

## Unexpected underestimation of primary productivity by $^{18}\text{O}$ and $^{14}\text{C}$ methods in a lake: Implications for slow diffusion of isotope tracers in and out of cells

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### Abstract

By use of in situ bottle incubations, we determined three variables for the characterization of plankton community primary productivity in Lake Kinneret, Israel: (1)  $\text{H}_2^{18}\text{O}$ -based primary productivity ( $^{18}\text{OP}$ ), measured by  $^{34}\text{O}_2$  ( $^{18}\text{O}^{16}\text{O}$ ) evolution; (2) radiocarbon-uptake-based primary productivity ( $^{14}\text{CP}$ ); and (3) net  $\text{O}_2$  production (NOP), calculated from the rate of  $\text{O}_2$ : Ar change. Six experiments were conducted in the fall, a period that is characterized by thermocline deepening, erosion of the hypolimnion, and eventual introduction of high concentration of reduced substances into the epilimnion. An additional experiment was conducted in spring. In comparison to net  $\text{O}_2$  evolution, the tracer-based methods severely underestimated primary productivity in the fall. This is indicated by unusually high NOP :  $^{18}\text{OP}$  and NOP :  $^{14}\text{CP}$  ratios (1–2.2 and 2–4, respectively). The latter is considerably different than the typical values of aquatic environments (~1.4). In contrast to these ratios, the  $^{18}\text{OP}$  :  $^{14}\text{CP}$  appears normal (1.6–2.9) and not significantly different from previous results in the lake. Ammonium was the major nitrogen source in the fall experiments, and there is no reason to assume that the cellular composition of phytoplankton was exceptional. It is therefore unlikely that the high NOP :  $^{14}\text{CP}$  ratio represents genuine ratios of oxygen and carbon metabolism. We suggest that an explanation accounting for the high values of both NOP :  $^{18}\text{O P}$  and NOP :  $^{14}\text{CP}$  can be slow diffusion of the tracers in and out of phytoplankton cells.

Primary productivity is the most common, and often the only, physiological process measured consistently in oceans, lakes, and reservoirs. It is measured either as the rate of oxygen evolution or as carbon uptake, but none of the available methods is free of producing ambiguous information. Determination of primary productivity by measuring time-dependent changes of oxygen concentration in light and dark bottles is a simple method for the estimation of net primary productivity (NOP) and respiration. However, the validity of the latter is questioned because the rate of respiration in dark bottles can be different than the respiration rate in light bottles. From general considerations, the dark rate should be lower than the light rate (Bender et al. 1987; Weger et al. 1989; Dickson and Orchardo 2001), and the result of that handicap is underestimation of gross primary productivity, which is calculated as the sum of net primary productivity

and respiration. An alternative simple method for the estimation of phytoplankton productivity is by  $^{14}\text{C}$  radioactive tracer ( $^{14}\text{CP}$ ). High sensitivity was apparently the main reason for the widespread acceptance of the radiotracer method, which has been in use for more than 50 yr and has been applied in most types of aquatic environments. Several drawbacks are intrinsic to the  $^{14}\text{C}$  method and were actually explicitly outlined in the original paper describing the method (Steeman-Nielsen 1952). Among them is the issue of recycling of the newly incorporated tracer as part of the cellular metabolism, and its export either in respiration or in a soluble form. It is therefore expected that the radiotracer method produces a variable intermediate between net and gross primary productivity, and that the proximity to either pure gross or net value is dependent on the duration of incubation (Laws et al. 2000; Marra 2002) and on its timing (Mingelbier et al. 1994). In our measurements in the productive Lake Kinneret, even 3-h incubations showed that radiocarbon-based productivity closely matches net primary productivity (Luz et al. 2002).

The introduction of a method based on the evolution of the  $^{18}\text{O}$  isotope after addition of  $\text{H}_2^{18}\text{O}$  to incubation bottles enabled direct measurement of oxygen evolution (Bender et al. 1987), thus omitting the uncertainties associated with the use of dark bottles. In the first publication dealing with the use of  $\text{H}_2^{18}\text{O}$  probe, primary productivity (PP) estimates were similar to those found by the light-dark bottle incubations (Bender et al. 1987), but such compatibility is certainly not universal (Bender et al. 1999; Ostrom et al. 2005). Comparisons of the oxygen

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isotope-based technique with the carbon radiotracer method showed that the former is mostly larger (e.g., Bender et al. 1987; Laws et al. 2000; Luz et al. 2002), although one researcher found the opposite to be true (Ostrom et al. 2005). The present consensus is that  $^{18}\text{OP}$  is an estimate of gross  $\text{O}_2$  production and therefore must be larger than NOP because various mechanisms in addition to ordinary respiration consume part of the photosynthetic  $\text{O}_2$  production. In most recent studies,  $^{14}\text{CP}$  is taken as a measure of net carbon fixation. In this case, the expected NOP :  $^{14}\text{CP}$  ratio is approximately 1.4 (Laws 1991).

Occasional comparisons of productivity characteristics by means of all three methods have been done in Lake Kinneret for almost a decade. Throughout that period, in incubations executed in fall, NOP :  $^{18}\text{OP}$  was considerably higher than in other seasons, and sometimes we obtained unexpected measurements in which  $^{18}\text{OP}$  was equal or smaller than NOP, and  $^{14}\text{CP}$  values were unusually low in comparison to NOP. In an attempt to understand whether these unexpected relationships indicate some special limnological situation in fall and are not just a result of experimental errors, we conducted systematic productivity measurements in the fall of 2003 and 2004.

