

Evaluation of community respiratory mechanisms with oxygen isotopes: A case study in Lake Kinneret

Boaz Luz, Eugeni Barkan, and Yftach Sagi

The Institute of Earth Sciences, Hebrew University, Jerusalem 91904, Israel

Yosef Z. Yacobi

Israel Oceanographic and Limnological Research, Kinneret Limnological Laboratory, POB 447, Migdal 14950, Israel

Abstract

Gross and net O₂ production between May 1996 and February 1999 was determined in bottle incubation experiments with H₂¹⁸O spike and from the change in O₂ concentration. Carbon fixation rates were obtained from ¹⁴C incubations. In general, production rates determined using the H₂¹⁸O-spike were about twice the primary production determined by the ¹⁴C method, where the latter was close to net oxygen evolution. These relationships are similar to results for the open ocean. During the spring bloom, when the dinoflagellate *Peridinium* was abundant, the ratio of gross O₂ production to carbon fixation was about 7.5, and net O₂ production was greater than carbon fixation. The difference between O₂ gross production and carbon fixation results, at least in part, from uptake by Mehler reaction and from recycling of the ¹⁴C tracer by dark respiration and the alternative oxidase (AOX). We used the difference in isotopic discrimination against ¹⁸O, occurring during O₂ consumption by various biological pathways, to place constraints on the relative engagement of these pathways. We estimated the overall discrimination against ¹⁸O in the lake from O₂ isotopic mass balance as 20.5–29‰. The only mechanism that can explain the strong overall fractionation in the lake is AOX, which strongly discriminates against ¹⁸O (~31‰). Our results show, for the first time, that uptake by AOX is widespread and quantitatively important to oxygen consumption in aquatic systems.

Oxygen exchange by photosynthesis and respiration is the largest biogeochemical cycle in aquatic systems. In order to understand this cycle, it is necessary to know the gross rates of the major processes involved in oxygen production and uptake. Production of O₂ is known to occur in one process in photosystem II, but O₂ consumption in aquatic organisms is possible by several reactions. These include ordinary respiration through the cytochrome oxidase pathway, respiration by the alternative oxidase pathway, Mehler reaction, and photorespiration. The first two processes take place in light as well as in dark conditions, whereas the latter two occur only under illumination. Although the presence of the above mechanisms has been established in different studies, their quantitative importance in the overall O₂ uptake in aquatic systems is not well known, and it is necessary to assess their role in natural environments. In this respect, ordinary O₂ incubation methods, which are very useful for the assessment of photosynthetic production from light- and dark-incubation experiments (e.g., Williams and Purdie 1991), do not provide the necessary information. The main drawback of these methods is their inability to measure the rate of O₂

uptake in the light, and it is unavoidably assumed that the rates of dark and light uptakes are equal. The rates of gross production as well as light O₂ uptake can be estimated in field incubation experiments using H₂¹⁸O as a spike (e.g., Bender et al. 1987, 1999; Luz and Barkan 2000). However, this method alone cannot help to characterize the type of the respiratory mechanisms involved in aquatic O₂ uptake.

In an attempt to evaluate the significance of the uptake mechanisms, we have taken a different approach, which is based on the difference in discrimination against ¹⁸O occurring during O₂ consumption by the various biological pathways. The discrimination against ¹⁸O associated with the cytochrome oxidase pathway is ~18‰ (Guy et al. 1992), but with the cyanide-resistant alternative oxidase pathway (AOX) it is much greater: ~31‰ in green tissues and ~26‰ in nongreen tissues (Robinson et al. 1992). The discrimination in the Mehler reaction is ~15‰ and in photorespiration ~21‰ (Berry 1992; Guy et al. 1993). Thus, if the isotopic discrimination of the entire plankton community is derived, depending on its magnitude, it is possible to evaluate the effects of the different respiratory mechanisms.

We have chosen to conduct our study in Lake Kinneret (the Sea of Galilee), where biological production and respiration are high and their chemical and isotopic signatures are easy to measure. In addition, wind speed is continuously monitored near the lake, and ¹⁴C primary production is determined routinely by the Kinneret Limnological Laboratory. We have used bottle incubation and lake surface variations in O₂/Ar along with lake-air gas exchange rates for estimating O₂ gross production, net production, and community O₂ uptake rates. These data together with the ratio of ¹⁸O/¹⁶O of dissolved O₂ were used as input parameters to an isotopic mass balance model in order to derive the isotopic fractionation of the

Acknowledgments

We thank Y. Geiffman and the Mekorot Watershed Unit for provision of wind data and extend special thanks to the Kinneret Limnological Laboratory skipper M. Hatab. We are grateful to Associate Editor R. J. Geider and two anonymous reviewers whose suggestions significantly improved the paper.

Support for this research was provided by grants from U.S.A.–Israel Binational Science Foundation, Israel Science Foundation, Germany–Israel cooperation in marine sciences (MARS2), and the Moshe Shilo Minerva Center. The work of Y.S. was facilitated by a scholarship from the Admiral Yohai Ben-Nun Foundation for Marine and Freshwater Research.

entire community. The study was conducted between May 1996 and February 1999.

The study site

Lake Kinneret is located in the Syrian–African Rift Valley at about 210 m below sea level. From December until mid-March the lake is homothermal, whereas during the rest of the year, it is stratified with a distinct seasonal thermocline. The epilimnion is characterized by relatively high pH (8.2–9.6) and high dissolved O₂ (200–250 μmol kg⁻¹). In summer, the hypolimnion is anoxic with lower pH (7.3–8.0). More details on the hydrography and chemistry of this lake are given in Eckert and Hambright (1996) and Nishri et al. (1997).

When the hypolimnion becomes anoxic, nitrate reducers are followed by sulfate-reducing bacteria that carry out the biodegradation of organic matter. This leads to hypolimnetic accumulation of H₂S and NH₄⁺ (Eckert and Truper 1993), and both compounds are rapidly oxidized when brought in contact with O₂-rich water during the winter overturn. Additional oxidation of reduced substances occurs in summer by mixing of epilimnetic with hypolimnetic water because of seiches in the lake (Ostrovsky et al. 1996).

Berman et al. (1995) described the seasonal abundance and distribution of phytoplankton in the lake. In general, the dominant phytoplankton from February through May is usually the dinoflagellate *Peridinium*, which forms thick blooms and comprises more than 90% of the algal biomass during the bloom and 59–90% on an annual basis. By the end of spring, the bloom usually crashes and the phytoplankton becomes dominated by diverse nanoplanktonic species and relatively low biomass community (20–40 g m⁻² [wet wt]). Chlorophytes, diatoms, cyanophytes, and dinoflagellates normally dominate the assemblage from June through January.

Field and laboratory methods

Water sampling and gas purification—Sampling was done at Sta. A located in the deepest part of Lake Kinneret (~43 m) and represents pelagic waters in the lake.

Lake water was collected with a 5-L Aberg–Rhode sampler. Water samples were transferred immediately into evacuated gas extraction vessels (300-ml flasks with Louwers Hapert® O-ring stopcocks) containing 1 ml of a HgCl₂-saturated solution. In the course of testing, we found that the stopcocks may leak slightly during long storage. To prevent these leaks, water was filled in the neck of the stopcocks and suction was applied to eliminate all air bubbles that tend to stick to the O-ring. The “water lock” was retained in place by covering the port of the stopcocks with flexible plastic caps. Flasks stored in this way can be safely kept for several months.

The following procedure was used for water sampling. (1) The flask was well evacuated and closed with a water lock. (2) At the site of water collection, the flask was attached to the water sampler, and the water from the sampler replaced the distilled water in the valve’s port. The flask was then opened, and about 150 ml water was sucked in while leaving

150 ml headspace. (3) The stopcock was closed and the port was refilled with distilled water and capped.

Following Emerson et al. (1991), with small modifications, the water and headspace in the sampling flasks were equilibrated for 24 h at room temperature. After equilibration, the water was sucked out of the flasks leaving only headspace gases. The flasks were then connected to a preparation system for the purification of O₂ and Ar (Luz et al. 1999). After purification, the O₂–Ar mixture was transferred to stainless steel holding tubes for further mass spectrometric measurements.

