

Seasonal variations in triple oxygen isotopes and gross oxygen production in the Sagami Bay, central Japan

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Abstract

We studied daily, diurnal, and seasonal variations in triple oxygen isotopes in the Sagami Bay from May to October 2002. The variations in composition of triple oxygen isotopes are in association with changes in concentrations of dissolved oxygen (DO) and chlorophyll *a* (Chl *a*). The lowest $^{17}\Delta$ anomaly (excess ^{17}O) was found during October and the highest in August. There was a prominent subsurface $^{17}\Delta$ anomaly maximum during June and August because of photosynthesis below the mixed layer and reduced gas exchange. The $^{17}\Delta$ anomaly was positively correlated with DO and Chl *a*, but with large scatter. The gross oxygen production (GOP) computed on the basis of triple oxygen isotopes is almost double the value given by the standard oxygen incubation method. This could possibly be because of inability to measure O_2 uptake rates in the light bottle and the consequent assumption that respiration is the same in light and dark bottles. GOP measured by a fast repetition rate fluorometer showed large daily variability, whereas GOP given by the $^{17}\Delta$ anomaly is within daily variability. This suggests that the $^{17}\Delta$ anomaly method measures average GOP over the residence time of DO in the mixed layer.

Primary production is defined as the amount of oxygen released by autotrophic organisms through the synthesis of organic matter, from inorganic compounds such as CO_2 , and nutrients with the use of energy derived from solar radiation (Falkowski and Raven 1997). The gross primary production can be defined as total amount of oxygen released by phytoplankton, which supports autotrophic respiration and heterotrophic activity (Riley 1940). The oxygen-based productivity can be converted to carbon units with the photosynthetic quotient (ratio of O_2 production to carbon uptake), and it varies from 0.5 to 5 (Burris 1981). Traditionally, gross photosynthetic oxygen production in marine waters is estimated from incubation of light and dark bottles, which gives net productivity and community respiration, respectively. However, these incubation experiments have two problems (Bender et al. 1987): (1) processes that take place in bottles are not entirely equivalent to those that occur in situ and (2) production and respiration rate estimates during in vitro incubations often give ambiguous results and they

fail to give a complete community metabolic rates. Bender et al. (1987) compared different incubation experiments, such as ^{14}C , O_2 , and CO_2 in light–dark bottles and the H_2^{18}O spike methods. They found that O_2 light respiration is several times higher than in the dark, and they suggested three potential processes to account for such higher rates: (1) oxidation of photosynthate produced during the light incubation, (2) photoenhancement of mitochondrial respiration, and (3) photorespiration. Recently, Dickson and Orardo (2001) found that both light and dark respiration rates are equal during spring, whereas light respiration far exceeds dark respiration rate during summer in the Antarctic Polar Front region. Luz et al. (2002) measured gross production using both ^{14}C and H_2^{18}O spike methods and found that the latter method was two to eight times higher than the former. Although Bender et al. (1987) suggested that H_2^{18}O spike measurement would be a comparatively better method than others to estimate gross oxygen production (GOP), this method also involves bottle incubation, and bottle effects cannot be avoided. Moreover, all these estimates give instantaneous production rates, but they miss short-term blooms because of heterogeneous distribution of plankton in time and space. Recently, Luz and Barkan (2000) developed a triple oxygen isotopic method to alleviate such inherent problems involved in the incubation methods. In this approach, GOP is estimated from the difference between triple isotopic composi-

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tions of atmospheric and dissolved oxygen (DO) and the rate of oxygen exchange at the air–water interface.

Triple oxygen isotope method—Natural oxygen is composed of three stable isotopes: ^{16}O , ^{17}O , and ^{18}O , with atomic abundances of 99.758%, 0.038%, and 0.204%, respectively. Their ratio in atmospheric O_2 primarily depends on the isotopic composition of photosynthetically produced O_2 from terrestrial and aquatic plants (Dongmann 1974; Farquhar and Lloyd 1993; Guy et al. 1993), and on isotopic fractionation from respiration (Lane and Dole 1956). The formation of a $^{17}\Delta$ anomaly in the DO with respect to atmospheric air is well discussed by Luz et al. (1999) and Luz and Barkan (2000). During photosynthesis, oxygen is produced from the water molecule (H_2O) with no fractionation and bears a mass-dependent signature. Thus, $\delta^{17}\text{O}$ of O_2 is produced solely by biological production; there is an excess of ^{17}O in the DO compared with air O_2 . This excess ^{17}O , called the $^{17}\Delta$ anomaly, varies from 0 to 249 per meg, and it is a tracer of GOP. The $^{17}\Delta$ anomaly is defined as (Angert et al. 2003):

$$^{17}\Delta = \ln(^{17}\text{R}/^{17}\text{R}_{\text{ref}}) - 0.518 \ln(^{18}\text{R}/^{18}\text{R}_{\text{ref}}) \quad (1)$$

where

$$\ln(^{17}\text{R}/^{17}\text{R}_{\text{ref}}) = \ln(\delta^{17}\text{O}/1,000 + 1) \quad \text{and} \quad (2)$$

$$\ln(^{18}\text{R}/^{18}\text{R}_{\text{ref}}) = \ln(\delta^{18}\text{O}/1,000 + 1) \quad (3)$$

and ^{17}R refers to the isotope ratio $^{17}\text{O}^{16}\text{O}/^{16}\text{O}_2$, ^{18}R refers to the isotope ratio $^{18}\text{O}^{16}\text{O}/^{16}\text{O}_2$, and the R_{ref} s are the reference ratios. The constant 0.518 represents the slope of $\delta^{17}\text{O}$ versus $\delta^{18}\text{O}$ for the mass-dependent processes. This value varies slightly from 0.506 to 0.521 for different processes, and 0.518 seems to be the most representative slope for the non-steady state system (Boaz Luz pers. comm.); hence, it is used in this study.