Lake Kinneret is a warm monomictic lake with a surface area of 170 km<sup>2</sup> and mean and maximum depths of 24 and 43 m, respectively. Homothermy occurs between late December and early March, with minimum water temperatures of <15°C. The lake is strongly stratified from about April to December with maximum epilimnetic temperatures reaching 29–30°C. With the onset of stratification, the hypolimnion becomes rapidly anoxic with high concentrations of sulfide (130–250  $\mu\text{mol L}^{-1}$ ) and ammonia (~30–100  $\mu\text{mol L}^{-1}$   $\text{N-NH}_4^+$ ). The dominant phytoplankton from February through May is often the dinoflagellate *Peridinium gatunense* Nygaard, which forms thick blooms and comprises more than 90% of the algal biomass during the bloom and 59–90% on an annual basis. In the recent decade, however, this bloom did not always occur, and other algae were prominent in the phytoplankton assemblage. Nanoplanktonic forms of chlorophytes, diatoms, cyanophytes, and dinoflagellates normally dominate the assemblage from June through January in Lake Kinneret. A detailed description of the lake phytoplankton was recently published (Zohary 2004).

## Methods

**Water sampling and bottle incubation**—Water samples were taken at a pelagic monitoring station (Sta. A) of Lake Kinneret with a 5-L Aberg-Rodhe sampler from several depths of the epilimnion. The details of the water sampling procedure are given in Luz et al. (2002). In short, at each sampled depth, 150-mL subsamples were immediately transferred into evacuated gas extraction vessels (300-mL flasks equipped with Louwers Hapert O-ring stopcocks containing 1 mL saturated  $\text{HgCl}_2$  solution to stop the biological activity), for determination of the initial oxygen isotopes and  $\text{O}_2$ :Ar ratios. Additional set of subsamples was used to fill 130 mL biological oxygen demand (BOD) bottles, and another set of subsamples was used to fill

60 mL poly-carbonate bottles. A duplicate of nonlabeled BOD bottles was deployed in situ, along with duplicates of BOD and poly-carbonate bottles labeled with either  $\text{H}_2^{18}\text{O}$  (~0.2 g, 98%  $^{18}\text{O}$ ) or  $^{14}\text{C}$ , respectively, and incubated at the sampling depths for approximately 3 h (~09:00–12:00 h). After the incubation termination, the content of the BOD bottles was transferred to gas extraction vessels, and the same procedure was used for the initial samples.

**Gas purification and mass spectrometry**—Sample preparation and mass-spectrometry measurements were carried out according to Luz et al. (2002) and Barkan and Luz (2003). The water and headspace in the flask were equilibrated for 24 h at room temperature, and afterwards the water was drawn out of the flasks leaving only headspace gases. The flasks were then connected to a preparation line for the purification of the  $\text{O}_2$ -Ar mixture.

The ratio  $^{18}\text{O}$ : $^{16}\text{O}$  in the purified oxygen-argon mixture (expressed as  $\delta^{18}\text{O}$ ) was measured by dual inlet mass spectrometry on a multicollector instrument (Finnigan MAT Delta<sup>plus</sup>). The ratio  $\text{O}_2$ :Ar was determined from peak switching between  $m/z$  (the mass-to-charge ratio) 32 and 40 and presented as

$$\delta\text{O}_2/\text{Ar} = \left[ \frac{(^{32}\text{O}/^{40}\text{Ar})_{\text{samp}}}{(^{32}\text{O}/^{40}\text{Ar})_{\text{ref}}} - 1 \right] 10^3 \quad (1)$$

The analytical precision of  $\delta^{18}\text{O}$  and  $\delta\text{O}_2/\text{Ar}$  measurements was 0.02‰ and 0.2‰, respectively. In the final results, we introduced corrections for the distribution of gases and isotopes between headspace and water in the sampling flask at room temperature (Luz et al. 2002). The isotopic and  $\text{O}_2$ :Ar ratios are reported with respect to atmospheric  $\text{O}_2$ .

**Net oxygen productivity**—Net  $\text{O}_2$  productivity (NOP) was calculated from  $\delta\text{O}_2/\text{Ar}$  values as in Luz et al. (2002):

$$\text{NOP} = [\text{O}_2]_{\text{initial}} \cdot t^{-1} \cdot \frac{(\delta\text{O}_2/\text{Ar})_{\text{final}} - (\delta\text{O}_2/\text{Ar})_{\text{initial}}}{(\delta\text{O}_2/\text{Ar})_{\text{initial}} + 1000} \quad (2)$$

where  $[\text{O}_2]_{\text{initial}}$  is the oxygen initial concentration; and  $t$  is the duration of the incubation. The values of  $[\text{O}_2]_{\text{initial}}$  were obtained by Winkler titration with precision of 0.2%.

**$^{18}\text{O}$ -based primary productivity**—We used the method of Bender et al. (1987) with modifications as described in Luz et al. (2002). Briefly, ~0.2 g of 98%  $\text{H}_2^{18}\text{O}$  was added to freshly sampled lake water, which was then incubated as described above. The newly produced photosynthetic  $\text{O}_2$  is highly enriched in  $^{18}\text{O}$  and thus causes the  $\delta^{18}\text{O}$  of the dissolved oxygen to increase in proportion to the rate of  $\text{O}_2$  production. Then, the  $\text{O}_2$  production ( $^{18}\text{OP}$ ) is calculated by the equation

$$\begin{aligned} ^{18}\text{OP} = & \left( \{ [\text{O}_2]_{\text{fin}} \times (\delta^{18}\text{O}_{\text{fin}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \} \right. \\ & \left. - \{ [\text{O}_2]_{\text{in}} \times (\delta^{18}\text{O}_{\text{in}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \} \right) \\ & \div (\delta^{18}\text{O}_{\text{w}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \cdot t^{-1} \end{aligned} \quad (3)$$

Table 1. Physical characteristics of Lake Kinneret throughout the period of experiment execution. Indicated is the daily entrainment rate of hypolimnetic water in the epilimnion.