Mass spectrometry—δ¹⁸O of O₂ in a purified oxygen–argon mixture was determined by dual inlet mass spectrometry (on Finnigan Delta-Plus or Balzers QMG-421 mass spectrometers). The O₂/Ar ratio was determined on the same samples by sequentially measuring m/z 32 and 40 in one collector. Corrections were applied in order to account for the sensitivity of ionization efficiencies of the isotope species of oxygen to variations in the O₂/Ar ratio. The analytical precision (standard error) of δ¹⁸O measurements was 0.01‰ (Delta-Plus) or 0.04‰ (QMG-421). The precision of δO₂/Ar was 0.1‰ on both instruments.

In the final results, we introduced corrections for the distribution of gases and isotopes between headspace and water in the sampling flasks at room temperature (~24°C)

$$\delta\text{O}_2/\text{Ar}_{\text{corr}} = [\text{Q}(10^{-3} \cdot \delta\text{O}_2/\text{Ar}_{\text{meas}} + 1) - 1]10^3 \quad (1)$$

where δO₂/Ar_{corr} and δO₂/Ar_{meas} are the corrected and measured variables. The value of Q is determined from the volume ratio of water to headspace (f):

$$\text{Q} = \frac{1 + 28.21 \cdot 10^{-3} \cdot f}{1 + 30.94 \cdot 10^{-3} \cdot f} \quad (2)$$

where 28.21 and 30.94 are the solubilities (ml L⁻¹ atm⁻¹ of the pure gas) of O₂ and Ar, respectively, at 24°C. In the present study, the ratio f was ~1 and the corresponding Q was ~0.997. The correction for δ¹⁸O can be calculated in a similar way; on average, it was ~0.02‰.

The isotopic and elemental ratios are reported with respect to an air standard—HLA (Luz and Barkan 2000). The integrity of the standard was checked every month by measuring samples of outside air.

Procedure verification—To check the accuracy of water sampling, gas extraction, (O₂ + Ar) purification, and mass spectrometric measurements, we have carried out experiments with distilled water equilibrated with air (n = 9). These experiments were conducted in an open 5-L flask. Fresh outside air was bubbled directly through water poisoned with saturated HgCl₂ solution (1 ml in 100 g of water). Water in the flask was constantly stirred and the temperature was kept at 25 ± 0.1°C. Atmospheric pressure was monitored and variations during the experiment were less than 1 mm Hg. The time needed to reach isotopic equilibrium was at least 16 h, but most of the experiments were carried out for 30–40 h. Bubbling was stopped 2 h before sampling. The measured δ¹⁸O in the equilibrium experiments agreed to within 0.05‰ with the equilibrium value of Benson and Krause (1984), and the O₂/Ar ratio was within 0.1% of the

equilibrium concentrations (Benson and Krause 1984; Krause and Benson 1989).

Bottle incubations—Incubation experiments were performed for measuring in situ gross production, isotopic fractionation during dark respiration, and isotopic fractionation during oxidation of reduced compounds in water from the hypolimnion (mostly hydrogen sulfide and ammonia).

Primary production was measured by two incubation techniques: ^{14}C assimilation and O_2 production from H_2^{18}O . Williams and Robertson (1989) reported that photosynthetic rates were 5- to 10-fold greater in samples taken with GoFlo bottles than with Niskin bottles; in contrast, there were no differences in community respiration. We compared incubation results of samples taken from the lake surface directly into the incubation bottles or by the standard procedure from the water sampler. No systematic differences were observed either in photosynthesis or in respiration rates.

Water for ^{14}C incubation experiments was collected from eight depths in the upper 15 m that covers the euphotic zone, which varies seasonally from 1.7 to 11 m. H_2^{18}O incubations were done only at three depths (1, 3, and 5 m). Before each H_2^{18}O incubation, glassware and silicone tubing were soaked in 10% HCl for at least 12 h followed by rinses with distilled water. The bottles were filled in a similar way as for BOD measurement. Each bottle was rinsed with sample water and then flushed with at least two bottle volumes. Care was taken to avoid bubbling and agitation. In both methods, duplicate samples were incubated in situ for 3 h according to routine procedure of the Kinneret Limnological Laboratory (Berman and Pollinger 1974; Berman et al. 1995).

^{14}C assimilation—Samples for ^{14}C productivity measurements— $\text{P}(^{14}\text{C})$ —were incubated in 60-ml polycarbonate bottles. Carbon uptake was measured with a modified ^{14}C uptake technique (Steeman-Nielsen 1952). A spike of approximately 8 μCi of [^{14}C] bicarbonate was added to each bottle. After incubation, the samples were filtered under light vacuum (about 100 mm Hg) onto 25-mm 0.45- μ filters (Millipore®), rinsed with 15 ml of filtered lake water, and briefly fumed in HCl vapor to eliminate any remaining traces of inorganic ^{14}C . Control samples poisoned by Lugol's iodine at time zero were run with each experimental series to compensate for nonbiological adsorption to filters. The total added ^{14}C was also checked for each sampling series by counting 0.1-ml portions directly from each of the incubated bottles. Total radioactivity and the radioactivity in the particulate fraction retained on the filters was determined by liquid scintillation with quench correction. The average difference between duplicates was $\sim 10\%$.

O_2 gross production— H_2^{18}O (~ 0.2 g, 98% ^{18}O) was added to freshly sampled lake water, which was incubated in situ. The newly added photosynthetic O_2 is highly enriched in ^{18}O and thus causes the $\delta^{18}\text{O}$ of the dissolved oxygen to increase in proportion to the rate of gross O_2 production.

We can write the following mass balance equation for $\delta^{18}\text{O}$ of dissolved oxygen in the incubation bottle.

$$[\text{O}_2]_{\text{in}} \delta^{18}\text{O}_{\text{in}} + \text{GP}(^{18}\text{O}) \delta^{18}\text{O}_{\text{w}} - \text{R}(\delta^{18}\text{O}_{\text{avg}} + \varepsilon_{\text{R}}) = [\text{O}_2]_{\text{fin}} \delta^{18}\text{O}_{\text{fin}} \quad (3)$$

$[\text{O}_2]_{\text{in}}$ and $[\text{O}_2]_{\text{fin}}$ are the initial and final O_2 concentrations ($\mu\text{mol kg}^{-1}$), respectively; $\delta^{18}\text{O}_{\text{w}}$ is the $\delta^{18}\text{O}$ of the enriched lake water (‰); $\text{GP}(^{18}\text{O})$ is gross O_2 production ($\mu\text{mol L}^{-1} \text{time}^{-1}$); R is the total O_2 consumption ($\mu\text{mol L}^{-1} \text{time}^{-1}$); $\delta^{18}\text{O}_{\text{in}}$ and $\delta^{18}\text{O}_{\text{fin}}$ are the initial and final $\delta^{18}\text{O}$ of dissolved O_2 (‰) respectively. The fractionation factor ε_{R} represents discrimination due to O_2 uptake in the bottle. Variations in this factor up to $\pm 20\%$ do not significantly affect the calculated $\text{GP}(^{18}\text{O})$, and in the present study, we used ε_{R} of -21.6% (the fractionation obtained in our dark respiration experiments, *see below*). For O_2 uptake (R) it was not possible to measure momentary $\delta^{18}\text{O}$ changes during incubation. Instead, we used an averaged value based on the final and initial $\delta^{18}\text{O}$. Sensitivity tests showed that the inaccuracy, which results from this approximation, is smaller than the differences between duplicate incubations.

