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Short-term variation of triple oxygen isotopes and gross oxygen production in the Sagami Bay, central Japan

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Abstract

We studied diurnal and daily variations in triple oxygen isotopes in the euphotic zone of the Sagami Bay during summer 2003 and 2004. There were sharp changes in physical processes, driven by changes in wind speeds, during the study period. Mixed-layer $^{17}\Delta$ anomalies were negatively correlated with wind speeds, whereas dissolved oxygen (DO) and chlorophyll *a* (Chl *a*) were positively correlated, suggesting that both physical and biological processes influence the $^{17}\Delta$ anomaly. Vertical mixing affected estimation of gross oxygen production (GOP) in the mixed layer, whereas horizontal advection was negligible in the Sagami Bay during the study period. Hourly GOP estimates are consistent with fast repetition rate fluorometer (FRRF) measurements; relatively higher estimates from the former method were due to the storing of past productivity signal. Daily integrated production estimated from $^{17}\Delta$ anomaly and FRRF were consistent during the entire study period, whereas the estimation from a ^{18}O spike incubation technique showed good agreement with the other two techniques only when the water mass structure was unchanged in the mixed layer.

Understanding the distribution of bioactive tracers in the sea is a fundamental challenge in oceanography. Gross oxygen production (GOP) is a fundamental but poorly known characteristic of the planktonic marine ecosystem. Most of our knowledge of primary production is based on data collected by the ^{14}C method (Steeman Nielsen 1952) and other techniques involving incubations in vitro. The ^{14}C method has significant problems associated with it, such as bottle effect (i.e., influence of lack of mixing on growth of bacteria and phytoplankton); loss of labeled ^{14}C as CO_2 and DOC; and assimilation of unlabeled CO_2 (e.g., Fitzwater et al. 1982; Bender et al. 1987; Howarth and Michaels 2000). Data derived using the ^{14}C method may seriously underestimate oceanic production. The light-dark (LD) bottle incubation method is free from tracer loss, as it measures changes in dissolved oxygen (DO) concentrations, but it is not free of bottle effect (Howarth and Michaels 2000). However, this method assumes equal respiration rates in dark and light. Several studies showed that respiration at light is higher than dark by a factor of 2 : 10 (Bender et al. 1987; Grande et al. 1989, 1991). The ^{18}O incubation method, which is also susceptible to the bottle effect, measures O_2 released at the photosystem II (PSII) and therefore directly reflects the GOP. Several investigators (Bender et al. 1987; Kiddon et al. 1995; Bender et al. 1999, 2000) found higher production by ^{18}O incubation than ^{14}C by a factor of two. On the contrary, recently Ostrom et al. (2005) found that primary production by ^{18}O incubation is significantly lower than ^{14}C and LD bottle incubations in the eutrophic and mesotrophic environment and attributed due to consumption of labeled O_2 within cells or evolution of extraneous O_2. In order to alleviate these problems, Luz and Barkan (2000) applied variations in ratios of all three stable O_2 isotopes to estimate changes in global GOP. This method requires no incubation, and average mixed-layer GOP over residence time of oxygen in the mixed layer can be estimated. Luz and Barkan (2000) estimated GOP at the Hawaii Ocean Time-series Station (HOTS) and the Bermuda Atlantic Time-series Station (BATS) using triple oxygen isotopes, and it was comparable with the same measured using the

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^{18}O spike incubation technique. Hendricks et al. (2004) measured net and gross oxygen production in the southern ocean using biological O_2 saturation and triple oxygen isotopes. Recently, Juranek and Quay (2005) estimated gross primary production (GPP) using ^{14}C, ^{18}O incubations, and triple oxygen isotopes at HOTS during winter and summer periods and found that 24–35% higher production by the latter method compared with the former two methods. They suggested that aperiodic bursts of production might contribute significantly to time-averaged mean production by triple oxygen isotopes.