Date	Surface temperature (°C)	Surface light ( $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ )	Mixed layer (m)	Euphotic depth (m)	Oxygen saturation (%)	Entrainment ( $\text{m d}^{-1}$ )
2003						
16 Nov	23.0	804	20	11.4	81	
30 Nov	21.0	628	24	10.8	73	0.16
16 Dec	19.1	920	29	10.8	71	0.13
28 Dec	17.4	809	40	9.6	69	0.20
2004						
5 Dec	19.8	1,050	23	12.6	76	
21 Dec	17.4	470	35	8.7	62	0.27
2005						
27 Mar	18.9	1,481	9	7.5	131	

where  $[\text{O}_2]_{\text{in}}$  and  $[\text{O}_2]_{\text{fin}}$  are the initial and final  $\text{O}_2$  concentrations ( $\text{mmol m}^{-3}$ ), respectively;  $\delta^{18}\text{O}_w$  is the  $\delta^{18}\text{O}$  of the enriched lake water (‰);  $\delta^{18}\text{O}_{\text{in}}$  and  $\delta^{18}\text{O}_{\text{fin}}$  are the initial and final  $\delta^{18}\text{O}$  of dissolved  $\text{O}_2$  (‰), respectively; and  $\delta^{18}\text{O}_{\text{av}} = (\delta^{18}\text{O}_{\text{in}} + \delta^{18}\text{O}_{\text{fin}})/2$ . The fractionation factor  $\epsilon_R$  represents discrimination due to  $\text{O}_2$  uptake in the bottle. Variations in this factor up to  $\pm 20\%$  do not significantly affect the calculated  $^{18}\text{OP}$ , and in the present study, we used  $\epsilon_R$  of  $-21.6\%$  (Luz et al. 2002).  $\delta^{18}\text{O}$  of the spiked water samples was determined by the  $\text{CO}_2$  equilibration method. In order to avoid contamination of the mass spectrometer with highly  $^{18}\text{O}$ -enriched  $\text{CO}_2$  ( $\sim 400\%$ ), the spiked water was diluted (about 1:10) with distilled water of known isotopic composition. This dilution was taken into account in the calculation of the  $\delta^{18}\text{O}$  of the spiked water samples. The  $\delta^{18}\text{O}$  of  $\text{CO}_2$ , equilibrated with diluted spiked water, was measured with a Gas Bench II—DELTA<sup>plus</sup>XL MS with precision better than 0.2‰. Overall, errors based on replicate analyses of  $^{18}\text{OP}$  were less than 10%.

**$^{14}\text{C}$  assimilation**—Carbon uptake rate ( $^{14}\text{CP}$ ) was measured by incubating subsamples of lake water with 0.296 MBq of  $^{14}\text{C}$ -labeled bicarbonate (Steeman-Nielsen 1952) in 60-mL polycarbonate bottles. After incubation, samples were filtered under reduced pressure (13 kPa) onto 25-mm Millipore filters (pore size 0.45  $\mu\text{m}$ ), rinsed with 15 mL of filtered lake water, and fumed in HCl vapor to remove inorganic  $^{14}\text{C}$ . Controls poisoned at zero time by Lugol iodine were run to compensate for nonbiological adsorption. The total  $^{14}\text{C}$  added to each bottle was measured in 0.1-mL portions. The radioactivity in the total and particulate fraction on the filters was determined by liquid scintillation counting with quench correction. The average difference between duplicate measurements of  $^{14}\text{CP}$  was  $\sim 10\%$ .

**Calculations of integrated values**—Areal primary productivity was calculated by integrating the weighed measurements at discrete samples over a water column of 0–15 m. The routine sampling and incubation depths in Lake Kinneret are 0, 1, 2, 3, 5, 7, 10, and 15 m, and those depths were used for the calculation of the radiocarbon-

based integral primary productivity in the euphotic zone. For operational reasons, the BOD bottles used for oxygen metabolism measurement were deployed only in the uppermost 5 or 7 m of the water column. For this reason, we could not directly assess  $^{18}\text{OP}$  at 10 and 15 m. On the basis of the 35-yr record of  $^{14}\text{CP}$  observations in the lake, the integrated rates below 7 m amount to  $<10\%$  of the total primary productivity, so the error introduced by not measuring  $^{18}\text{OP}$  at 10 and 15 m is small. Nevertheless, in an attempt to account for  $^{18}\text{OP}$  in the deeper part of the photic zone, we estimate the rates there by extrapolation from the 1- to 7-m measurements, assuming that the proportion of integrated  $^{18}\text{OP}$  between 7 and 15 m to the overall  $^{18}\text{OP}$  (0 to 15 m) is similar to the same proportion in  $^{14}\text{CP}$ . The daily primary productivity was calculated by multiplying the hourly average by the appropriate photoperiod.

**Chlorophyll *a***—For the determination of chlorophyll *a* (Chl *a*), samples were processed in the laboratory  $\sim 1$  h after collection. Particulate matter was collected by filtration of water samples onto glass-fiber filters (Whatman GF/C), ground in 90% acetone, and left overnight at 4°C in the dark. Chl *a* concentration was determined fluorometrically (Holm-Hansen et al. 1965) after clarification of the extract by centrifugation of 3 min at  $1,100 \times g$ .

## Results

Six experiments were conducted throughout the period of thermocline deepening in the lake (fall 2003 and 2004) and one in March 2005, when the thermocline just started to reform after the winter holomixis period. All the data are listed in Tables 1, 2, and 3.