Given that $\text{R} = \text{GP} - ([\text{O}_2]_{\text{fin}} - [\text{O}_2]_{\text{in}})$, Eq. 3 can be rewritten in the following form.

$$\begin{aligned} \text{GP}(^{18}\text{O}) = & \{ [\text{O}_2]_{\text{fin}} \cdot (\delta^{18}\text{O}_{\text{fin}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \\ & - [\text{O}_2]_{\text{in}} (\delta^{18}\text{O}_{\text{in}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \} \\ & \div (\delta^{18}\text{O}_{\text{w}} - \delta^{18}\text{O}_{\text{avg}} - \varepsilon_{\text{R}}) \end{aligned} \quad (4)$$

The CO_2 equilibration method was used for measuring $\delta^{18}\text{O}$ of spiked water samples. In order to avoid contamination of the mass spectrometer with highly ^{18}O -enriched CO_2 ($\sim 900\%$), the spiked water was diluted (about 1:25) 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 CO_2 equilibrated with diluted spiked water was measured using an upgraded Micromass 602 mass spectrometer with precision better than 0.1‰.

$[\text{O}_2]_{\text{in}}$ was determined by the Winkler method with precision of $\pm 0.2 \mu\text{mol kg}^{-1}$. $[\text{O}_2]_{\text{fin}}$ was calculated from $[\text{O}_2]_{\text{in}}$ and the initial and final $\delta\text{O}_2/\text{Ar}$ measurements

$$[\text{O}_2]_{\text{fin}} = [\text{O}_2]_{\text{in}} \frac{10^{-3}(\delta\text{O}_2/\text{Ar})_{\text{fin}} + 1}{10^{-3}(\delta\text{O}_2/\text{Ar})_{\text{in}} + 1} \quad (5)$$

where $\delta\text{O}_2/\text{Ar}$ is expressed in the usual δ -notation form.

$$\delta\text{O}_2/\text{Ar} = \left[\frac{(\text{O}_2/\text{Ar})_{\text{samp}}}{(\text{O}_2/\text{Ar})_{\text{HLA}}} - 1 \right] 10^3 \quad (6)$$

The same data were used for calculating net O_2 production ($\text{NOP} = [\text{O}_2]_{\text{fin}} - [\text{O}_2]_{\text{in}}$) and light O_2 uptake ($\text{LR} = \text{GP}(^{18}\text{O}) - \text{NOP}$).

In the first several cruises, incubations for ^{18}O productivity determinations were done both in 120-ml Pyrex and in 110-ml quartz bottles. There were no systematic differences between the two types of bottles; therefore, in the rest of the study, we used only Pyrex bottles. At the end of the incubation, the samples were transferred into evacuated flasks (described above), which were kept for further processing in the laboratory. Variations in replicate analyses of GP were less than 10%.

Table 1. Changes in the remaining fraction of O₂ (f) and δ¹⁸O (‰ vs. HLA) of dissolved oxygen in dark respiration and hypolimnion oxidation experiments.

Date	Time (h)	f	δ ¹⁸ O	Date	Time (h)	f	δ ¹⁸ O	
Mar 97	0	1	0.78	Jan 99	0	1	1.91	
	24	0.9658	1.47		24	0.9665	2.61	
	72	0.9025	2.98		54	0.9361	3.35	
	96	0.8639	3.91		70	0.8961	4.31	
		$\epsilon_{\text{dark}} = -21.4 \pm 0.1$				$\epsilon_{\text{dark}} = -21.9 \pm 0.3$		
Apr 97	0	1	-0.88	Aug 99	0	1	-1.05	
	23	0.9735	-0.19		25	0.9624	-0.18	
	46	0.8893	1.65		48	0.9487	0.11	
	73	0.8846	1.76		70	0.9266	0.59	
	98	0.8375	3.03		104	0.9011	1.21	
		$\epsilon_{\text{dark}} = -21.6 \pm 0.4$				$\epsilon_{\text{dark}} = -21.5 \pm 0.3$		
May 97	0	1	-2.01	Dec 96	0	1	-7.72	
	24	0.9537	-0.94		18	0.8409	-4.98	
	49	0.9218	-0.29		18	0.8417	-4.79	
	77	0.8883	0.55		22	0.8071	-4.01	
		$\epsilon_{\text{dark}} = -21.4 \pm 0.4$			22	0.8043	-3.81	
					22	0.7502	-3.32	
							$\epsilon_{\text{hyp}} = -16.1 \pm 0.9$	

Dark respiration and respiratory fractionation of O₂ isotopes—Incubation bottles were filled with water from 3 m depth and incubated (24–96 h) in a thermostat at in situ temperature. In recent studies, Quay et al. (1995) and Benner et al. (1995) showed that changes in incubation bottle volumes did not influence respiration rate or isotopic fractionation. Thus, in the present study, we used 300-ml BOD bottles, from which duplicate samples were taken for isotopic measurements. The dark respiratory fractionation coefficient (ϵ_{dark}) was calculated from the changes in δ¹⁸O_{diss} and O₂ concentration using the “Rayleigh” fractionation relationship (Kroopnick and Craig 1976).

$$\epsilon_{\text{dark}} = \frac{10^3 \cdot \ln(R_{\text{fin}}/R_{\text{in}})}{\ln([O_{\text{fin}}]/[O_{\text{in}}])}$$

$$= \frac{10^3 \cdot \ln[(10^{-3} \cdot \delta^{18}\text{O}_{\text{fin}} + 1)/(10^{-3} \cdot \delta^{18}\text{O}_{\text{in}} + 1)]}{\ln([O_{\text{fin}}]/[O_{\text{in}}])} \quad (7)$$

[O_{fin}]/[O_{in}] was determined from δO₂/Ar measurements as in Eq. 5.

Isotopic fractionation in hypolimnetic oxidation processes—The fractionation associated with oxidation of H₂S, and NH₄⁺ in the hypolimnion (ϵ_{hyp}) was determined by mixing nine parts surface water (3 m) with one part hypolimnetic water (35 m) followed by a period of dark incubation. The calculation of ϵ_{hyp} was done in the same way as for ϵ_{dark} . Control experiments were done with mercuric chloride added in order to stop bacterial activity. The changes in O₂ concentration in this case were negligible, indicating that oxidation processes in the lake are mostly biological.

Results and discussion

The field and laboratory data were obtained during two periods: May 1996–May 1997 and February 1998–February 1999. Notably, *Peridinium* dominated the community during

the bloom of spring 1998, but it was absent in the spring bloom of 1997. All the data are given in Web Appendix 1 (http://www.aslo.org/lo/toc/vol_47/issue_1/0033al.pdf) and in Table 1. Each data point in the appendix represents an average of two determinations with a precision (absolute difference from the average) of 0.1°C for temperature, 0.2 μmol kg⁻¹ for [O₂], 0.02‰ for δ¹⁸O_{diss}, 1‰ for δO₂/Ar, 6‰ for GP(¹⁸O), and 5‰ for P(¹⁴C). The database was used for deriving the input parameters in order to evaluate the respiratory fractionation of the epilimnetic plankton community and in the incubation experiments.

Isotopic fractionation in dark respiration and in oxidation of hypolimnetic reduced substances—Five series of dark incubation experiments were run from March 1997 to August 1999 (Table 1). The average rate of O₂ uptake in these experiments was 0.52 ± 0.11 μmol L⁻¹ h⁻¹, and the average fractionation factor (ϵ_{dark}) was -21.6 ± 0.3‰. Kiddon et al. (1993) determined the isotopic fractionation in cultures of marine organisms and found that discrimination by phytoplankton ($\epsilon_{\text{dark}} \sim -22$ ‰) was stronger than by bacteria ($\epsilon_{\text{dark}} \sim -19$ ‰). The plankton community in Lake Kinneret is dominated by phytoplankton (~90%), with bacteria and zooplankton comprising about 10% (Hadas and Berman 1998), and the determined fractionation factor is as expected in a phytoplankton-dominated community.

Kroopnick (1975), in experiments similar to the present study, obtained an average ϵ_{dark} of -20.8‰ for surface ocean community, but weaker discrimination ($\epsilon_{\text{dark}} \sim -17.6$ ‰) was reported by Quay et al. (1995) for the Amazon River. The latter value, as pointed out by the authors, represents bacterial respiration and is in good agreement with the experiments of Kiddon et al. (1993) with bacteria. Notably, fractionation in bacteria is consistent with that of the cytochrome oxidase, whereas the stronger discrimination determined in dark experiments with phytoplankton might indicate some engagement of the AOX pathway.