In all these estimations, the influence of physical processes, such as the lateral and vertical mixing, were assumed to be negligible. Sarma et al. (2005) suggested that ~3% to 15% of GOP was overestimated by the triple oxygen isotope method as the result of ignoring the influence of vertical mixing in the Sagami Bay. Sarma et al. (2005) compared the estimation of GOP by triple oxygen isotopes with the LD bottle incubation technique and FRRF in the Sagami Bay during different seasons. They found that triple oxygen isotopes estimated relatively higher production compared with the LD incubation technique; however, it was sometime consistent with the average production measured by FRRF over residence time of oxygen (3–4 d) in the mixed layer. Another possible reason for the difference was due to change in water mass structure with time at the study site. Sarma et al. (2005) collected only one profile of $^{17}\Delta$ anomaly data per day in the photic zone, whereas FRRF measured in situ production over nine times per day (i.e., from 04:00 h to 20:00 h at 2-h intervals). If water mass structure changes significantly on a diurnal scale, one to one correlation between these two techniques is unlikely. Moreover, the $^{17}\Delta$ anomaly technique gives average production over residence time of oxygen in the mixed layer of a water mass sampled, whereas FRRF gives daily production of a site where the instrument was moored. In order to compare GOP by these two techniques, $^{17}\Delta$ anomaly has to be measured at the same time frequency of FRRF measurements (i.e., 2-h intervals). Our aim of this study is to examine (1) how physical processes, such as mixing caused by sudden changes in wind speeds, and advection of different water masses would change triple oxygen isotope composition in the mixed layer; (2) to derive hourly/daily GOP in the mixed layer using time-series $^{17}\Delta$ anomaly data; and (3) compare hourly/daily production with the same measured by in situ (FRRF) and in vivo (^{18}O spike incubation) techniques.

Triple oxygen isotopes systematics—The stable isotopes of natural oxygen, ^{16}O, ^{17}O, and ^{18}O, exist with atomic abundances of 99.758%, 0.038%, and 0.204%, respectively. The ratios among these three isotopes in the atmospheric O_2 depend primarily on the isotopic composition of photosynthetically produced O_2 and their fractionation during respiration. These biologically mediated processes fractionate O isotopes in a mass-dependent way such that ^{17}O enrichment is about half of ^{18}O relative to ^{16}O. On the contrary, photochemical reactions involving O_3, O_2, and CO_2 in the stratosphere fractionate O isotopes in mass-independent ways, and both ^{17}O and ^{18}O are equally

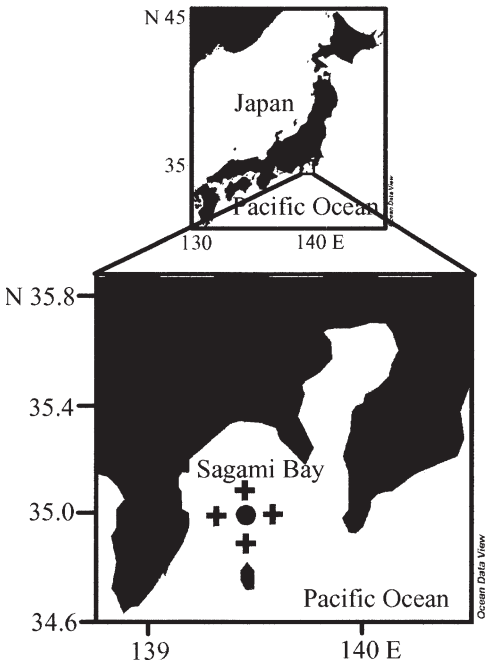

Fig. 1. The map shows the study region, Sagami Bay. The closed circle denotes time-series station S3; plus signs are neighboring stations surveyed to examine the influence of horizontal advection.

lowered (Thiemens 2001). Therefore, at any given $\delta^{18}O$ of O_2, which is the result of biological production and consumption, there is always an excess of ^{17}O in comparison to that produced by stratospheric processes. This excess $\delta^{17}O$ is called $^{17}\Delta$ anomaly, which varies from 0 to 249 mg^{-1} in the mixed layer (Luz and Barkan 2000). In the case of no biological activity, $^{17}\Delta$ anomaly in the mixed layer is equal to 16 mg^{-1}, instead of zero, due to the difference in the $\delta^{17}O/\delta^{18}O$ slope during gas exchange from that of other processes (Luz and Barkan 2000). Therefore, the influx of O_2 from the atmosphere reduces the magnitude of $^{17}\Delta$ of DO toward 16 mg^{-1}, which is the $^{17}\Delta$ of dissolved O_2 at equilibrium solubility, whereas in situ biological O_2 production increases to 249 mg^{-1}, which represents the value of photosynthetic O_2. Therefore, its value depends on the ratio of the rates of GOP and the influx of O_2 from the atmosphere. Thus, GOP can be estimated from $^{17}\Delta$ of DO, and influx of O_2, using the following equation (Luz and Barkan 2000):