**Limnological background**—The experiments in 2003 and 2004 were done throughout periods of epilimnetic temperature decline and thermocline deepening (Fig. 1, Table 1). Pertaining to those characteristics of Lake Kinneret, in the fall is the decline of oxygen saturation level in the epilimnion. That decline is attributed mainly to the reaction of dissolved oxygen with reduced substances—hydrogen sulfide, ammonia, and organic matter, entrained in

Table 2. Chlorophyll *a* (Chl *a*, mg m<sup>-2</sup>) and primary productivity (mmol m<sup>-2</sup> d<sup>-1</sup>) integrated over the euphotic depth in Lake Kinneret. Maximal photosynthetic rate (mg C mg Chl<sup>-1</sup> h<sup>-1</sup>) is calculated from the radiocarbon-based primary productivity estimate.

Date	Chl <i>a</i>	Max. photosynthetic rate	NOP	<sup>14</sup> CP	<sup>18</sup> OP	NOP : <sup>14</sup> CP	NOP : <sup>18</sup> OP
2003							
16 Nov	43	4.9	147	72	117	2.0	1.3
30 Nov	65	3.7	301	75	139	4.0	2.2
16 Dec	73	4.5	196	84	150	2.3	1.3
28 Dec	75	4.5	242	112	196	2.2	1.2
2004							
5 Dec	88	2.0	87	35	82	2.5	1.1
21 Dec	149	1.7	183	64	184	2.9	1.0
2005							
27 Mar	440	0.6	157	158	373	1.0	0.4

epilimnetic waters with the erosion of the hypolimnion (Nishri et al. 1998). The overall oxygen reservoir showed a temporal trend of increase in the two fall periods, despite the trend of reduction in oxygen saturation level, a result of the increase in epilimnion volume with the deepening of the thermocline. The experiment in March 2005 was done when the epilimnion was still relatively narrow, as is usually the case with onset of thermal stabilization in the lake.

*Productivity measurements*—The experiments in fall 2003 were conducted when Chl *a* concentrations were relatively low and close to the multiannual average recorded in Lake Kinneret. The same Chl *a* concentration prevailed on 5 December 2004, but on 21 December, Chl *a* concentration was high (Table 2) and was composed mainly of dinoflagellates. On 27 March 2005, Chl *a* concentration was very high, composed mainly of the filamentous chlorophyte *Debarya* sp. Photosynthetic rate, calculated on the basis of radioactive carbon uptake, showed that considering photosynthetic efficiency the phytoplanktonic population throughout the fall of 2003 were at least a factor of two higher than those of the fall of 2004 and a factor of more than eight higher than in March 2005 (Table 2).

The vertical distribution of productivity showed a typical subsurface peak located at 1 or 2 m (Fig. 1). Exceptional was the experiment on 12 December 2004, when productivity was highest at the lake surface (Fig. 1b), probably due to the low solar energy on that day (Table 1). <sup>18</sup>O-based productivity exceeded the radiocarbon based measurements in all pairwise comparisons (Figs. 1 and 2a), save one exception on 28 December 2003 (Fig. 1a). The average ratio of <sup>18</sup>OP : <sup>14</sup>CP was 1.9, a value not conspicuously different from a previous record in Lake Kinneret (Luz et al. 2002). The average in the years 1996–1999 and 2000–2001 was 2.1 and 2.6, respectively. However, exceptionally high ratios (~8) were recorded when almost a pure and dense crop of the dinoflagellate *Peridinium gatunense* prevailed in the lake (Chl *a* concentrations were in the range of tens and hundreds) in 1996–1999 (Luz et al. 2002). A high ratio of <sup>18</sup>OP : <sup>14</sup>CP (>10) was also found in January 2001, when the large diatom *Aulacoseira granulata* dominated the phytoplankton (Yacobi et al. 2002). In the current set of

experiments, we did not find a prominently high value of <sup>18</sup>OP : <sup>14</sup>CP, but the highest ratio of 2.9 was on 21 December 2004 (Fig. 1b), when *P. gatunense* was the dominant phytoplankton.

NOP : <sup>14</sup>CP ratio was close to 1 in March (Fig. 2b), conforming to the idea that the radiocarbon-based method is often an estimate of net O<sub>2</sub> primary productivity (Bender et al. 1999; Luz et al. 2002). On the other hand, in 2003 and 2004, fall measurements, NOP : <sup>14</sup>CP was ≥1, and in average 2.2 (Fig. 2b; Table 2). An even more unusual outcome of the fall experiments presented herein is that NOP was either greater or equal to <sup>18</sup>OP in all six experiments performed in the fall of 2003 and 2004 (Figs. 1 and 2c; Table 2), with an average NOP : <sup>18</sup>OP of 1.1. The result of the March experiment was conspicuously different, with a ratio of 0.4. The ratios of NOP to both <sup>18</sup>OP and <sup>14</sup>CP showed a trend of increase with depth in all fall experiments, but not in March (Fig. 3). The increase of these ratios was relatively slight but constant in the 0–3-m water column, and it showed a more prominent decline with the deepening of the incubation depth from 3 to 5 m, and even more conspicuously in the comparison of 5- and 7-m incubations that were done in 2003.

## Discussion

In our fall experiments, the ratio between primary productivity estimated in experiments with artificial spikes and net O<sub>2</sub> evolution was unusually low. This may indicate underestimation of primary production by the spike methods or overestimation of O<sub>2</sub> evolution. Alternatively, we can question the quality of our experimental results. However, on the basis of our experience in both field and laboratory methods, we tend to eliminate the possibility of operational errors. Clear evidence supporting this statement comes from independent experiments in seasons other than fall, where we have previously shown normal trends (Luz et al. 2002), as we do here.