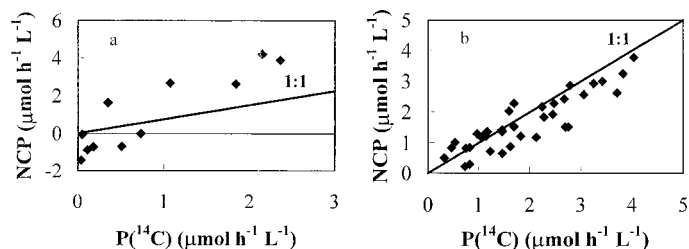


Fig. 1. Comparison of net organic carbon production (NCP) and ^{14}C fixation [$\text{P}(^{14}\text{C})$]: (a) *Peridinium* bloom; (b) non-*Peridinium*.

In addition to dark respiratory mechanisms, considerable amounts of O_2 are removed from Lake Kinneret during its winter overturn by oxidation of hypolimnetic reduced compounds ($\sim 0.045 \text{ mol m}^{-2} \text{ d}^{-1}$). The overall fractionation factor for oxidation of hypolimnetic water (ε_{hyp}) was determined in December 1996 as $-16.1 \pm 0.9\text{‰}$ (Table 1). In Lake Kinneret, H_2S is conspicuously more abundant than NH_4^+ ; thus, the determined ε_{hyp} reflects mostly hydrogen sulfide oxidation.

Net production—In Fig. 1, we compare rates of net carbon production obtained from bottle incubations (NCP) with the rate of ^{14}C fixation $\text{P}(^{14}\text{C})$. The NCP values were calculated from the measured oxygen net production (NOP) using the relationship: $\text{NCP} = \text{NOP}/1.4$ (Laws 1991). During the non-*Peridinium* period, NCP rates were close to or somewhat lower than the $\text{P}(^{14}\text{C})$ rates. This is in good agreement with results from oceanic experiments (e.g., Bender et al. 1999). During the *Peridinium* bloom, the dense plankton population considerably limited light penetration to the deep incubation bottles (3 and 5 m). As a result, uptake exceeded gross production, and net production was negative. On the other hand, at 1 m, where illumination was higher, the calculated NCP rates were about twice the $\text{P}(^{14}\text{C})$ rates. The exceptionally low $\text{P}(^{14}\text{C})/\text{NCP}$ ratios could result from either an unusually high rate of ^{14}C excretion in dissolved organic matter or from a large internal inorganic carbon pool unlabeled with ^{14}C . The former possibility seems unlikely in view of Berman (1976), who showed that the excretion of ^{14}C -labeled dissolved organic carbon was by no means higher throughout the *Peridinium* bloom than otherwise. On the other hand, Berman-Frank and Erez (1996) experimentally simulated

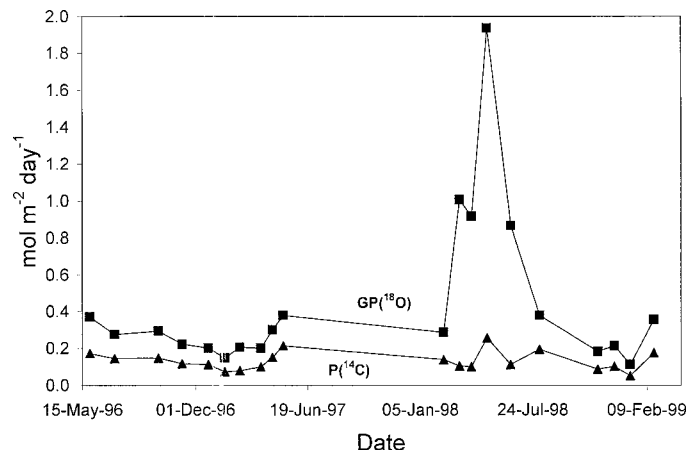


Fig. 2. Seasonal variations of integrated O_2 gross production [$\text{GP}(^{18}\text{O})$] and ^{14}C fixation [$\text{P}(^{14}\text{C})$].

bloom conditions and clearly demonstrated the presence of a large intracellular pool of inorganic carbon in *Peridinium*.

It should be emphasized that the net rates determined in the bottle experiments must overestimate the true rates in the lake. Bottle O_2 consumption was affected only by micro- and nanoplankton, but not by large zooplankton and nekton respiration or by O_2 uptake due to sediment resuspension and entrainment of hypolimnetic water. Thus, in our treatment of the isotopic fractionation by the entire lake community, we did not use the results from bottle incubations. Instead, we estimated net production rates for the whole lake from $\delta\text{O}_2/\text{Ar}$ and wind speed measurements (see below).

Gross production—Integrated gross O_2 production rates for the light period over the entire euphotic zone are given in Table 2 and Fig. 2. These rates were calculated by trapezoidal integration of data from discrete depths. Because H_2^{18}O incubations were made only at three depths, which did not cover the entire euphotic zone, the missing $\text{GP}(^{18}\text{O})$ values (below 5 m) were estimated from $\text{P}(^{14}\text{C})$ data using the relationships between the two variables (Fig. 3). It should be noted that these relationships may not be constant with depth, but the contribution of the gross production below 5 m to the total gross production is small, and even in an extreme case, where the uncertainty in correlation between $\text{P}(^{14}\text{C})$ and

Table 2. Summary of $\delta^{18}\text{O}$ of dissolved O_2 ($\delta^{18}\text{O}_{\text{diss}}$), $\delta\text{O}_2/\text{Ar}$ ratio (both in ‰ vs. HLA), fraction of biological O_2 saturation (f, calculated from $\delta\text{O}_2/\text{Ar}$), and integrated gross production ($\text{GP}(^{18}\text{O})$, $\text{mol m}^{-2} \text{ d}^{-1}$) in the epilimnion.

Date	$\delta^{18}\text{O}_{\text{diss}}$	$\delta\text{O}_2/\text{Ar}$	f	$\text{GP}(^{18}\text{O})$	Date	$\delta^{18}\text{O}_{\text{diss}}$	$\delta\text{O}_2/\text{Ar}$	f	$\text{GP}(^{18}\text{O})$
5 May 96	-3.1	158.2	1.27	0.37	15 Feb 98	-0.6	-94.1	1.00	0.29
11 Jul 96	-0.6	-102.2	0.99	0.28	15 Mar 98	-2.7	193.4	1.31	1.01
26 Sept 96	-0.4	-89.9	1.00	0.29	4 Apr 98	-0.3	10.3	1.11	0.92
7 Nov 96	0.4	-215.0	0.86	0.22	3 May 98	-0.9	163.8	1.28	1.94
24 Dec 96	1.8	-315.4	0.75	0.21	14 Jun 98	-3.3	111.5	1.22	0.86
22 Jan 97	1.9	-214.1	0.88	0.16	4 Aug 98	-2.7	-72.7	1.02	0.31
18 Feb 97	-2.4	24.5	1.13	0.20	15 Nov 98	-1.9	-175.6	0.91	0.20
27 Mar 97	0.7	-142.2	0.94	0.19	14 Dec 98	0.2	-243.7	0.83	0.21
17 Apr 97	-0.8	-47.3	1.05	0.31	11 Jan 99	3.7	-449.3	0.61	0.11
6 May 97	-0.9	26.5	1.13	0.39	21 Feb 99	0.5	-252.1	0.82	0.34