$$\mathrm{GOP} = k\mathrm{Co}(^{17}\Delta - \Delta\mathrm{eq})/(\Delta\mathrm{max} - {}^{17}\Delta) \qquad (1)$$

where k is the piston velocity of O_2 (Wanninkhof 1992), Co is O_2 saturation concentration at in situ temperature and salinity (Garcia and Gordon 1992), Δeq is the $^{17}\Delta$ of dissolved O_2 at equilibrium solubility (16 ± 5 mg^{-1}), Δmax is the $^{17}\Delta$ of dissolved O_2 when affected only by photosynthesis and respiration (249 ± 15 mg^{-1}; Luz and Barkan 2000), and $^{17}\Delta$ is average mixed-layer $^{17}\Delta$ anomaly.

Study area—We studied the diurnal and daily variations of triple oxygen isotopes in the upper 40 m of the water

column in the Sagami Bay, central Japan, at fixed location S3 (35°N, 139.35°E; Fig. 1). The Sagami Bay is located in the east coast of Japan and is connected to the western North Pacific. The bay exchanges waters from low-saline Tokyo Bay and high-saline North Pacific. The bay waters show large seasonal variability in surface salinity due to advection of waters from the coastal regions, and it varies from 33 to 35 at the study site. The Kuroshio current has a strong influence on nutrient distribution in the Sagami Bay, and it runs along the east coast of Japan. The intrusion of the Kuroshio water into Sagami Bay is strongly related to the pattern of the mainstream path of the Kuroshio current. When the Kuroshio path is straight and parallel to the southern coasts of Honshu Island, the amount of pelagic water that flows into the bay is small and the coastal water mass dominates the surface water mass of the bay. On the other hand, when the Kuroshio meanders and flows north to Honshu Island, the pelagic water flows into the bay more strongly and dominates the surface water mass (Kawabe and Yoneno 1987). As a result, Chl *a* concentration shows large seasonal variations (Satoh et al. 2000). Phytoplankton bloom events, with relatively shorter timescales, were observed in the Sagami Bay by satellite images (Kanda et al. 2003) due to episodic winds. This study was conducted under the project entitled Profiling Ocean Primary Productivity Study (POPPS) under the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency.

Sampling technique

Water sampling and processing of sample—Sampling was carried out at station S3 in the Sagami Bay (Fig. 1) during 29 August 2003, 18–19 June 2004, and 01–02 August 2004 in the upper 40 m at 2-h intervals from 04:00 to 20:00 h. Seawater samples were collected with 5-liter Niskin bottles mounted to the Sea-Bird CTD rosette system. The water sampling methodology was adopted from Luz et al. (2002). In brief, ~150 mL of water sample was drawn into pre-evacuated gas extraction vessels (300 mL flasks with Louwers Hapert O-ring stopcocks) containing 250 μL of saturated $HgCl_2$ solution. The sampling bottle port was filled with distilled water and capped to avoid leakage during storage.

In order to extract dissolved gases in the seawater-to-gas phase, the sampling flasks were equilibrated for 24 h at room temperature. After equilibration, the water was carefully drawn out from the flasks to leave only headspace gases in the flask. The flask was then connected to a preparation system for separation of O_2 from N_2, Ar, CO_2, N_2O, and H_2O following Sarma et al. (2003).

Separation and purification of DO—DO was separated from other dissolved gases using a chromatographic column (8 m × 2 mm inside diameter) packed with 45/60 mesh 5 Å molecular sieve at −90°C. The separation was initiated by chilling the equilibrated sample flask to liquid nitrogen temperature to freeze CO_2, N_2O, and water in the vacuum line. Sample was then injected to the gas chromatography column using high-purity helium as a carrier gas. First Ar releases followed by O_2 and the latter were collected on a molecular sieve at liquid nitrogen temperature and was admitted into the dual-inlet of the mass spectrometer (Finnigan Delta Plus) for further analysis. Each reported value is the result of 10 analyses for each sample, and each analysis consisted of 10 comparisons between the reference and sample gases to obtain sufficient precision. The standard error of mean for $\delta^{17}O$ and $\delta^{18}O$ are 6 and 3 mg^{-1} and the computed $^{17}\Delta$ anomaly is 5 mg^{-1}, respectively.