Starting with NOP, we are not aware of any mechanism other than operational error that can introduce O<sub>2</sub> whose origin is other than photosynthesis. So overestimation of NOP is unlikely. On the other hand, we cannot rule out the

Table 3. Database for primary productivity estimates in Lake Kinneret.  $[O_2]$  ( $\text{mol m}^{-3}$ );  $\delta^{18}O_{\text{diss}}$  and  $\delta O_2/\text{Ar}$  ratio ( $\text{‰}$  vs air  $O_2$ );  $^{14}\text{CP}$ ,  $^{18}\text{OP}$ , and  $\text{NOP}$  in units of  $\text{mmol m}^{-3} \text{h}^{-1}$ .

Date	Depth	$[O_2]$	$\delta^{18}O_{\text{diss}}$	$\delta O_2/\text{Ar}$	$^{14}\text{CP}$	$^{18}\text{OP}$	$\text{NOP}$
16 Nov 03	0	223.7	0.32	-260.7	1.27	1.28	1.18
	1	224.2	0.25	-259.2	1.54	2.68	2.95
	2	223.3	0.22	-262.1	1.68	2.82	2.91
	3	221.7	0.26	-267.4	1.25	2.33	2.65
	5	221.1	0.35	-269.5	0.53	0.78	1.28
	7	221.0	0.35	-269.6	0.27	0.50	0.87
	10				0.10		
30 Nov 03	15				0.04		
	0	211.4	1.51	-328.0	1.66	2.59	3.27
	1	210.5	1.71	-331.3	1.85	2.76	4.18
	2	211.4	1.40	-327.6	1.73	2.92	5.20
	3	211.1	1.46	-328.4	1.40	2.86	4.65
	5	208.2	1.65	-337.8	0.59	1.44	3.19
	7	207.1	1.65	-341.3	0.24	0.65	2.70
16 Dec 03	10				0.11		
	15				0.04		
	0	212.4	2.33	-349.0	2.47	3.03	4.40
	1	212.8	2.23	-347.7	2.40	4.35	4.78
	2	212.8	2.29	-347.9	2.16	3.60	4.65
	3	212.9	2.22	-347.5	1.35	2.90	3.46
	5	210.5	2.42	-356.3	0.55	1.04	1.47
28 Dec 03	7	211.4	2.19	-353.4	0.23	0.50	0.92
	10				0.06		
	15				0.03		
	0	213.1	2.53	-369.1	2.65	1.99	4.58
	1	211.4	3.15	-373.9	3.08	5.38	5.87
	2	211.4	2.77	-374.0	2.93	5.04	5.51
	3	211.3	2.72	-374.3	1.96	3.66	4.05
05 Dec 04	5	210.7	2.90	-376.1	0.82	2.07	2.39
	7	209.0	3.13	-381.1	0.36	0.75	1.27
	10				0.12		
	15				0.05		
	0	223.1	1.88	-306.7	0.61	1.40	1.66
	1	221.3	1.82	-312.2	0.86	2.18	2.43
	2	222.7	1.66	-306.7	1.08	2.31	2.10
21 Dec 04	3	220.7	1.82	-312.8	0.66	1.44	1.31
	5	220.3	1.78	-314.2	0.22	0.64	0.71
	7	222.6	1.60	-306.9	0.11		
	10				0.04		
	15				0.02		
	0	193.7	4.85	-426.6	2.89	7.67	7.68
	1	192.8	4.01	-429.2	2.31	6.24	5.49
27 Mar 05	2	190.5	4.32	-435.8	1.67	3.74	3.54
	3	189.7	4.25	-438.2	0.85	2.67	3.37
	5	189.7	4.36	-432.4	0.27	1.66	1.18
	7				0.09		
	10				0.04		
	15				0.03		
	0	393.7	-4.07	201.76	2.84	6.42	2.07
27 Mar 05	1	396.7	-4.16	208.40	3.80	8.37	3.75
	2	400.1	-4.21	214.00	3.23	7.92	3.48
	3	400.4	-4.213	209.9	2.24	6.21	2.72
	5	386.7	-3.028	120.5	1.03	2.46	0.66
	7				0.40		
	10				0.10		
	15				0.01		