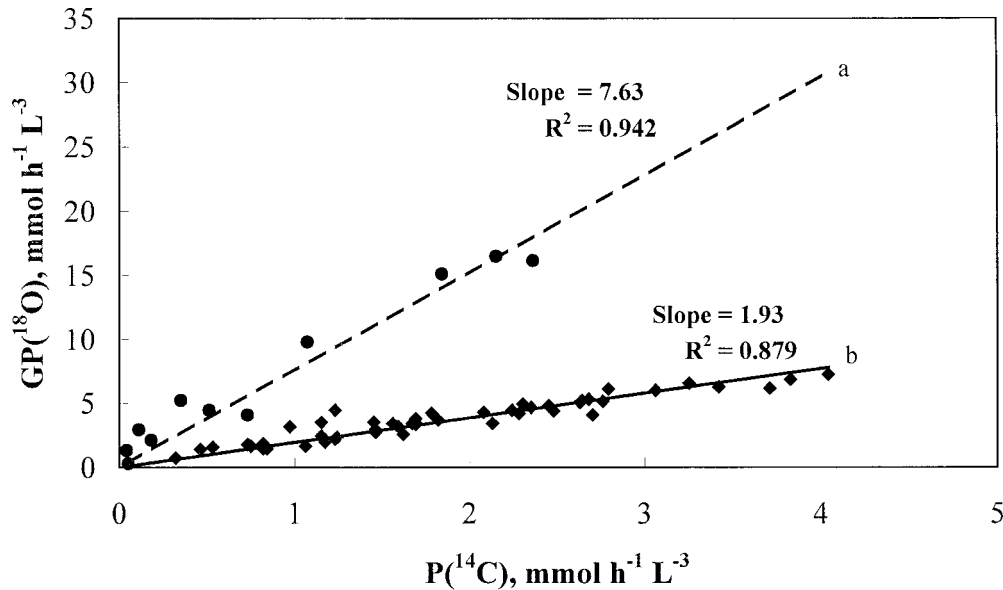


Fig. 3. Comparison of gross O₂ production [GP(¹⁸O)] and ¹⁴C fixation [P(¹⁴C)]: (a) *Peridinium* bloom; (b) non-*Peridinium*.

GP(¹⁸O) is taken as 50%, the maximum error in integrated O₂ production will not exceed 10%. The 3-h ¹⁴C incorporation rates and GP(¹⁸O) rates were converted to daily rates by using empirically determined conversion factors (Berman and Pollinger 1974). The overall error in the integrated GP(¹⁸O) is estimated at about 15%.

Seasonal variations in GP(¹⁸O) and P(¹⁴C) are plotted in Fig. 2. Both parameters show systematic variations with high productivity peaks during spring and early summer blooms. GP(¹⁸O) reached exceptionally high values during the spring bloom of 1998 when the community was dominated by *Peridinium*. A plot of GP(¹⁸O) against P(¹⁴C) (Fig. 3) and the relationships between GP(¹⁸O) and light O₂ uptake (LR, Fig. 4) show distinct patterns during the *Peridinium* bloom and during the rest of the study period when the lake was dominated by nanoplanktonic species. In the latter case, the GP(¹⁸O)–P(¹⁴C) slope was 1.9, implying that P(¹⁴C) was ~53% of GP(¹⁸O) and LR was ~34% of GP(¹⁸O) (Fig. 4b

and Web Appendix 1). According to Laws (1991), the ratio of GP(¹⁸O) to gross carbon fixation is 1.1, and about 13% of P(¹⁴C) are excreted in dissolved organic carbon as DO¹⁴C (Baines and Pace 1991; Laws et al. 2000). This will explain ~21% of the 47% difference between GP(¹⁸O) and P(¹⁴C). The remaining 26% can be explained by some combination of (1) respiratory recycling of the ¹⁴C tracer, (2) photorespiration for which three moles of O₂ are consumed for each mole of CO₂ produced, and (3) uptake by Mehler reaction that does not involve any carbon exchange. We argue that during the period under discussion, uptake by photorespiration was unlikely in the lake. Photorespiration, as a rule, becomes significant when the supply of inorganic carbon is limiting (Badger 1980). However, because inorganic carbon (as HCO₃⁻) was plentiful (2–2.5 mmol kg⁻¹) and most aquatic organisms possess mechanisms for efficient utilization of dissolved bicarbonate (Kaplan and Reinhold 1999; Tchernov et al. 1998), photorespiration was most probably suppressed.

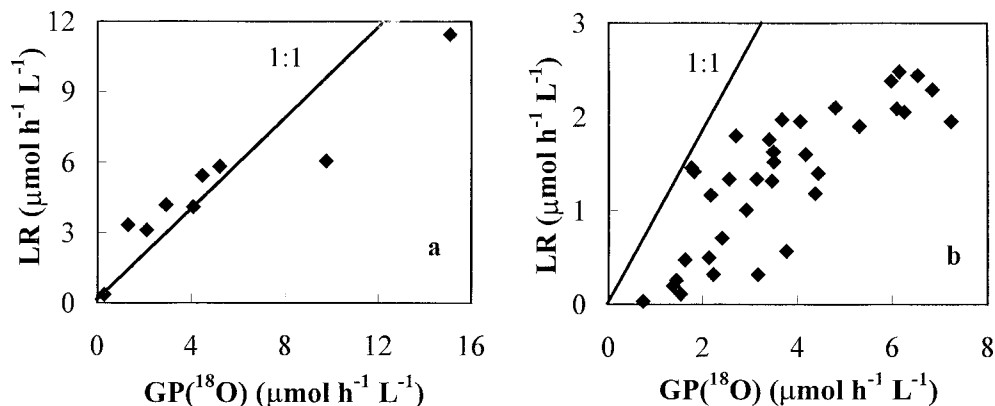


Fig. 4. Comparison of light O₂ uptake (LR) and gross O₂ production [GP(¹⁸O)]: (a) *Peridinium* bloom; (b) non-*Peridinium*.

To compare the results of the present research with similar studies in the ocean (e.g., Bender et al. 1992, 1999; Laws et al. 2000), we divide the 1.9 GP(^{18}O)–P(^{14}C) slope by a factor of 0.85. This correction is necessary, because the figures we report are based on measurements in the photoperiod, whereas the reported oceanic values were based on 24-h incubations in which a fraction of about 0.15 of the ^{14}C tracer was recycled at night by dark uptake. The corrected slope (2.2) falls in the range obtained for various oceanic regions (2.0–2.8). Bender et al. (1999) and Laws et al. (2000) explained the GP(^{18}O)–P(^{14}C) slope by making a priori assumptions on the proportions of Mehler reaction and respiratory ^{14}C recycling. We take an alternative approach and use isotopic mass balance to place constraints on possible proportions of these processes.

During the massive *Peridinium* bloom, the GP(^{18}O)–P(^{14}C) slope was much greater than in the rest of the study. As shown in Fig. 4a and Web Appendix 1, most gross production and all net production during this bloom were confined to the upper 1 m of the lake. At that depth, ~70% of gross production was taken up by light O_2 consumption. Below the surface, production was restricted by light limitation and was completely recycled. In this case, it is possible to explain the high GP(^{18}O)/P(^{14}C) ratios by making an extreme assumption that all respired carbon was newly fixed, which implies recycling of all labeled carbon or that all the uptake took place by Mehler reaction and photorespiration. The latter is expected in *Peridinium* blooms due to inorganic carbon limitation (Berman-Frank and Erez 1996). However, at the surface even with this extreme assumption, the maximum possible GP(^{18}O)/P(^{14}C) ratio is only 3.7 (1.1GP(^{18}O)/[GP(^{18}O) – LR]) and not 7.5. A likely explanation of the very high slope is a presence of an intracellular pool of inorganic carbon unlabeled with ^{14}C (see above).

Evaluation of the significance of O_2 uptake mechanisms—The proportions among the different uptake mechanisms can be evaluated from the isotopic discrimination of the entire plankton community in the epilimnion (ε_{epi}), because the discriminations of the various uptake processes are different (see above). ε_{epi} can be determined from a mass balance of $\delta^{18}\text{O}$ of dissolved O_2 ($\delta^{18}\text{O}_{\text{diss}}$). Such mass balance should take into account O_2 gains and losses due to photosynthesis, respiration, and air–lake gas exchange as well as O_2 consumption due to oxidation of hypolimnetic reduced substances in the water column and in the sediment. In a complete mass balance it is necessary to take account of temporal changes in the depth of the epilimnion as well as variations in O_2 concentration and $\delta^{18}\text{O}_{\text{diss}}$. However, the available data set is not detailed enough for such calculations. A simplified mass balance is possible, if quasi steady state is assumed in the epilimnion. Such an assumption is justified for the spring bloom and summer when the residence time of oxygen is short (3–5 d) because of the high gross production rates and the shallow mixed layer.