The rate of air–sea O_2 exchange decreases the magnitude of $^{17}\Delta$ of DO as it equilibrates with the atmospheric air, whereas in situ biological O_2 production increases $^{17}\Delta$ to a maximum value of pure biological O_2 (Δ_{max}). In natural aquatic systems, $^{17}\Delta$ varies between these two extremes, and its value depends largely on the ratio of the rates of GOP and air–sea O_2 exchange. Thus, GOP can be calculated from $^{17}\Delta$ of DO, if the rate of air–sea exchange is known, by Eq. 4 (Luz and Barkan 2000).

$$\text{GOP} = K\text{Co}(\Delta^{17}\text{O} - \Delta_{\text{eq}})/(\Delta_{\text{max}} - \Delta^{17}\text{O}) \quad (4)$$

GOP is the gross O_2 production, K is piston velocity, Co is equilibrium O_2 concentration, Δ_{eq} is the equilibrium $^{17}\Delta$ anomaly (16 ± 2 per meg), and Δ_{max} is 249 ± 15 per meg (Luz and Barkan 2000). Equation 4 is valid only for a uniform mixed layer with zero exchange with the water below.

Study area—We studied triple oxygen isotopes in the upper 100 m of the water column in Sagami Bay, central Japan, for 4 months at the fixed location S3 (35°N , 139.35°E ; Fig. 1). This Bay is situated on the east coast of Japan and is connected to the North Pacific. The Bay waters are saline, with surface salinity ranging from 33 to 35 and highly variable by season. The water mass of Sagami Bay is strongly influenced by the Kuroshio Current, which runs along the

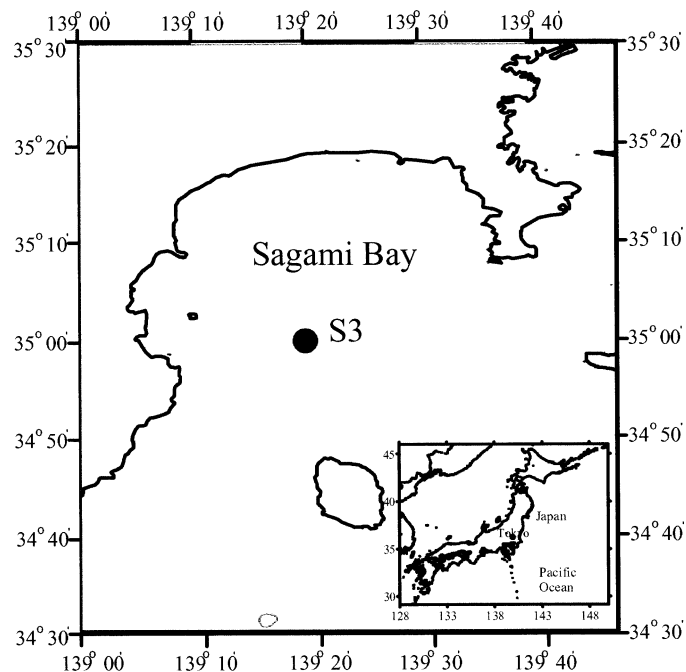


Fig. 1. Station location (S3) in Sagami Bay, central Japan.

east coast of Japan. Oligotrophic oceanic waters of the bay mix with eutrophic coastal waters and lead to an increase in chlorophyll a (Chl a) levels. Chl a concentration ranges from 0.2 (April) to 59 mg m^{-3} (July) in the coastal regions, which is 15 times higher than in the central Bay. Peak Chl a in the central Bay is $\sim 4 \text{ mg m}^{-3}$ (Satoh et al. 2000). The seasonal variations in the amount of Chl a , integrated over the upper 50 m in the Sagami Bay, ranged from 10 to 200 mg m^{-2} , with three peaks during winter, early summer, and autumn (Hogetsu and Taga 1977). The winter Chl a peak showed strong interannual variability with the highest (200 mg m^{-2}) in April. The total amount of Chl a in the upper 50-m layer varied from 2 to 262 mg m^{-2} (mostly within 70 mg m^{-2}) in 1971 and $4\text{--}55 \text{ mg m}^{-2}$ in 1972. Since 2000 in the Sagami Bay, water column physical and biogeochemical properties have been continuously measured at monthly intervals under the CREST (Core Research for Evaluation of Science and Technology) program. This study is also a part of the CREST program, primarily aimed at studying diurnal, daily, and seasonal variability in triple oxygen isotopes and estimating GOP in the Sagami Bay.

Sampling technique

Water sampling and processing of sample—Sampling was carried out at station S3 in the Sagami Bay (Fig. 1) during May, June, August, and October 2002 in the upper 100 m. Seawater samples were collected with 5-L Niskin bottles mounted to the Sea-Bird CTD rosette system. The water sampling methodology was adopted from Luz et al. (2002). About 150 ml of water sample was collected into pre-evacuated gas extraction vessels (300-ml flasks with Louwers Hapert® O-ring stopcocks) containing $250 \mu\text{l}$ of saturated HgCl_2 solution. To avoid leakage during storage, the sample

port of the bottle was filled with distilled water and capped. In this way, samples can be stored up to 6 months without contamination.

The water and headspace in the sampling flasks were equilibrated for 24 h at room temperature. After equilibration, the water was carefully removed from the flasks by leaving only headspace gases. The flask was then connected to a preparation system for separation of the O₂-Ar mixture from N₂, CO₂, N₂O, and H₂O following Barkan and Luz (2003).

Separation and purification of the O₂-Ar mixture—The O₂-Ar mixture was separated from other dissolved gases with a chromatographic column (8 × 2 mm inside diameter) packed with a 45/60-mesh 5A molecular sieve at zero degrees. The separation is initiated by chilling the equilibrated sample flask to liquid nitrogen temperature to freeze CO₂, N₂O, and water in the vacuum line. Then sample is injected onto the GC column with high-purity helium as a carrier gas. Ar and O₂ release within 30 min, and they were collected on a molecular sieve at liquid nitrogen temperature and admitted to the mass spectrometer (Finnigan Delta Plus) for further analysis. Each sample was run for 10 cycles, with 10 runs per cycle, to obtain good precision. The standard error of the mean for δ¹⁷O and δ¹⁸O are 7 and 3 per meg and the computed ¹⁷Δ anomaly is 5.5 per meg, respectively.