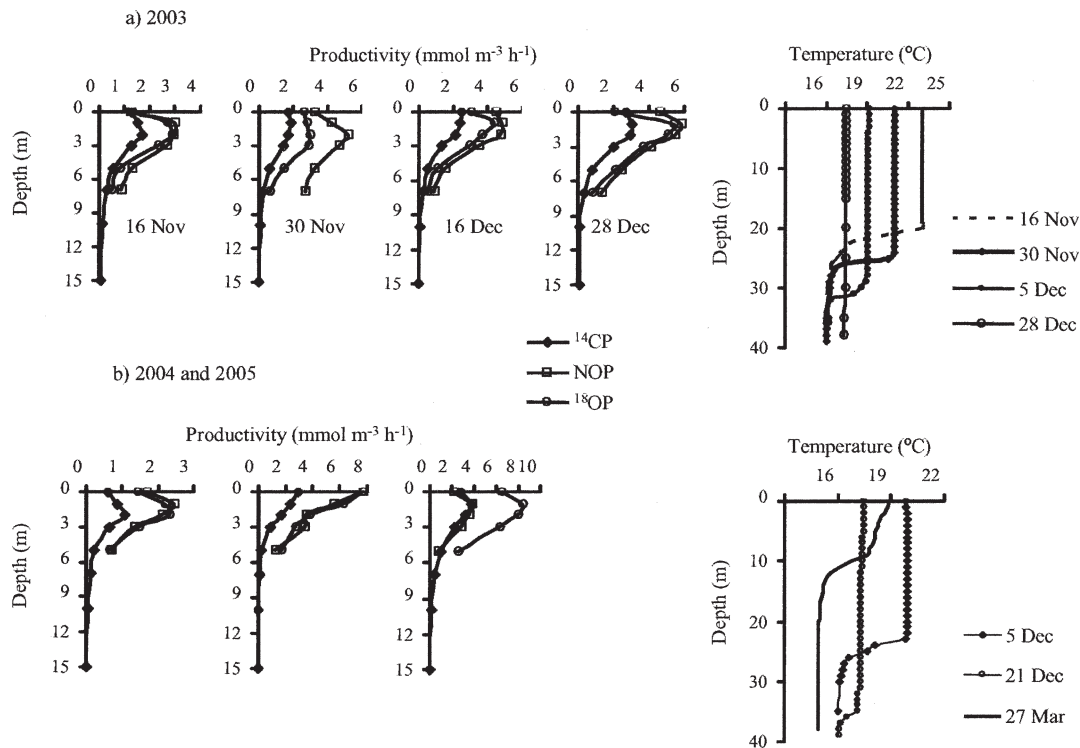


Fig. 1. Primary productivity and temperature profiles in Lake Kinneret. Primary productivity was estimated by means of three different methods: (1)  $^{18}\text{O}$ -based primary productivity ( $^{18}\text{OP}$ ), using  $\text{H}_2^{18}\text{O}$  tracer; (2) net primary productivity (NOP), calculated as time-dependent difference between initial and final oxygen concentration; and (3) radiocarbon-uptake-based primary productivity ( $^{14}\text{CP}$ ), using labeled sodium bicarbonate.

possibility that our measurements underestimate NOP if some of the dissolved  $\text{O}_2$  in the bottles was used for oxidation of dissolved organic carbon in addition to plankton respiration. In this case, both  $\text{NOP}:\text{P}^{14}\text{C}$  and  $\text{NOP}:\text{NOP}^{18}\text{OP}$  ratios will become even higher. Therefore, to explain our observation, we will look for possible mechanisms that will lead to underestimation of both  $\text{P}^{14}\text{C}$  and  $^{18}\text{OP}$ .

The  $\text{NOP}:\text{P}^{14}\text{C}$  ratios in fall range from 2 to 4. According to Laws (1991) and Williams and Robertson (1991), the ratio of net  $\text{O}_2$  production to net carbon fixation (PQ) in many aquatic systems is about 1.4. Relatively small (up to  $\pm 0.3$ ) deviations from the 1.4 ratio are possible as a result of changes of cellular composition of phytoplankton under different environmental conditions and also as a result of variations in the available nitrogen source. For example, if the source of nitrogen is ammonia, the expected PQ is  $\sim 1$ , whereas a PQ of  $\sim 1.3$  is expected if the nitrogen source is nitrate. Somewhat higher ratios are possible if cells contain large amounts of lipids. Therefore, the very high  $\text{NOP}:\text{P}^{14}\text{C}$  ratios in our fall experiments are unlikely to represent a genuine relationship between  $\text{O}_2$  evolution and carbon fixation. It is far above the highest constraint of chemical cell composition, and there is no particular reason to assume that the phytoplankton cell composition is conspicuously exceptional during fall in the lake. Moreover, it should be skewed toward the lower values, because the most abundant nitrogen form in Lake Kinneret in the fall is ammonium after the deepening of the thermocline and entrainment of hypolimnetic water in the euphotic zone (McCarthy et al. 1982).

The  $\text{NOP}:\text{NOP}^{18}\text{OP}$  ratios of 1–2.2 in the fall experiments certainly are not values that represents genuine oxygen metabolism because it is highly unlikely that in a productive ecosystem like Lake Kinneret, oxygen consumption is zero, and certainly NOP greater than gross  $\text{O}_2$  production is absurd. It is interesting that the  $\text{NOP}:\text{NOP}^{18}\text{OP}$  ratios we measured in the fall of 1998 (Luz et al. 2002) were on average about 0.77. Although this value is less than in the present study, it is considerably larger than the average (0.43) during other seasons in 1997 and 1998 (the 0.43 value was calculated after excluding samples incubated at low illumination where NOP was negative). This again suggests underestimation of primary productivity in fall, although the discrepancy was not as extreme as in 2003–2004. In turn, the 0.43 value falls in the range of 0.32 to 0.48 obtained during upwelling in the Arabian Sea (Dickson et al. 2001); Equatorial Pacific (Bender et al. 1999); in the North Atlantic spring bloom (Bender et al. 1992; Kiddon et al. 1993); and in the Ross Sea (Bender et al. 2000). Much lower values (0–0.2) were observed during the termination of the spring diatom bloom on the west Florida shelf (Hitchcock et al. 2000), in the oligotrophic offshore regions of the Arabian Sea (Dickson et al. 2001) during the austral summer across the Antarctic Polar Front (Dickson and Orchardo 2001) and in the Southern Ocean (Hendricks et al. 2004). Thus, fall measurements in Lake Kinneret stand out as an extreme case of low  $^{18}\text{OP}$  in comparison to NOP.