Bottle incubations—Incubation experiments were performed for measuring in situ GOP at six depths in the upper 40 m (0, 5, 10, 15, 25, and 40 m). Two samples per depth were collected for initial $\delta^{18}O$ of dissolved O_2, and two samples per depth were spiked with 100 μL of 95 atom% enriched ^{18}O-enriched water (Cambridge Isotope Laboratories) and incubated for 24 h in the primary production array (Bender et al. 1987). After incubation, ~100 mL of subsample was drawn into pre-evacuated gas extraction vessels and capped as mentioned above.

Daily GOP was calculated from the isotopic composition of the spiked DO in initial and incubated samples using the following equation (Bender et al. 2000):

$$^{18}\text{O gross } O_2 \text{ production} = [\delta^{18}O(O_2)_f - \delta^{18}O(O_2)_i]/[\delta^{18}O_{water} - \delta^{18}O(O_2)_i] \times (O_2)_i$$

where the subscripts i and f refer to the isotopic composition of O_2 in initial and final samples, $(O_2)_i$ is the oxygen concentration of the initial water sample, and $\delta^{18}O_{water}$ is the isotopic composition of the spiked water. The CO_2 equilibration method was used for measuring $\delta^{18}O$ of spiked water samples with precision better than 0.1 mL. $(O_2)_i$ was determined by the Winkler method with precision of ±0.2 μmol kg^{-1}. Dark community respiration rates were measured from changes in the O_2 in triplicate initial and incubated in dark bottles for 24 h in the mooring array. The analytical precision, expressed as standard deviations, was ±0.07%.

Estimation of GOP using FRRF—The GOP measurements were carried out by FRRF (FASTtrack, Chelsea Technologies Group) using an underwater profiling buoy system and measured at 2-h intervals in the upper 40 m. The FRRF delivers a series of rapid high-frequency (200 kHz) flashes and enables the in situ measurements of the functional absorption cross section of Photosystem II (σ_{PSII}), the rate of photosynthetic electron transport and the level of photochemical quenching. Simultaneous measurements of photosynthetically active radiation (PAR) allow the estimation of GOP. The evolved O_2 was computed using the Kolber and Falkowski (1993) equation

$$P_{O2}{}^{B}(E) = \sigma_{PSII}\, \phi_{RC}\, q_p(E)\, \phi_e(E) f\, n_{PSII}\, E \qquad (2)$$

where $P_{O2}{}^{B}(E)$ is the rate of gross photosynthetic O_2 evolution per unit Chl *a* [mol O_2 evolved (g Chl *a*)$^{-1}$