Estimation of gross oxygen production by fast repetition rate fluorometer—Fast repetition rate fluorometer (FRRF) measurements were carried out in situ by deploying FAST-track^{ca} (Chelsea Technologies Group) via the underwater profiling buoy system. Our buoy system consists of an underwater winch, placed below the euphotic zone, and a profiling buoy equipped with a FRRF and external photosynthetically active radiation (PAR), pressure, temperature, and salinity sensors. The buoy moves up and down between the winch depth (60 m) and surface at 2-h interval from 0400 to 2000 h. Fluorescence parameters, such as initial and maximum in vivo fluorescence yields in the dark-adapted state in the absence of nonphotochemical quenching (F_o and F_m, respectively), initial and maximum in vivo fluorescence yield at ambient irradiance in the presence of nonphotochemical quenching (F and F_m'), maximum and functional photochemical energy conversion efficiency ($\phi = [F_m - F_o]/F_m$, $\phi' = [F_m' - F]/F_m'$), functional absorption cross section of photosystem II (PSII) in the dark-adapted state and at ambient irradiance (σ_{PSII} and $\sigma_{\text{PSII}'}$), and extent of energy transfer between RCII (Reaction centers of PSII) in the dark-adapted state and at ambient irradiance (p and p') were measured. The instantaneous P^B (biomass specific production) values were extracted from FRRF data with the equations given by Kolber and Falkowski (1993). The FRRF was set to deliver 100 saturation flashlets (1 μs duration, 1 μs interval) to reduce the primary electron acceptor (Q_a) of phytoplankton PSII and a subsequent 20 relaxation flashlets (1 μs duration, 50 μs interval) to analyze the reoxidation processes of the PSII acceptor side. Acquisition of a series of 16 flash sequences was internally averaged and repeated five times with a 500-μs interval between iterations. The evolution of oxygen was computed by Eq. 5 from Kolber and Falkowski (1993).

$$P_{\text{O}_2}^{\text{B}}(z) = E(z)f(z)n_{\text{PSII}}\sigma_{\text{PSII}}(z)q_p(z)\phi_e(z) \quad (5)$$

P_{O₂}^B(z) is the rate of gross photosynthetic oxygen evolution per unit Chl *a* (mol O₂ evolved [mg Chl *a*]⁻¹ time⁻¹), *E*(z) is underwater irradiance at depth (quanta m⁻¹ s⁻¹), *f*(z) is the fraction of PSII reaction centers that are capable of evolving oxygen, and *n*_{PSII} is the ratio of PSII reaction centers to Chl *a*, for which we used a typical value of 1/500 suggested by Kolber and Falkowski (1993), *q_p*(z) is photochemical quenching ($= [F_m - f']/[F_m - F_o]$), and ϕ_e is quantum yield of electron transport, accounting for losses from thermal dissipation and electron cycling around PSII, a dimensionless number between 0 and 1, approaching 1. ϕ_e is calculated following Kolber and Falkowski (1988):

$$E\sigma_{\text{PSII}}q_p \leq 1/\tau_p \quad \text{then} \quad \phi_e = 0.25 \quad (6)$$

$$E\sigma_{\text{PSII}}q_p \geq 1/\tau_p \quad \text{then} \quad \phi_e = 0.25/(E\sigma_{\text{PSII}}q_p\tau_p) \quad (7)$$

where τ_p is turnover time for whole-chain electron flow in steady-state photosynthesis, and it is assumed to be constant (4.3 ms).

The FRRF was calibrated in the laboratory against short-time (20 min) incubation of phytoplankton cultures with a ¹⁴C radioactive tracer at artificial light intensity. Both ¹⁴C- and FRRF-based production displayed strong positive correlation ($r^2 = 0.98$).

Although vertical profiles of photosynthetic parameters were measured from the surface to 60 m deep, we used the values measured in the mixed layer for comparison with gross production on the basis of the ¹⁷Δ anomaly.

Results and discussion

Hydrography of Sagami Bay during the study period—The temperatures in the upper 10 m showed large variability, with low temperatures observed during May and June (~20.0–20.4°C) followed by October (24.6°C) and August (27.2°C; Fig. 2a). Shallow mixed layer depth was found during June and August (~15–20 m), whereas it was deeper during May and October (~20–40 m). The depth of the euphotic zone (on the basis of 1% PAR) is also deeper during October (43 m) compared with June (38 m) and August (31.5 m). The mixed layer nitrate concentrations were higher during October (0.3–0.8 μmol kg⁻¹) compared with May–August (0.15–0.22 μmol kg⁻¹). Similarly, higher silicate concentrations were found during October (5.5–6 μmol kg⁻¹) than in August (2.75–3.5 μmol kg⁻¹) or June (0.01–0.14 μmol kg⁻¹) because of convective mixing during October.

Vertical distributions of Chl *a* showed low and uniform concentrations (0.48–0.56 mg m⁻³) in the upper 50 m during October. On the contrary, the highest Chl *a* concentrations were observed during August (1–3 mg m⁻³) in the euphotic zone, whereas it was 0.3–1.5 mg m⁻³ during May and June (Fig. 2c). The prominent subsurface Chl *a* maximum was evident below the mixed layer with 1.2 and 3.1 mg m⁻³ during June and August, respectively, when the water column stratified.