Figure 5 shows the major fluxes (production, consumption, and gas exchange) used in a simple steady state model. O_2 exchange across the lake thermocline is insignificant (Nishri et al. 1997) and was neglected. At steady state, the isotopic mass balance for $\delta^{18}\text{O}$ in the epilimnion is given as

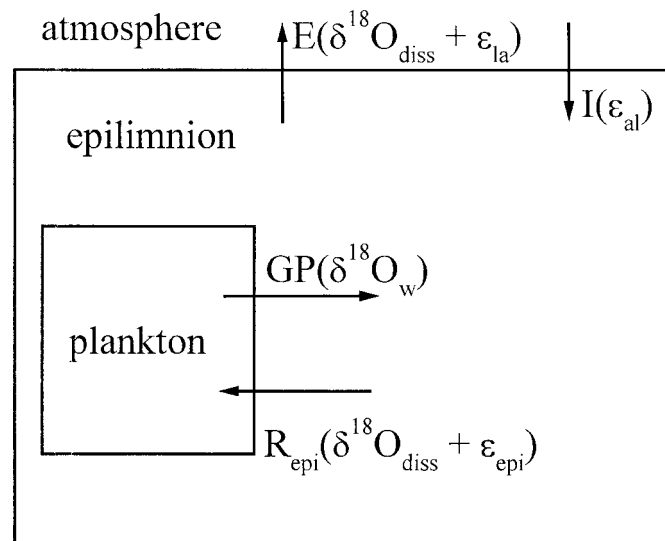


Fig. 5. Schematic representation of reservoirs and fluxes used in the isotope mass balance model.

$$\begin{aligned} \text{GP}(^{18}\text{O})\delta^{18}\text{O}_w + I\varepsilon_{\text{al}} \\ = R_{\text{epi}}(\delta^{18}\text{O}_{\text{diss}} + \varepsilon_{\text{epi}}) + E(\delta^{18}\text{O}_{\text{diss}} + \varepsilon_{\text{la}}) \end{aligned} \quad (8)$$

where GP(^{18}O) is gross O_2 production; R_{epi} is overall respiration in the epilimnion; I and E are O_2 air–lake influx and efflux, respectively; $\delta^{18}\text{O}_w$ is the $\delta^{18}\text{O}$ of photosynthetic O_2 and is identical to the $\delta^{18}\text{O}$ of the water (Guy et al. 1993); and ε_{epi} , ε_{al} , and ε_{la} are the fractionation factors (‰) for total respiratory consumption by plankton, air–lake invasion, and lake–air evasion, respectively. The units of GP(^{18}O), R_{epi} , I, and E are $\text{mol m}^{-2} \text{d}^{-1}$.

By substituting $E = \text{GP}(^{18}\text{O}) + I - R_{\text{epi}}$ and rearranging Eq. 8, we obtain the following expression for ε_{epi} .

$$\begin{aligned} \varepsilon_{\text{epi}} = \frac{\text{GP}(^{18}\text{O})(\delta^{18}\text{O}_w - \delta^{18}\text{O}_{\text{diss}} - \varepsilon_{\text{la}}) + I(\varepsilon_{\text{al}} - \varepsilon_{\text{la}} - \delta^{18}\text{O}_{\text{diss}})}{R_{\text{epi}}} \\ + \varepsilon_{\text{la}} \end{aligned} \quad (9)$$

The magnitudes of ε_{al} and ε_{la} are known (–2.8 and –3.5‰, respectively; Knox et al. 1990); $\delta^{18}\text{O}_w$, $\delta^{18}\text{O}_{\text{diss}}$, and GP(^{18}O) were determined experimentally; and R_{epi} and I were calculated as discussed below.

The rate of O_2 uptake in the epilimnion (R_{epi}) can be determined from the difference between gross production and net air–lake O_2 flux due to biological forcing alone (NF). In a steady state situation, NF equals net O_2 production and was determined from the measured O_2/Ar ratio. Craig and Haywart (1987) first suggested the logic for such an application of oxygen and argon data. The temperature dependence of the solubility of these gases is almost identical (Benson and Krause 1984; Krause and Benson 1989); thus, deviations from the equilibrium ratio reflect biological consumption/production. The biologically forced flux of O_2 from or into the lake is calculated as

$$\text{NF} = kC_o[(C/C_o)_{\text{bio}} - 1] \quad (10)$$

where $(C/C_o)_{\text{bio}} = (\text{O}_2/\text{Ar})_{\text{lake}}/(\text{O}_2/\text{Ar})_o$ and is the O_2 satura-

Table 3. Overall epilimnetic respiratory–fractionation factors (ϵ_{epi} , ‰) calculated from the steady state model. O_2 solubility (C_o , mol m^{-3}) was corrected for the partial pressure of O_2 at the elevation of the surface of Lake Kinneret (~ 210 m below sea level); piston velocity (k , m d^{-1}) is a monthly average. All O_2 fluxes are given in units of mol $\text{m}^{-2} \text{d}^{-1}$.

Date	C_o	k	GP (^{18}O)	NF	R_{epi}	ϵ_{epi}
5 May 96	0.260	0.91	0.37	0.06	0.31	-22.9 ± 0.5
11 Jul 96	0.250	1.26	0.28	0.00	0.28	-22.4 ± 0.5
26 Sept 96	0.253	0.79	0.29	0.00	0.29	-23.7 ± 0.3
17 Apr 97	0.307	0.73	0.31	0.01	0.29	-23.6 ± 0.4
6 May 97	0.290	0.73	0.39	0.03	0.35	-23.1 ± 0.4
4 Apr 98	0.308	0.68	0.92	0.03	0.89	-24.9 ± 0.1
3 May 98	0.281	0.91	1.94	0.07	1.87	-24.5 ± 0.2
14 Jun 98	0.261	1.13	0.86	0.07	0.80	-21.6 ± 0.4
4 Aug 98	0.248	0.83	0.31	0.00	0.38	-20.5 ± 0.7

tion due to biological consumption or production; $(\text{O}_2/\text{Ar})_o$ is the equilibrium solubility ratio for oxygen and argon, k is piston velocity, and C_o is oxygen solubility. In the present study, $(C/C_o)_{\text{bio}}$ was determined directly by mass spectrometry.

$$(C/C_o)_{\text{bio}} = \frac{\delta(\text{O}_2/\text{Ar})_{\text{lake}} + 1,000}{\delta(\text{O}_2/\text{Ar})_o + 1,000} \quad (11)$$

Determination of $(C/C_o)_{\text{bio}}$ in this way has a major advantage—it involves only a single and very precise mass spectrometric measurement of the O_2/Ar ratio, rather than two separate and less precise measurements of O_2 and Ar concentrations. The value of $\delta(\text{O}_2/\text{Ar})_o$ was obtained from published solubility data (Benson and Krause 1984; Krause and Benson 1989) as -88.8‰ , and it is almost constant over the range of temperature variations in the lake. The calculated rates of NF and R_{epi} (equals $\text{GP}(^{18}\text{O}) - \text{NF}$) are given in Table 3.

Air– O_2 invasion (I) was estimated from the relationship: $I = kC_o$. Oxygen solubilities (C_o , Table 3) were taken from Benson and Krause (1984) and corrected for the partial pressure of O_2 at the elevation of the surface of Lake Kinneret. The piston velocity (k , Table 3) was estimated from measured wind speeds using the empirical relationship of Clark et al. (1995).