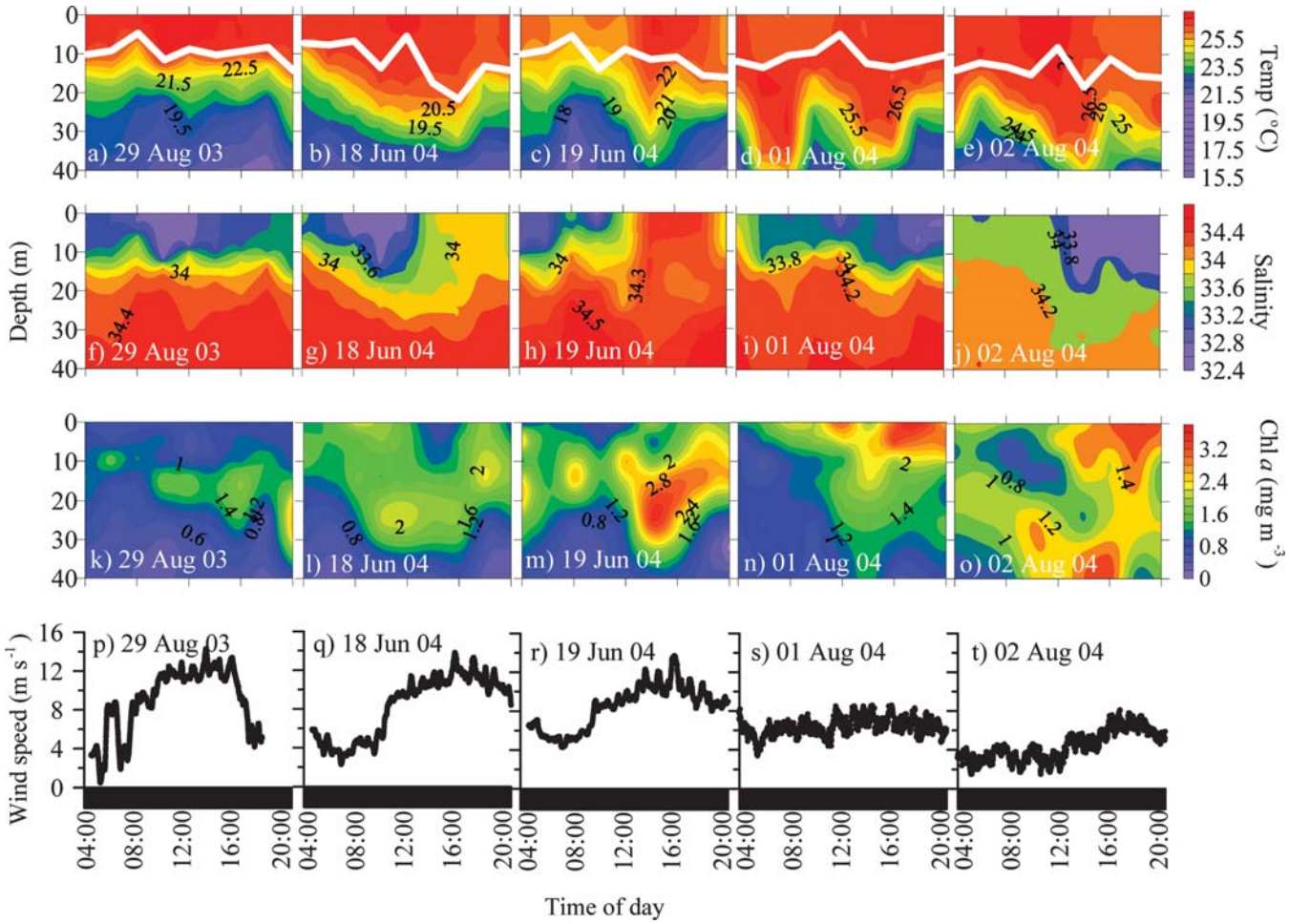

Fig. 2. Vertical distribution of temperature (a–e, first row); salinity (f–i, second row); Chl *a* (k–o, third row); and wind speed (p–t, bottom row) during study period in the Sagami Bay. The mixed-layer depth is shown in the top panel as a white line.

time^{-1}], ϕ_{RC} is the quantum yield of photochemistry within PSII, photochemical quenching (q_p) and quantum yield of electron ϕ_e) are irradiance-dependent, f is the fraction of PSII reaction centers that are capable of producing oxygen, and n_{PSII} is the ratio of PSII reaction centers to Chl *a*. Sarma et al. (2005) used an average n_{PSII} value of 1/500 suggested by Kolber and Falkowski (1993). Recently Suggett et al. (2004) suggested that it is species-dependent and varies; for instance, 1/200 for diatoms and dianoflagetes to 1/600 for Cyanobacteria (Suggett et al. 2004). The n_{PSII} value was derived according to Suggett et al. (2004) using phytoplankton composition by high-performance liquid chromatography. The derived n_{PSII} was found to vary from 1/360 to 1/420 during the Sagami Bay study period.

Results and discussion

Hydrography of the Sagami Bay during the study period—Figure 2a–t depicts diurnal variations in temperature, salinity, Chl *a*, and wind speed during the study period. Both mixed-layer temperature and salinity showed marginal variations, by <1°C and 0.4, respectively, during 29 August 2003 (Fig. 2a,f). On the other hand, mixed-layer Chl *a* was increased by 0.15 to 0.20 mg m^{-3} after 12:00 h compared with 04:00 h due to mixing of subsurface Chl *a* maximum with surface waters (Fig. 2k). This could possibly be the result of enhanced vertical mixing driven by increased wind speed (Fig. 2p). The wind speed increased from 1 to 3 m s^{-1} at 04:00 h to 13 m s^{-1} by 12:00 h (Fig. 2p). As a result of mixing, the mixed-layer depth (depth where increase in density by 0.125 was from that of the surface) was deepened from 6 to 13 m at the end of the day (20:00 h; Fig. 2a). All of these features strongly suggest that vertical mixing was significant in the afternoon during 29 August 2003. Low salinity warm waters were found between 08:00 and 10:00 h, which could possibly be the result of advection of water mass from the coastal region (Fig. 2f).