DO concentrations showed strong variability in the upper 30 m. DO concentrations in the mixed layer are supersatu-

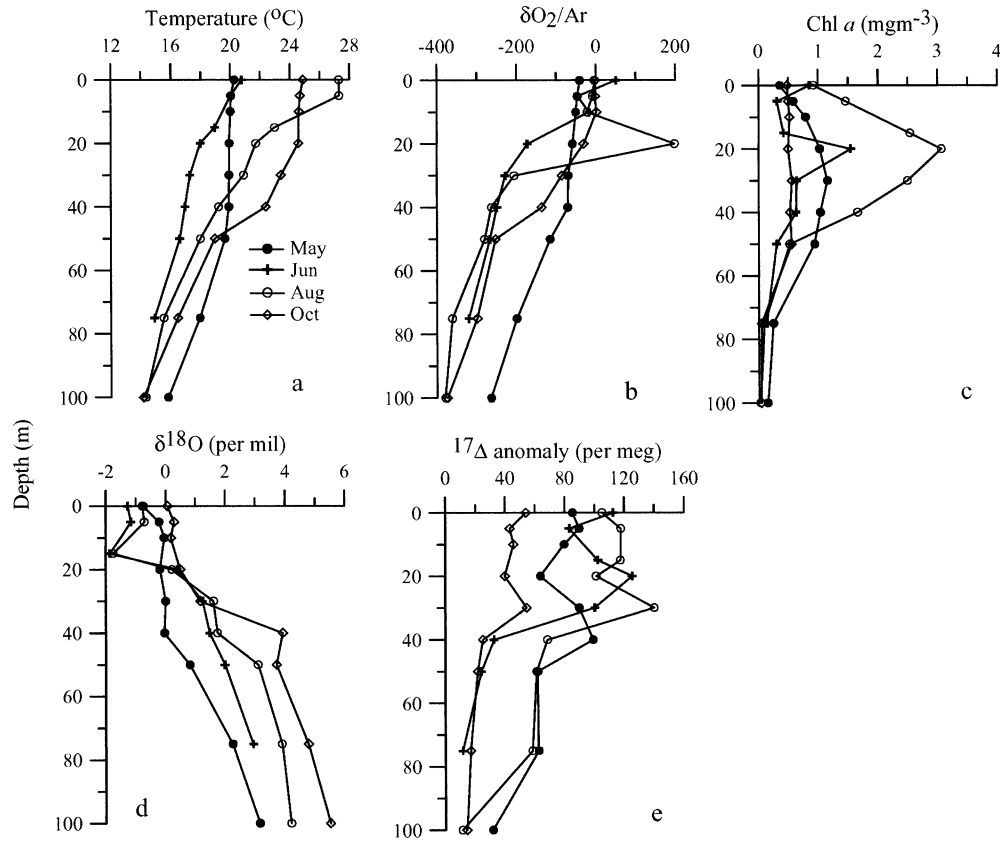


Fig. 2. Monthly variability in (a) temperature, (b) $\delta(\text{O}_2/\text{Ar})$, (c) Chl *a*, (d) $\delta^{18}\text{O}$, and (e) the $^{17}\Delta$ anomaly in Sagami Bay.

rated (102–114%) during all months, except October when waters are close to saturation (98–100%). Subsurface maximum in DO concentrations can be observed during June and August at the depth of 10–20 m, and it is associated with a subsurface Chl *a* maximum. On the contrary, DO concentrations decreased gradually with increase in depth during May and October, when no Chl *a* maximum was found because of deep mixing. To examine the potential reasons for a subsurface DO maximum as a consequence of changes in temperature, salinity, or biological production, $\delta(\text{O}_2/\text{Ar})$ values (deviation of the O_2/Ar ratio of dissolved gases from the O_2/Ar ratio in air) were measured.

Three main processes affect DO concentrations and isotopic ratios in the mixed layer. They are air–sea gas transfer, biological processes (photosynthesis and respiration), and mixing with waters below the mixed layer. The measurement of $\delta(\text{O}_2/\text{Ar})$ provides information about potential causes for changes in oxygen concentrations, such as physical processes (solubility effect by temperature and salinity) or biological processes (phytoplankton productivity/respiration). It is because argon (Ar) is a biologically inert analog for O_2 because of the similarity in Henry's constants and the diffusion rates of these two gases (Emerson et al. 1991). The main difference in these gases is that Ar concentration increases with depth because of an increase in solubility, whereas O_2 concentration decreases because of consumption by biological activity; thus, $\delta(\text{O}_2/\text{Ar})$ decreases with depth. Hence, it is

possible to examine whether the changes in DO concentrations are a result of physical or biological processes by variations in $\delta(\text{O}_2/\text{Ar})$ values. Vertical variations in $\delta(\text{O}_2/\text{Ar})$ during 4 months were plotted in Fig. 2b. This figure shows that $\delta(\text{O}_2/\text{Ar})$ in the mixed layer is low during October, and the highest in August. The subsurface maximum in $\delta(\text{O}_2/\text{Ar})$ during June and August suggests that the changes in oxygen concentrations in the euphotic zone were the result of predominantly biological processes during June and August.

Variations in triple oxygen isotope and $^{17}\Delta$ anomaly in the water column—The $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values are low at the surface and increased sharply to 100 m depth in Sagami Bay during all sampling months (Fig. 2d; Web Appendix 1 at http://www.aslo.org/lo/toc/vol_50/issue_2/0544a1.pdf). The lighter $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ in the surface waters are a result of the dominance of phytoplankton production compared with community respiration. The lighter $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ were found in the upper 30 m during June and August compared with May and October, and comparatively, the heaviest oxygen isotopes are found during the latter month. Three different processes, such as air–sea exchange, mixing with deep waters, and biological production/respiration, can cause differences in $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values in the mixed layer. The mean gross flux of oxygen from the atmosphere was found to be 498.2, 518.5, 524.7, and 520.3 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ in May, June, August, and October, respectively. An average O_2 flux

Table 1. Mixed layer properties in the Sagami Bay during different months, comparison of gross oxygen production by light- and dark-bottle incubation, and comparison of triple oxygen isotopes. GOP is integrated production in the mixed layer.