The results of the calculated discriminations of the entire community uptake (ϵ_{epi}) are given in Table 3. The ϵ_{epi} figures represent both dark and light O_2 uptake; thus, fractionation during the photoperiod must have been stronger. Only in June and August 1998, ϵ_{epi} values were similar to those obtained in the dark incubation experiments ($\epsilon_{\text{dark}} = -21.6 \pm 0.3\text{‰}$). During the rest of the study, the epilimnetic discrimination was significantly stronger ($\epsilon_{\text{epi}} < -23\text{‰}$) than dark discrimination, and exceptionally low ϵ_{epi} values were obtained for the *Peridinium* bloom ($\sim -25\text{‰}$).

As mentioned above, the quasi steady state assumption does not apply to fall and winter in Lake Kinneret, and the data set is not sufficient for rigorous treatment. Nevertheless, it is interesting to make a crude calculation of ϵ_{epi} for this period. For example, between December 1998 and January 1999, the dissolved O_2 inventory increased from 5.074 mol

m^{-2} to 5.757 mol m^{-2} and $\delta^{18}\text{O}_{\text{diss}}$ changed from 0.2 to 3.7‰; 4.931 mol $\text{m}^{-2} \text{d}^{-1}$ were added by gross production with $\delta^{18}\text{O}$ of -24.8‰ (the $\delta^{18}\text{O}$ of Lake Kinneret water), 5.179 mol $\text{m}^{-2} \text{d}^{-1}$ were added by O_2 invasion with $\delta^{18}\text{O}$ of -2.8‰ , and 3.196 mol $\text{m}^{-2} \text{d}^{-1}$ were removed by O_2 evasion with $\delta^{18}\text{O}$ of -1.6‰ (the average $\delta^{18}\text{O}_{\text{diss}} + \epsilon_{\text{la}}$). During the same period, about 1.246 mol $\text{m}^{-2} \text{d}^{-1}$ were removed by H_2S oxidation due to entrainment of hypolimnetic water. The $\delta^{18}\text{O}$ of O_2 removed by this reaction was -14.2‰ (the average $\delta^{18}\text{O}_{\text{diss}} + \epsilon_{\text{hyp}}$). The rate of respiratory O_2 removal is calculated as 4.182 mol $\text{m}^{-2} \text{d}^{-1}$. The $\delta^{18}\text{O}$ of this respired O_2 is calculated as -27.1‰ (the average $\delta^{18}\text{O}_{\text{diss}} + \epsilon_{\text{epi}}$) and shows that the average discrimination against ^{18}O was about 29‰ ($\epsilon_{\text{epi}} = -29\text{‰}$). Similar low values of ϵ_{epi} can be calculated for other time intervals during fall and winter, but it is necessary to conduct a more detailed survey of the lake in order to derive an accurate estimate of ϵ_{epi} for this period.

The results of a simple steady state model, as well as of our crude mass balance calculation for fall and winter, show that over most of the study period, ϵ_{epi} was smaller than -23‰ . The magnitude of the overall discrimination in the epilimnion (ϵ_{epi}) depends on the relative proportions of O_2 uptake by four possible mechanisms: ordinary dark respiration, photorespiration, Mehler reaction, and the alternative oxidase (AOX). Discriminations by the first three mechanisms (-21.6 ± 0.3 , -21.3 ± 0.4 , and $-15.3 \pm 0.5\text{‰}$, respectively, Table 1; Guy et al. 1993) cannot explain ϵ_{epi} values smaller than -22‰ . The only mechanism that can account for the smaller ϵ_{epi} values is uptake by AOX, which in green tissues discriminates by $31 \pm 1\text{‰}$ (Robinson et al. 1992).

As discussed above, with the exception of the *Peridinium* bloom, photorespiration was unlikely in the lake. In this case, various proportions of the Mehler reaction, dark respiration, and AOX can explain ϵ_{epi} of -23‰ . In one extreme case when the Mehler reaction is absent, 15% of LR is accounted for by AOX and 85% by dark respiration. In another extreme case, where dark respiration is absent, Mehler reaction and AOX consume about equal amounts of O_2 . If we follow Laws et al. (2000) and assume that the Mehler reaction was 10% of $\text{GP}(^{18}\text{O})$ (or 29% of LR), the same ϵ_{epi} can be explained by removal of 34% of LR by AOX and 37% by dark respiration. During the *Peridinium* bloom in 1998 ϵ_{epi} was about -25‰ . Photorespiration was likely during this bloom, but because the discriminations by dark respiration and photorespiration are similar, their relative proportions cannot be determined from isotopic mass balance. Assuming again that the Mehler reaction was 29% of LR, the derived ϵ_{epi} is explained by 56% uptake by AOX and 15% by both dark respiration and photorespiration.

Our isotopic mass balance shows that, in most cases at least, 15% of O_2 uptake should go through the AOX pathway. Uptake by this mechanism must be more important when the Mehler reaction is present. Combined engagement of increased proportions of both pathways can explain, at least part of, the observed $\text{GP}(^{18}\text{O})\text{--P}(^{14}\text{C})$ slope without the need to invoke very substantial recycling of the ^{14}C tracer by dark respiration.

Recently, it has been shown that AOX is widespread and quantitatively important among diverse aquatic phytoplank-

ton and can be stimulated by various environmental factors (McIntosh et al. 1998; Eriksen and Lewitus 1999). In particular, Maxwell et al. (1999) demonstrated the engagement of this mechanism as a response to the formation of reactive oxygen species that resulted in oxidative stress. They suggested that the role of AOX was to decrease this stress. Oxidative stress was likely to develop during the *Peridinium* bloom because of inorganic carbon limitation in the lake (Berman-Frank and Erez 1996). In turn, oxidative stress and carbon limitation are also favorable for photorespiration (Badger 1980). Goyal and Tolbert (1996) demonstrated inhibition of glycolate oxidation (an important process in photorespiration) by SHAM—an inhibitor of AOX. Their observation may suggest some linkage between photorespiration and AOX. However, as our results indicate, over most of the study period, AOX was not associated with photorespiration and its origin must be related to other causes.

Determining how O₂ uptake is partitioned among various respiratory mechanisms is central to the understanding of global and local aquatic systems. Based on oxygen isotope budgets, we have demonstrated, for the first time, a large-scale O₂ uptake by the alternative oxidase pathway in an aquatic environment. Similar future studies are necessary for evaluating the importance of this mechanism in fresh water systems and in the ocean.