The most conspicuous difference between NOP estimation in our study and both  $^{14}\text{CP}$  and  $^{18}\text{OP}$  is that the latter two are based on penetration of tracers into cells and then

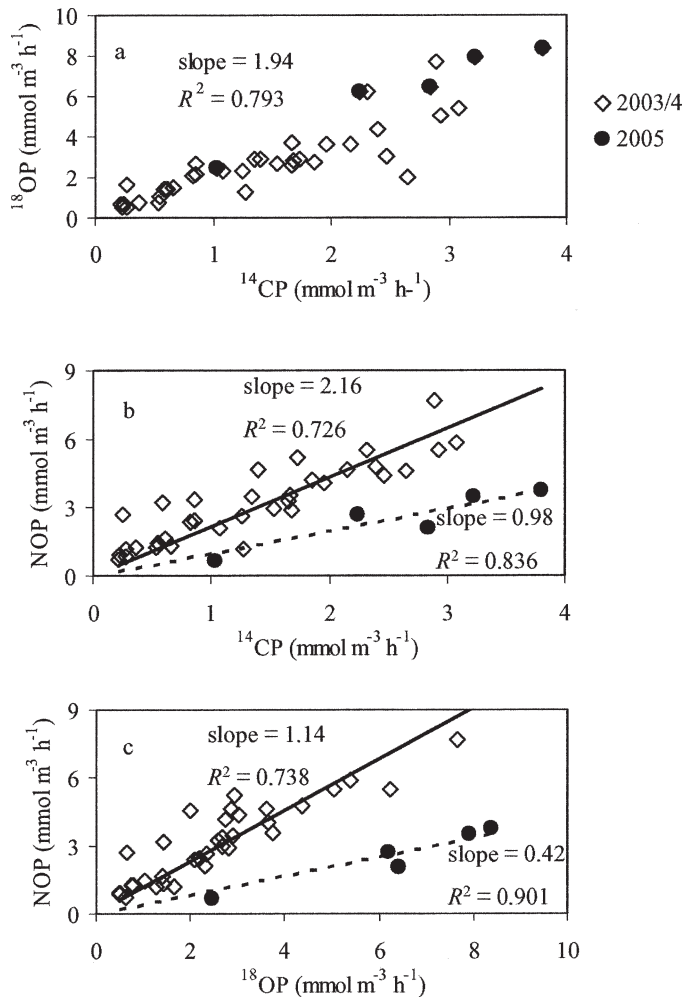


Fig. 2. Pairwise comparison between (a) radiocarbon-uptake-based primary productivity ( $^{14}\text{CP}$ ) to  $^{18}\text{O}$ -based primary productivity ( $^{18}\text{OP}$ ), using  $\text{H}_2^{18}\text{O}$  tracer; (b) net primary productivity (NOP), calculated as the time-dependent difference between initial and final oxygen concentration, to radiocarbon-uptake-based primary productivity ( $^{14}\text{CP}$ ), and (c) net primary productivity (NOP) to  $^{18}\text{O}$ -based primary productivity, in March 2005, and in the fall experiments of 2003 and 2004.

on either  $^{14}\text{C}$  fixation or  $^{34}\text{O}_2$  ( $^{18}\text{O}^{16}\text{O}$ ) evolution. Implicit in methods based on tracer addition is the assumption that the added moiety is uniformly distributed, and its uptake/release proceeds linearly with time. If, however, there is an obstacle to tracer penetration into cells such that the intracellular concentrations of labeled bicarbonate and water are less than in the medium, calculated rates assuming uniform tracer distribution will underestimate the true rates. This is quite similar to the explanation of carbon fixation underestimation caused by intracellular pools of unlabeled carbon (e.g., Berman-Frank and Erez 1996). Such pools, however, will not explain underestimation by the  $^{18}\text{O}$  method where the label is  $^{18}\text{O}$ -enriched water. Admittedly, the difficulty with our explanation is that a diffusion barrier to tracer penetration is not expected to have equal effect on water and dissolved bicarbonate.

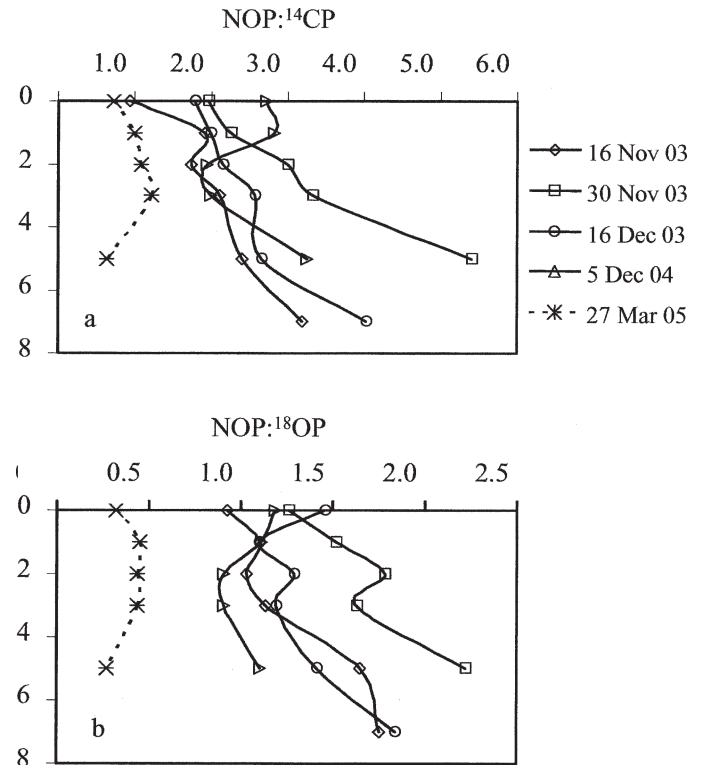


Fig. 3. Depth-dependent change of the ratio of net primary productivity (NOP), calculated as the time-dependent difference between initial and final oxygen concentration to the tracer methods: (a) radiocarbon-uptake-based primary productivity ( $^{14}\text{CP}$ ), and (b) to  $^{18}\text{O}$ -based primary productivity, in: 16 November 2003, 30 November 2003, 16 December 2003, 05 December 2004, and 27 March 2005.