Month	MLD (m)*	Mixed layer Chl <i>a</i> (mg m ⁻²)	Average anomaly before correction (per meg)	Average anomaly after correction (per meg)	Average gross O ₂ flux (mmol m ⁻² d ⁻¹)	GOP by O ₂ incubation (mmol m ⁻² d ⁻¹)	GOP by triple O ₂ isotopes (mmol O ₂ m ⁻² d ⁻¹)		GOP by FRRF (mmol O ₂ m ⁻² d ⁻¹)
							Before correction	After correction	
22 May	20	17.2	81.8	80.1	498.2	—	196.1	189.1±58	—
23 May	40	14.9	82.7	82.0	498.2	105.8±10	199.8	196.9±57	146.9±58
24 May	20	17.2	81.8	79.8	498.2	—	196.0	187.8±59	114.6±46
3 Jun	10	—	—	—	—	—	—	—	189.0±75
4 Jun	15	21.5	99.3	92.3	518.5	132.5±13	288.5	252.5±62	143.9±57
5 Jun	12	—	—	—	—	329.2±33	—	—	305.9±92
Aug	20	44.0	118.2	109.9	524.7	162.7±16	409.9	354.2±63	—
Oct	20	10.1	51.8	51.0	520.3	84.3±8	94.4	91.9±22	—

* MLD, mixed layer depth.

of 5 mmol O₂ m⁻² d⁻¹ would change δ¹⁸O by 0.05 per mil from the air saturation value (Emerson et al. 1991). The difference in air flux between October and May is ~22 mmol m⁻² d⁻¹, and it is expected to enrich the δ¹⁸O value by 0.22 per mil. Hence, the difference in O₂ air–sea exchange alone cannot explain the observed O₂ enrichment of ~0.3–0.6 per mil during October compared with May–August. Higher respiration might have enriched δ¹⁷O and δ¹⁸O during October. It is possible because O₂ in the euphotic zone is close to saturation in October (98–100%), whereas it is oversaturated during May–August (102–114%). This suggests that either respiration is higher than production during October or O₂ has been input to the epilimnion by mixing with deeper water that is enriched in δ¹⁷O and δ¹⁸O as a result of respiration in the absence of production. During this study, we measured community dark respiration at different depths. The rate of dark community respiration (24 mmol O₂ m⁻² d⁻¹) in the upper 20 m is lower than production (91 mmol O₂ m⁻² d⁻¹) during October and suggests that enrichment in the heavier isotopes is not the result of respiration in excess of production. Mixing of waters at the base of the mixed layer drives the third possibility. On the basis of computed vertical mixing at the base of the mixed layer with the use of time-series mixed layer depth data, mixing increases δ¹⁷O and δ¹⁸O by ~0.04‰ and 0.08‰ in the mixed layer, respectively. Thus, the depletion of δ¹⁷O and δ¹⁸O in the mixed layer during June and August might be caused by higher production compared with October. This is consistent with the Chl *a* distribution, which is lowest during October. In addition, a minimum ¹⁷Δ anomaly occurred below the mixed layer (~15–20 m), in association with subsurface Chl *a* and δ(O₂/Ar) maxima during June and August from production and reduced gas exchange. This further suggests that the changes in δ¹⁷O and δ¹⁸O resulted mainly from production and respiration. Respiration discriminates in a mass-dependent way, and the relative increase in δ¹⁷O is about half (~0.52) of the relative increase in δ¹⁸O (Luz and Barkan 2000). Angert et al. (2003) found that the slope of δ¹⁷O versus δ¹⁸O varies in different respiration mechanisms, such as in the cytochrome oxidase pathway (0.518 ± 0.001), alternative oxidase pathway (0.5179 ± 0.00031), and in photorespiration (0.506 ± 0.005). The first two processes take

place in light as well as dark conditions, whereas the latter occurs only under illumination.

The vertical distribution of the ¹⁷Δ anomaly, computed with Eq. 1, is depicted in Fig. 2e. The ¹⁷Δ anomaly in the mixed layer is low during October (40–54 per meg), which is 64–118 per 10⁶ during other months, and the highest anomaly was found during August (Table 1). The variations in the ¹⁷Δ anomaly are consistent with the Chl *a* distribution (i.e., high anomaly is associated with high Chl *a* and vice versa). More interestingly, an accumulation of the ¹⁷Δ anomaly is found at the depth of a subsurface Chl *a* maximum below the mixed layer depth during June and August, whereas a uniform anomaly is observed in October when the mixed layer was deep. The ¹⁷Δ anomaly showed two important features: (1) higher ¹⁷Δ anomaly than equilibrium in the mixed layer because, in addition to the dominance of gas exchange, the effect of gross production is also significant and (2) in waters of the sunlit thermocline, where both δ¹⁸O and δ¹⁷O are high and DO concentration is low, the ¹⁷Δ anomaly is elevated, which could be because of photosynthesis and a reduced gas exchange.

Diurnal and daily variability in triple oxygen isotopes and the ¹⁷Δ anomaly—The ¹⁷Δ anomaly in DO is expected to distribute uniformly in a given water mass. Hence, the influence of short-term events of low and high production on the ¹⁷Δ anomaly is expected to average out over the residence time of oxygen (Luz and Barkan 2000). The residence time of oxygen in Sagami Bay is found to be about a week according to time series oxygen flux data collected by a gas tension device (GTD; Pro-Oceanus Systems Inc., Canada). To examine daily variations in the ¹⁷Δ anomaly in the mixed layer, we have collected samples from the upper 100 m during 3 d (22–24 May 2002) from station S3. The vertical distribution of temperature, salinity, and depth of mixed layer displayed slight variations in the upper 50 m, which could be a result of diurnal variations in the physical properties of the seawater. This is possible because the sampling time was different during these 3 d (Fig. 3a). For instance, sampling was done on 22 May at 1200 h, whereas it was 1400 h and 1600 h on 23 and 24 May, respectively. DO concentrations displayed significant differences between 10 and 40 m. Com-