Physical and biological properties showed strong diurnal variability during 18 and 19 June 2004. Similar to 29 August 2003, mixed-layer temperature did not show significant diurnal variations (i.e., <0.5°C), whereas salinity increased from 33.1 to 34.0 from 04:00 to 20:00 h (Fig. 2b,g). The subsurface Chl *a* maximum, found

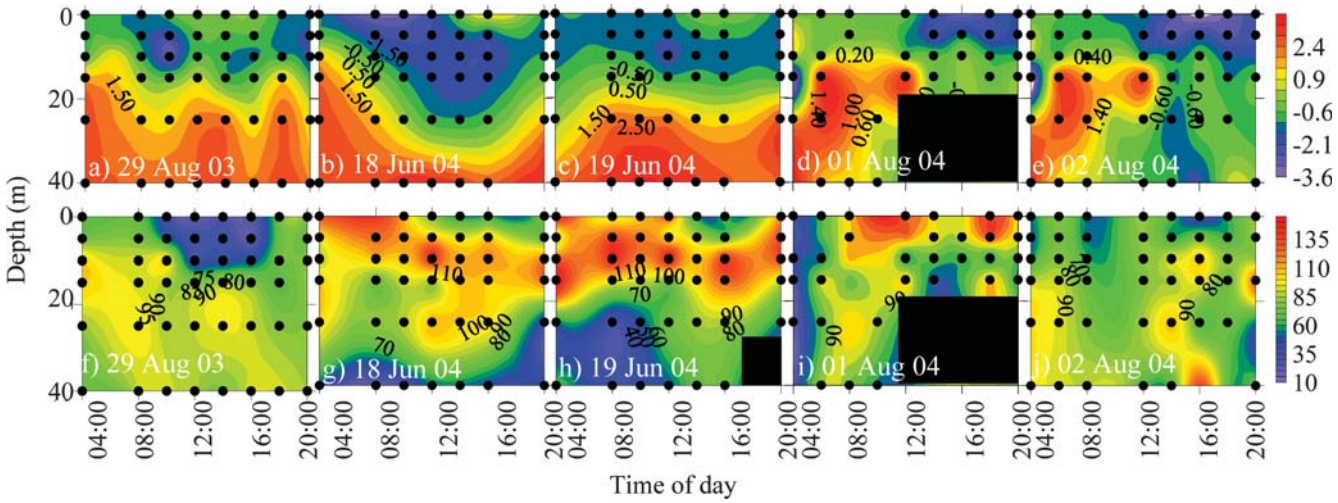

Fig. 3. Vertical distribution of $\delta^{18}O$ (mL^{-1}; a–e, first row) and $^{17}\Delta$ anomaly (mg^{-1}; f–i, bottom row) during study period in the Sagami Bay.

generally between 15 and 25 m depths, was shallowed at 18:00 h (Fig. 2l). These features are consistent with wind speed, which increased from 4 m s^{-1} at 04:00 h to 12 m s^{-1} at 16:00 h during 18 June 2004 (Fig. 2q). As a result, depth of the mixed layer deepened from 9 m at 04:00 h to 20 m at 16:00 h and then shallowed to 12 m by 20:00 h (Fig. 2b). The low salinity warm water patch, found between 08:00 to 12:00 h, was associated with high Chl *a* (~1.8 mg m^{-3}), suggesting advection of coastal waters to the study region (Fig. 2g,l). Similar features were found even on the next day (19 June 2004) as well. The mixed-layer temperature was uniform (21.6–22.5°C) on 19 June 2004, whereas salinity was also uniform until 10:00 h (33.3), then sharply increased to 34.4 (Fig. 2c,h). The depth of the mixed layer varied rapidly from 10 to 16 m and then increased toward end of the day (Fig. 2c). The wind speed structure was exactly the same as in the previous day (18 June 2004; i.e., it increased from 5 m s^{-1} at 04:00 h to 12 m s^{-1} at 14:00 to 20:00 h; Fig. 2r). Upward migration of subsurface Chl *a* can be seen at the end of the day during 19 June 2004 (Fig. 2m).

