells. Considering the low light availability in our systems (high turbidities) and the relatively low O_2 concentrations (all sites were undersaturated with respect to O_2), an important O_2 pathway via photorespiration seems unlikely. Fig. 7 shows the variation in O_2-GPP:^{18}O-GPP ratios as a function of salinity, and its relation to O_2 concentration (water column averages in Randers Fjord). There is a strong correlation between O_2 concentration and the O_2-GPP:^{18}O-GPP ratio ($r^2 = 0.8$; $p < 0.01$). It is therefore possible that due to the relatively low oxygen concentrations in the upper estuary, intracellular O_2 cycling becomes increasingly important towards the upper estuary and results in an underestimation of the rates determined from the increase of $\delta^{18}O$-O_2 in the water.

In conclusion, the ^{14}C and O_2-LD methods gave consistent results in both estuaries. The difference between the 2 methods seems to increase with increasing heterotrophic mineralization, leading to an increased recycling of ^{14}C-labeled organic matter. In the most brackish station of the Scheldt estuary in April, the production of $DO^{14}C$ represented 65% of the total ^{14}C production and explained the strong discrepancy between the 2 methods at this station. Surprisingly, the

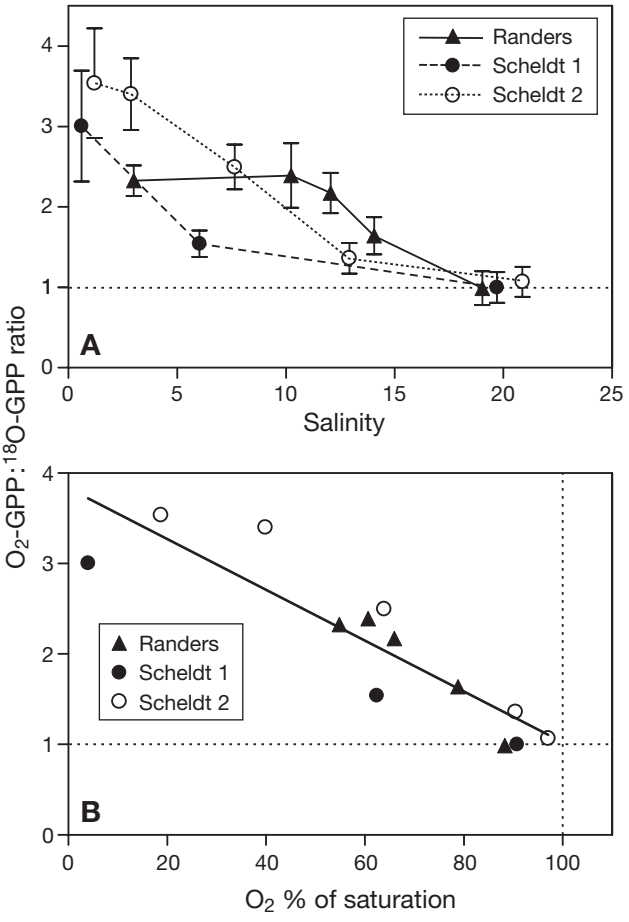

Fig. 7. (A) Ratio between gross primary production measured by the O_2 light-dark method (O_2-GPP) and by the ^{18}O method (^{18}O-GPP) along the salinity gradient in the 2 estuaries. (B) Relationships between O_2-GPP:^{18}O-GPP and O_2 percentages of saturation for Randers Fjord and the Scheldt estuary

^{18}O method, which was expected to provide the highest rates of primary production, provided rates that were often lower than those obtained using the O_2-LD method and even lower than the ^{14}C-based rates. This discrepancy can be attributed to intracellular O_2 recycling in low oxygen waters. Our results are consistent with those obtained in 2 lakes that showed an underestimation of the ^{18}O technique (Ostrom et al. 2005, Yacobi et al. in press) and suggest that the ^{18}O method must be used with extreme care in nutrient-rich, low oxygen systems.

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