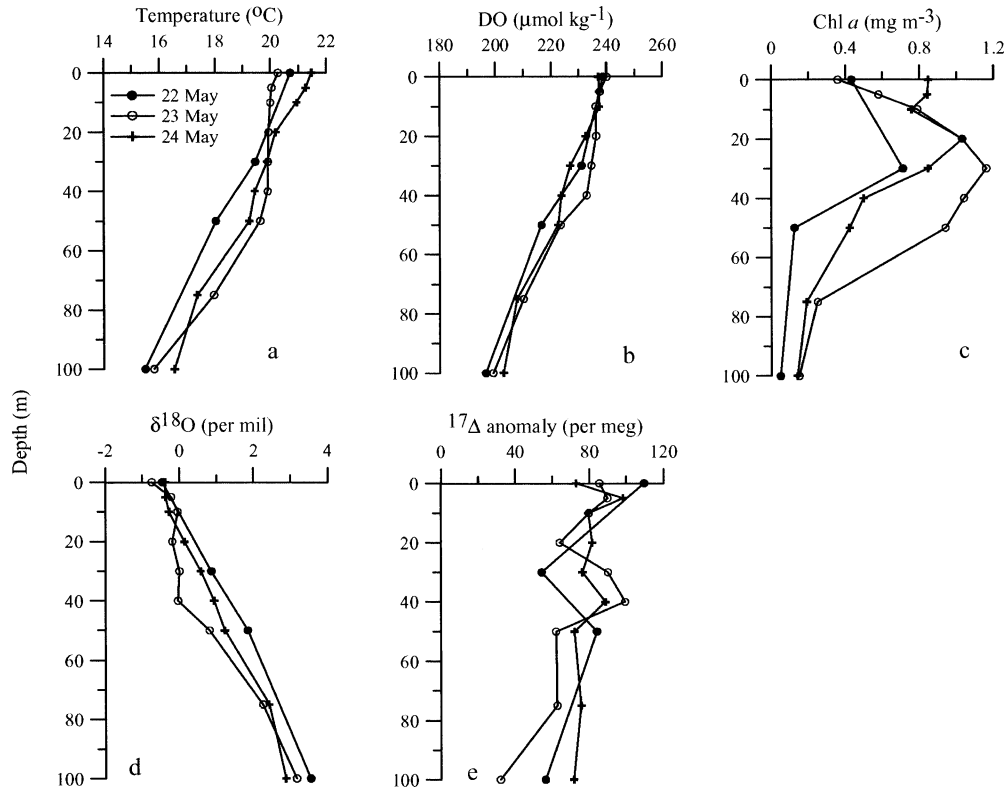


Fig. 3. Diurnal variations in (a) temperature (b) DO (c) Chl *a*, (d) $\delta^{18}\text{O}$, and (e) the $^{17}\Delta$ anomaly.

paratively higher DO concentrations were found on 23 May than on 22 or 24 May (Fig 3b). Chl *a* showed significant differences during 3 d at all depths (Fig. 3c). Variations in DO and Chl *a* concentrations might be driven by diurnal variations in phytoplankton production because of changes in light intensity, nutrient availability, and zooplankton grazing on phytoplankton, for example. However, no relation was found between the $^{17}\Delta$ anomaly and Chl *a*. Although Chl *a* varied significantly at all depths during 3 d (Fig. 3c), column integrated Chl *a* over the mixed layer was uniform (Table 1). The vertical variations in both $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ are uniform with respect to DO and Chl *a*; for instance, low $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ are consistent with high DO and Chl *a* concentrations. The $\Delta^{17}\text{O}$ anomaly in the mixed layer varied from 72 to 108 per meg during 3 d (Fig 3e; Web Appendix 1). However, the peak of the $^{17}\Delta$ anomaly was unchanged during 3 d, but their magnitude was different. For instance, the maximum $^{17}\Delta$ anomaly was found at 5 m during 3 d, but a higher value (97 per meg) was found during 24 May, which is again consistent with the highest Chl *a* concentration. However, the surface anomaly did not show such a relation, in which a lower $^{17}\Delta$ anomaly was found during 24 May when high Chl *a* was observed. As stated before, a strong correlation between Chl *a* and the $^{17}\Delta$ anomaly is unlikely because the latter is the time-integrated signal over the residence time of oxygen. Moreover, Chl *a* does not relate quantitatively with productivity because the latter depends on several other parameters (Balch et al. 1992). To determine whether changes in surface the $^{17}\Delta$ anomaly was a result of variation in productivity, surface samples were

collected for 2 d from 0400 to 2000 h every 3 h, and the variations were compared with FRRF-based continuous primary productivity measurements (Fig. 4c). The surface $^{17}\Delta$ anomaly showed maxima at noon (~ 1200 h) for 2 d that is consistent with maxima in FRRF-based primary production (Fig. 4b,c). This suggests that vertical variability in the $^{17}\Delta$ anomaly could be due to diurnal variations in productivity at different depths (Fig. 3e). Nevertheless, the average mixed layer anomaly is more or less uniform for 3 d (Table 1). As it is described above, the $^{17}\Delta$ anomaly is a time-integrated rate signal of GOP in the mixed layer over the residence time of oxygen (Luz and Barkan 2000). Hence, short time variations in the $^{17}\Delta$ anomaly were averaged out in the mixed layer. This strongly suggests that although this method responds to short-term changes in the water column properties, the average mixed layer $^{17}\Delta$ anomaly represents GOP integrated to the residence time of oxygen in the mixed layer.

A positive relation was found between the $^{17}\Delta$ anomaly and DO in Sagami Bay, but the slope of the relation varied slightly during different months (Fig. 5a). The statistical *F*-test on the entire data set suggests that the relation is significant ($p = 0.0037$) at the $\alpha = 0.01$ level. Although both DO and the $^{17}\Delta$ anomaly store time-integrated signals over the residence time of oxygen in the mixed layer, scatter in the relation is possible because of their different controlling parameters. Despite the major controlling processes, flux of oxygen from atmosphere, horizontal advection, mixing with subsurface waters, and biological production are similar for both DO and the $^{17}\Delta$ anomaly, but biological respiration and flux to atmosphere changes DO concentrations significantly.