References

- BADGER, M. R. 1980. Kinetic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase from *Anabaena variabilis*. Arch. Biochem. Biophys. **200**: 247–54.
- BAINES, S. B., AND M. L. PACE. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol. Oceanogr. **36**: 1078–1090.
- BENDER, M. L., AND OTHERS. 1987. A comparison of four methods for the determination of planktonic community metabolism. Limnol. Oceanogr. **32**: 1085–1098.
- , H. DUCKLOW, J. KIDDON, J. MARRA, AND J. MARTIN. 1992. The carbon balance during the 1989 spring bloom in the North Atlantic Ocean, 47°N, 20°NW. Deep-Sea Res. **39**: 1707–1725.
- , J. ORCHARDO, M. L. DICKSON, R. BARBER, AND S. LINDLEY. 1999. In vitro O₂ fluxes compared with ¹⁴C production and other rate terms during the JGOFS Equatorial Pacific experiment. Deep-Sea Res. I **46**: 637–654.
- BENNER, R., S. OPSAHL, G. CHIN-LEO, J. E. RICHEY, AND B. R. FORSBERG. 1995. Bacterial carbon metabolism in the Amazon River system. Limnol. Oceanogr. **40**: 1262–1270.
- BENSON, B. B., AND D. K. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with atmosphere. Limnol. Oceanogr. **29**: 620–632.
- BERMAN, T. 1976. Release of dissolved organic carbon by photosynthesizing algae in Lake Kinneret, Israel. Freshw. Biol. **6**: 13–18.
- , AND U. POLLINGER. 1974. Annual and seasonal variations of phytoplankton, chlorophyll and photosynthesis in Lake Kinneret. Limnol. Oceanogr. **19**: 31–55.
- , L. STONE, Y. Z. YACOBI, M. SHLICHTER, A. NISHRI, AND U. POLLINGER. 1995. Primary production and phytoplankton in Lake Kinneret: A long term record (1972–1993). Limnol. Oceanogr. **40**: 1064–1076.
- BERMAN-FRANK, I., AND Y. EREZ. 1996. Inorganic carbon pools in the bloom-forming dinoflagellate *Peridinium gatunense*. Limnol. Oceanogr. **41**: 1780–1789.
- BERRY, J. A. 1992. Biosphere, atmosphere, ocean interactions: A plant physiologist's perspective, p. 441–453. In P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biochemical cycles in the sea. Plenum Press.
- CLARK, J. F., P. SCHLOSSER, H. J. SIMPSON, M. STUTE, R. WANINKHOF, AND D. T. HO. 1995. Relationship between gas transfer velocities and wind speeds in the tidal Hudson River determined by dual tracer technique, p. 785–800. In B. Jaehne and E. C. Monahan [eds.], Air–water gas transfer. Aeon Verlag and Studio.
- CRAIG, H., AND T. HAYWART. 1987. Oxygen supersaturation in the ocean: Biological versus physical contributions. Science **235**: 199–201.
- ECKERT, W., AND K. D. HAMBRIGHT. 1996. Seaside vertical distributions of temperature, pH, oxygen and sulfide in Lake Kinneret. Limnologica **26**: 345–351.
- , AND H. G. TRUPPER. 1993. Microbially-related redox changes in sub-tropical lake. I. In situ monitoring of the annual redox cycle. Biochemistry **21**: 1–19.
- EMERSON, S., P. QUAY, C. STUMP, D. WILBUR, AND M. KNOX. 1991. O₂, Ar, N₂, and ²²²Rn in surface waters of the subarctic Pacific Ocean: Net biological O₂ production. Glob. Biochem. Cycles **5**: 49–69.
- ERIKSEN, N. T., AND A. J. LEWITUS. 1999. Cyanide-resistant respiration in diverse marine phytoplankton. Evidence for the widespread occurrence of the alternative oxidase. Aquat. Microb. Ecol. **17**: 145–152.
- GOYAL, A., AND N. E. TOLBERT. 1996. Association of glycolate oxidation with photosynthetic electron transport in plant and algal chloroplasts. Proc. Natl. Acad. Sci. USA **98**: 3319–3324.
- GUY, R. D., J. A. BERRY, M. L. FOGEL, D. H. TURPIN, AND H. G. WEGER. 1992. Fractionation of the stable isotopes of oxygen during respiration by plants—the basis of a new technique to estimate partitioning to the alternative path, p. 444–453. In H. Lambers and L. H. W. van der Plas [eds.], Molecular, biochemical and physiological aspects of plant respiration. SPB Academic Publishing.
- , M. L. FOGEL, AND J. A. BERRY. 1993. Photosynthetic fractionation of stable isotopes of oxygen and carbon. Plant Physiol. **101**: 37–47.
- HADAS, O., AND T. BERMAN. 1998. Seasonal abundance and vertical distribution of Protozoa (flagellates, ciliates) and bacteria in Lake Kinneret, Israel. Aquat. Microb. Ecol. **14**: 161–170.
- , AND R. PINKAS. 1992. Sulfate reduction process in Lake Kinneret, Israel. Hydrobiologia **235/236**: 295–303.
- KAPLAN, A., AND L. REINHOLD. 1999. The CO₂ concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 539–570.
- KIDDON, J., M. L. BENDER, J. ORCHARDO, D. A. CARON, J. C. GOLDMAN, AND M. DENNETT. 1993. Isotopic respiration of oxygen by respiring marine organisms. Glob. Biogeochem. Cycles **7**: 679–694.
- KNOX, M., P. D. QUAY, AND D. WILBUR. 1990. Isotopic fractionation during air–water transfer of oxygen and nitrogen, p. 184–199. In S. C. Wilhelms [ed.], Air–water mass transfer. American Society of Civil Engineers.
- KRAUSE, D. K., AND B. B. BENSON. 1989. The solubility and isotopic fractionation of gases in dilute aqueous solution. IIa. Solubilities of the noble gases. J. Chem. Sol. **18**: 823–873.
- KROOPNICK, P. 1975. Respiration, photosynthesis, and oxygen isotope fractionation in oceanic surface water. Limnol. Oceanogr. **20**: 988–992.
- , AND H. CRAIG. 1976. Oxygen isotope fractionation in dis-

- solved oxygen in the deep sea. *Earth Planet. Sci. Lett.* **32**: 375–388.
- LAWS, E. A. 1991. Photosynthetic quotients, new production and net community production in the open ocean. *Deep-Sea Res.* **38**: 143–167.
- , M. R. LANDRY, R. T. BARBER, L. CAMPBELL, M. L. DICKSON, AND J. MARRA. 2000. Carbon cycling in primary production bottle incubations: inferences from grazing experiments and photosynthetic studies using ^{14}C and ^{18}O in the Arabian Sea. *Deep-Sea Res. II* **47**: 1339–1352.
- LUZ, B., AND E. BARKAN. 2000. Assessment of oceanic productivity with the triple-isotope composition of dissolved oxygen. *Science* **288**: 2028–2031.
- , E. BARKAN, M. L. BENDER, M. H. THIEMENS, AND K. A. BOERING. 1999. Triple-isotope composition of atmospheric oxygen as a tracer of biosphere productivity. *Nature* **400**: 547–550.
- MAXWELL, D. P., Y. WANG, AND L. MCINTOSH. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proc. Natl. Acad. Sci. USA* **96**: 8271–8276.
- MCINTOSH, L., T. EICHLER, G. GRAY, D. MAXWELL, R. NICKELS, AND Y. WANG. 1998. Biochemical and genetic controls exerted by plant mitochondria. *Biochim. Biophys. Acta—Bioenerget.* **365**: 278–284.
- NISHRI, A., T. ZOHARY, M. GOPHEN, AND D. WYNNE. 1997. Lake Kinneret dissolved oxygen regime reflects long term changes in ecosystem functioning. *Biogeochemistry* **42**: 253–283.
- OSTROVSKY, I., Y. Z. YACOBI, P. WALLINE, AND I. KALIKHMAN. 1996. Seiche-induced mixing: Its impact on lake productivity. *Limnol. Oceanogr.* **41**: 323–332.
- QUAY, P. D., D. O. WILBUR, J. E. RICHEY, A. H. DEVOL, R. BENNER, AND B. R. FORSSBERG. 1995. The ^{18}O : ^{16}O of dissolved oxygen in rivers and lakes in the Amazon Basin: Determining the ratio of respiration to photosynthesis rates in freshwaters. *Limnol. Oceanogr.* **40**: 718–729.
- ROBINSON, S. A., D. YAKIR, M. RIBAS-CARBO, L. GILES, C. B. OSMOND, J. N. SIEDOW, AND J. A. BERRY. 1992. Measurements of the engagement of cyanide-resistant respiration in the Crasulacean acid metabolism plant *Kalanchoe daigremontiana* with the use of on-line oxygen isotope discrimination. *Plant Physiol.* **100**: 1087–1091.
- STEEMAN-NIELSEN, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Int. Explor. Mer.* **18**: 117–140.
- TCHERNOV, D., M. HASSIDIM, A. VARDI, B. LUZ, A. SUKENIK, L. REINHOLD, AND A. KAPLAN. 1998. Photosynthesizing marine microorganisms can constitute a source of CO_2 rather than a sink. *Can. J. Bot.* **76**: 1109–1118.
- WILLIAMS, P. J. LEB., AND D. A. PURDIE. 1991. In vitro and in situ derived rates of gross production, net community production and respiration of oxygen in the oligotrophic subtropical gyre of the North Pacific Ocean. *Deep-Sea Res.* **38**: 891–910.
- , AND J. E. ROBERTSON. 1989. A serious inhibition problem from a Niskin sampler during plankton productivity studies. *Limnol. Oceanogr.* **34**: 1330–1305.

Received: 5 February 2001

Accepted: 19 September 2001

Amended: 16 October 2001