Another way to explain underestimation of primary production by the  $^{18}\text{O}$  method was put forward by Ostrom et al. (2005). They suggested internal consumption of newly formed  $^{18}\text{O}$  labeled  $\text{O}_2$  inside the cells. For effective operation of this mechanism, diffusion of  $\text{O}_2$  in and out of cells must be slow such that the internal concentration of labeled  $\text{O}_2$  is considerably greater than in the ambient water. So in either way, some barrier to diffusion of tracers into or out of the cell must exist.

Another peculiar feature in fall is the increasing NOP: $^{18}\text{OP}$  ratios with depth, which is unlikely to represent accurate metabolic rates. The observed vertical trend cannot be explained by light variation with depth. If anything, it should induce an opposite effect as a result of increasing proportion of the respiration component with depth. The question then is why the rate of increase in  $\delta^{18}\text{O}$  of dissolved  $\text{O}_2$  in the fall incubation experiments is slower than expected and why the discrepancy tends to become more pronounced with depth. We suggest that the unusual results seen in the current study may be the existence of an obstacle to the penetration of water and dissolved  $\text{CO}_2$  (and thus the  $^{18}\text{O}$  and  $^{14}\text{C}$  tracers) into cells, as well as the escape of newly formed labeled  $\text{O}_2$  into the medium.

A situation of slowing down of solutes transfer in and out a cell may occur after the formation of diffusive

boundary layers as happens when cells aggregate and/or are coated by extracellular polymers (Ploug et al. 1999). Aggregation, and cell coating is fostered by the presence of transparent polysaccharide and proteinaceous particles, which are found in natural aquatic systems (Long and Azam 1996; Simon et al. 2002). The polysaccharide particles are sulfated carbohydrates and known to be surface-active and inducing coagulation (Zhou et al. 1998). Much has been learned about their role as agents of aggregate formation (Kepkay 1994), but they well may be also blockers for membrane permeability, coating cell surface.

In a study done many years ago in Lake Kinneret, the highest percentage of freshly fixed  $^{14}\text{C}$  found in the reservoir of dissolved organic matter occurred in the fall (Berman 1976). It is interesting that in the same season, concentrations of transparent exopolymer particles found in the lake epilimnion were highest (Berman and Viner-Mozzini 2001). We note that in the fall, the lake overturns and the epilimnion becomes enriched in hydrogen sulfide (and other reduced substances) from the hypolimnion (Nishri et al. 1998). Hydrogen sulfide supply was documented as an inducer of transparent exopolymer particles formation in diatoms in a marine system (Ciglencki et al. 2003), and it also may be a factor that enhances the formation of the transparent particles in Lake Kinneret.

It is noteworthy that both  $\text{NOP} : ^{14}\text{CP}$  and  $\text{NOP} : ^{18}\text{OP}$  increased with depth in our experiments (Fig. 3). This depth trend, as discussed above, cannot be explained by changes in light intensity. In turn, it is compatible with proximity to the hypolimnion, the source of reduced compounds and associated organisms. The depth trends then are another indication, in addition to the seasonal variations, for the effect of mixing of surface communities with hypolimnetic water. We also note that  $\text{O}_2$  consumption by reduced substances in incubation bottles should be proportional to the depth of sampling and cause a decrease in the value of NOP. If that actually occurred in our fall experiments the trend of increase of  $\text{NOP} : ^{18}\text{OP}$  with depth even more emphasizes the underestimation of  $^{18}\text{OP}$  in our study.

To summarize, on the basis of measurements done in the past (Luz et al. 2002) and experiments presented in the current study, it was shown that  $\text{NOP} : ^{18}\text{OP}$  in fall is always high and sometimes reaches extremely high values (1.0–2.2). We suggest that a possible reason for these high values and, in general, underestimation of primary production from  $^{18}\text{O}$  and  $^{14}\text{C}$  experiments is slow tracers diffusion in and out of phytoplankton cells induced by lakes overturn and contact of epilimnetic plankton with low  $\text{O}_2$  levels and reduced compounds from the hypolimnion. It is important to mention that underestimation of primary productivity by the  $^{18}\text{OP}$  method has been recently recorded in two European estuaries and the apparent underestimation was positively correlated with the level of oxygen deficit (Gazeau F. pers. comm.). Thus, the association of discrepancies among proxies of primary productivity with contact of surface plankton with reduced compounds may be a general phenomenon. However, further study in other aquatic systems is necessary in order

to verify this association. In addition, it will be necessary to study the cause of the discrepancy and underestimation in controlled laboratory experiments.

We have shown that in comparison to net  $\text{O}_2$  evolution, both  $^{14}\text{CP}$  and  $^{18}\text{OP}$  severely underestimated primary productivity in Lake Kinneret during fall. This is indicated by exceptionally high  $\text{NOP} : ^{18}\text{OP}$  and  $\text{NOP} : ^{14}\text{CP}$  ratios. It is unlikely that the high  $\text{NOP} : ^{14}\text{CP}$  ratio in the fall experiments results from neither unusually highly oxygenated nitrogen source (because ammonium was the main available nitrogen resource) nor from exceptional cellular composition. An explanation accounting for the high values of both  $\text{NOP} : ^{18}\text{OP}$  and  $\text{NOP} : ^{14}\text{CP}$  may be slow diffusion of the tracers in and out of phytoplankton cells. A possible obstacle to tracer diffusion may be excretion and coating of cells by extracellular polymers.

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