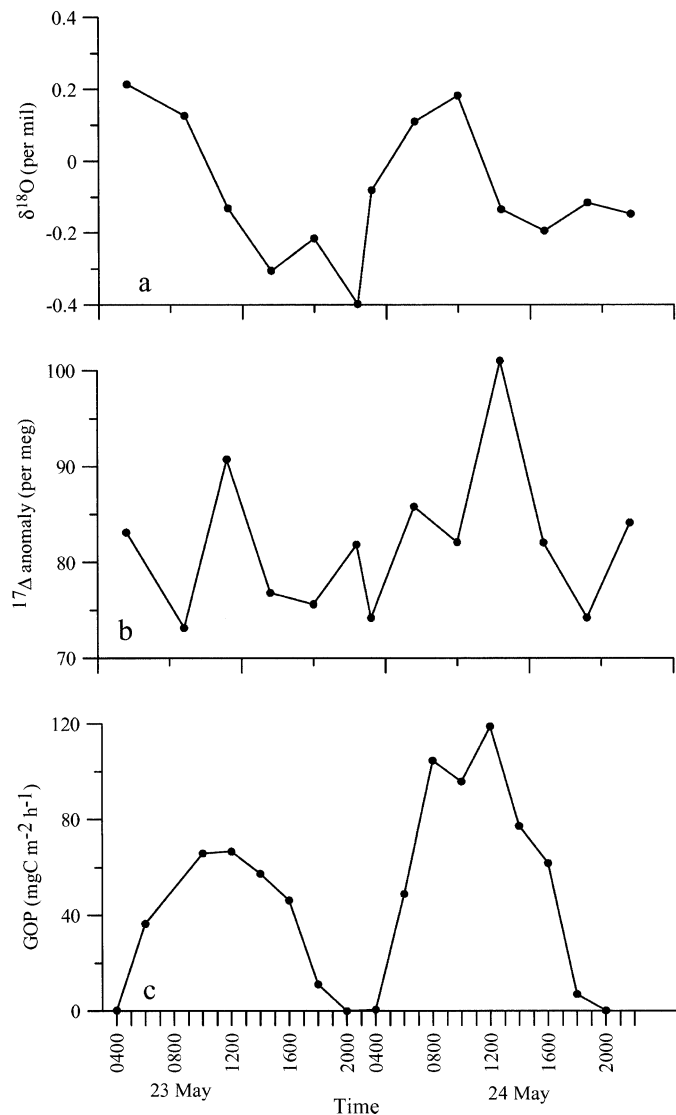


Fig. 4. Diurnal variations in (a) $\delta^{18}\text{O}$ (b) the $\Delta^{17}\text{O}$ anomaly, and (c) gross primary production estimated with FRRF in the surface waters of Sagami Bay during May.

Moreover, the latter processes have no effect on the $^{17}\Delta$ anomaly. These could be the potential reasons for scatter in the relation. On the contrary, the $^{17}\Delta$ anomaly displayed a positive relation with Chl *a* with large scatter (Fig. 5b), and the statistical *F*-test yields a significant relationship between them ($p = 0.0085$). Like the DO– $^{17}\Delta$ anomaly relation, Chl *a* concentrations vary rapidly because their concentration is controlled by temperature, salinity, stability of the water column, micro- and macronutrients, zooplankton grazing, and so on, and none of these parameters change the $^{17}\Delta$ anomaly. Moreover, flux of oxygen from atmosphere, which is one of the prime controlling factors on the $^{17}\Delta$ anomaly, does not affect Chl *a* concentration. In addition to this, Chl *a* concentration does not represent primary production quantitatively (Campbell and O'Reilly 1988; Balch et al. 1992).

Estimation of GOP with triple oxygen isotopes—The air–sea exchange of oxygen is one of the prime parameters used

to estimate GOP in the mixed layer with the $^{17}\Delta$ anomaly. To estimate the air–sea exchange of O_2 , we have monitored the changes in surface temperature, salinity, and DO and nitrogen partial pressures with a GTD–CTD couple (McNeil et al. 1995) in Sagami Bay every 5 min. With this data and piston velocities computed with Wanninkhof (1992) equations, the O_2 flux into the mixed layer was computed. On the basis of air–sea fluxes of oxygen, the residence time of O_2 in the mixed layer was found to be about 1 week in Sagami Bay. Because our experiments were conducted for about 1 week, an average oxygen flux for 1 week was used in the computation of GOP by Eq. 4. The GOP in the mixed layer of Sagami Bay, computed according to Luz and Barkan (2000), ranged from 94.4 to 409.9 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$, with low production during October and higher production in August (Table 1; column 8). The Luz and Barkan (2000) model is applicable only if waters are in the steady state. They assumed in their estimates that vertical mixing is negligible on changes in the $^{17}\Delta$ anomaly in the mixed layer. However, we believe that it might be true if there is no subsurface Chl *a* maximum and the $^{17}\Delta$ anomaly is uniform even below the mixed layer depth as seen, for instance, during May and October in Sagami Bay. On the contrary, vertical mixing would significantly influence the mixed layer $^{17}\Delta$ anomaly if there were a significant gradient between the mixed layer and the water underneath. To compute the $^{17}\Delta$ anomaly derived by phytoplankton production in the mixed layer, it has to be corrected for the possible exchange of the $^{17}\Delta$ anomaly with subsurface waters. The mixing of the subsurface $^{17}\Delta$ anomaly with the surface is evaluated by time series changes in depth of the mixed layer. During our experiments, vertical temperature and salinity structure was measured at a 2-h interval. The mixed layer depth during different months changed by 3–10 m. The mixing effect on the $^{17}\Delta$ anomaly is calculated with Eq. 8.

$$^{17}\Delta_m = (^{17}\Delta_d - ^{17}\Delta_s) \times \delta Z / Z_m \quad (8)$$

$^{17}\Delta_m$ is change in the $^{17}\Delta$ anomaly by mixing; $^{17}\Delta_d$ and $^{17}\Delta_s$ are the anomalies below and above the mixed layer, respectively; δZ is the change in the mixed layer depth, and Z_m is the depth of the mixed layer. During the residence time of oxygen, if the mixed layer deepens, it is likely that the $^{17}\Delta$ maximum would mix with surface layers and can be quantified by Eq. 8.

It was found that the $^{17}\Delta$ anomaly in the mixed layer increased by 0.7 to 8.3 per meg, due to mixing with subsurface waters (Table 1). However, complicated mixing is possible and might not be captured in this computation. Hence, three-dimensional modeling efforts are needed to understand the influence of mixing. Nevertheless, the mixing influence is especially high during June and August (7–8.3 per meg) because of a high $^{17}\Delta$ anomaly below the mixed layer. After applying this correction, GOP was computed with the Luz and Barkan (2000) model and GOP was found to change by 2.5–56 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ in the mixed layer of Sagami Bay (Table 1; columns 8 and 9). Consistently, GOP estimated with the $^{17}\Delta$ anomaly is higher than that from oxygen bottle incubation in light and dark and FRRF. Two potential reasons are possible for this difference. Rates of gross primary production by (1) both the O_2 incubation and FRRF measure-

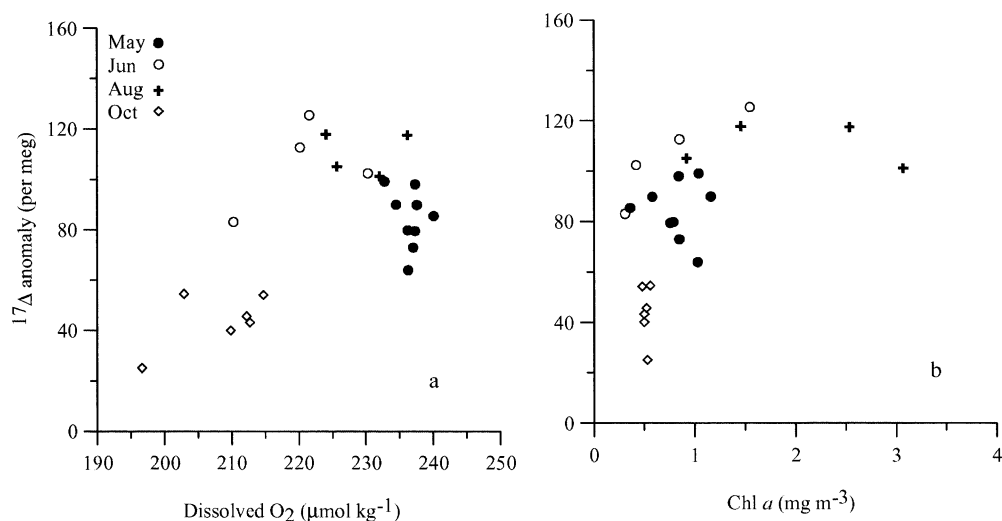


Fig. 5. Relationship of the $\Delta^{17}\text{O}$ anomaly with (a) DO and (b) Chl *a* in Sagami Bay during different months.

ment are underestimates, (2) there is large daily variability in GOP in Sagami Bay, or both. The first reason is possible, for instance, in the case of oxygen bottle incubation, with the inability to measure the rate of O_2 uptake in the light, so it is assumed to be equal to dark incubation. Bender et al. (1987) and Dickson and Orchardo (2002) found that rates of O_2 light respiration are about an order of magnitude greater than that of O_2 dark respiration. Therefore, it is possible that GOP by the oxygen incubation experiment might have been underestimated. On the other hand, the estimation of oxygen evolved with the use of FRRF assumes that some parameters are constant. In particular, the estimate of n_{PSII} for natural phytoplankton communities are scarce. Values of n_{PSII} from 1/800 to 1/500 have been used for eukaryotic microalgae (Kolber and Falkowski 1993; Falkowski and Kolber 1995; Boyd et al. 1997), and this number is species dependent. We used a typical value of 1/500 in our calculations that was suggested by Kober and Falkowski (1993). Changing this value by 1/100 would introduce an uncertainty in GOP estimates of 30%. In addition, Raateoja et al. (2004) recently found that scattered ambient red light reaching the detector side of the FAST^{tracka} impedes the interpretation of the variable fluorescence data by flattening the variable fluorescence transients in the near-surface layers. They attributed that the sensitivity of the FRRF technique to the ambient red light in near-surface layers weakens the reliability of the results (Raateoja et al. 2004). However, it is difficult to quantify the influence of the red light effect in our data. In addition, the second possibility, daily variability in GOP, could also be a possible causative factor for the differences found between GOPs estimated by different methods. We have measured daily GOP for 2 and 3 d consecutively during May and June, respectively, with FRRF. The GOP ranged from 114.6 to 147 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ during 23–24 May, whereas it ranged from 144 to 306 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ during 3–5 June by FRRF (Table 1; column 10). The GOP estimated with the $^{17}\Delta$ anomaly seems to fall within the daily variability in Sagami Bay. This study further suggests that GOP estimated with

the $^{17}\Delta$ anomaly would reflect average primary production over the residence time of oxygen; hence, the influence of the short-term bloom effect can be captured by this method.

The diurnal and seasonal variations in triple oxygen isotopes were studied in Sagami Bay from May to October 2002. The changes in triple oxygen isotopes are consistent with variations in DO and Chl *a*. The subsurface Chl *a* and $^{17}\Delta$ anomaly maxima are found during June and August, and they were absent during another 2 months. The $^{17}\Delta$ anomaly responded to short-term variations (~ 3 h) in productivity in the surface layers. Although the $^{17}\Delta$ anomaly changed with time over 3 d at station S3 at a given depth, the averaged mixed layer $^{17}\Delta$ anomaly is more or less constant. This strongly suggests that the $^{17}\Delta$ anomaly stores GOP signal over the residence time of oxygen (1 week in the case of Sagami Bay). The influence of vertical mixing on the mixed layer $^{17}\Delta$ anomaly was found to be 0.7–8.3 per meg. By the Luz and Barkan (2000) model, the GOP in Sagami Bay was computed to be almost double that of the oxygen incubation technique, whereas it is within the daily variability in GOP over 3 d as measured by FRRF. Inconsistency in the bottle incubation method could be because of the inability to measure O_2 uptake rates in the light bottle and assuming it to be equal to the rates in dark bottles. This suggests that the $^{17}\Delta$ anomaly method measures average GOP over the residence time of DO and that the effect of short-term blooms can be captured.

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