Unlike 29 August 2003 and 18–19 June 2004, water column structure was relatively stable during 01 and O_2 August 2004. Both mixed-layer temperature and salinity varied by 0.6°C and 0.5, respectively (Fig. 2d,e,i,j). On the contrary, mixed-layer depth was uniform (~12 m) during the entire day on 01 August 2004, except at 12:00 h when it was shallowed to ~6 m (Fig. 2d,e). Wind speeds were relatively stable, with an average wind speed of 6.5 m s^{-1} on 01 August, whereas it was ~3 m s^{-1} at 04:00 h and increased to ~7 m s^{-1} at the end of the day on 02 August 2004 (Fig. 2s,t). Mixed-layer Chl *a* increased sharply from <1.0 to ~1.8 mg m^{-3} at 20:00 h (Fig. 2n,o). The high mixed-layer Chl *a* is consistent with low-salinity waters.

DO concentrations show strong variability in the mixed layer. DO concentrations in the mixed layer were mostly super-saturated during 29 August 2003 (123–130%) and 18–19 June 2004 (112–142%), whereas they were close to saturation during 01–02 August 2004 (95–114%). Subsurface maximum in DO concentrations were observed during the entire study period at a depth of 10 to 20 m and was associated with subsurface Chl *a* maximum, suggesting the occurrence of a significant amount of production.

Short-term variations in triple oxygen isotope in the water column—$\delta^{18}O$ values were low in the surface and increased sharply to 40 m depth in the Sagami Bay during all sampling periods (Fig. 3). The smaller $\delta^{18}O$ in the surface waters were the result of the dominance of phytoplankton production compared with community respiration. On the other hand, air-sea exchange of oxygen equilibrated and was close to the atmospheric value (0 mg^{-1} relative to air; 1,000 mg^{-1} mL^{-1}). Thus, the value of $\delta^{18}O$ in the mixed layer mainly depends on three processes such as air-sea exchange, phytoplankton production, and respiration. Interestingly, mixed-layer $\delta^{18}O$ were close to the atmospheric value when wind speeds were >10 to 12 m s^{-1}, suggesting the dominant effect of air-sea exchange on oxygen isotopes in the mixed layer. Below the mixed layer, $\delta^{18}O$ was increased gradually to the depth and mainly resulted in dominant respiration (Fig. 3). During 29 August 2003, patches of smaller $\delta^{18}O$ were seen at 10:00 h and 14:00 h at 10 m depth (Fig. 3a), associated with high Chl *a* (Fig. 2k), suggesting higher subsurface production. Relatively smaller $\delta^{18}O$ were found during 18 June 2004 in the upper 30 m (Fig. 3b) compared with the other study period and could possibly be the result of higher phytoplankton production during the former period when high Chl *a* concentrations were observed (Fig. 2l). As a whole, the $\delta^{18}O$ vertical distribution pattern mimics Chl *a* distribution. However, inconsistency was also found during 19 June 2004 (Fig. 3c) when the highest subsurface Chl *a* (>3 mg m^{-3}; Fig. 2 m) was not associated with lower $\delta^{18}O$. However, lower values of $\delta^{18}O$ were found at 12:00 h when a sudden increase in subsurface Chl *a* (2.5 mg m^{-3}) was observed. Nevertheless, production need not always correlate with subsurface Chl *a* because intensity of light is also an important factor to determine production at these depths (Balch et al. 1992).

Relatively large $\delta^{18}O$ values were found during 01–02 August 2004 compared with the other study periods (Fig. 3d,e). This could possibly be due to three processes: (1) higher respiration than production, (2) mixing with deep waters, and (3) high air-sea exchange. The rate of dark community respiration in the mixed layer was higher ($\sim$78.2 mmol O_2 m^{-2} d^{-1}) during 01–02 August 2004 compared with that of other study periods ($\sim$61 mmol O_2 m^{-2} d^{-1}). The LD bottle incubation method suggested that net production was heterotrophic during 01–02 August 2004 (Dr. S. Hashimoto pers. comm.). This is also consistent with oxygen saturation levels, which were lower during 01–02 August 2004 (95–114%) compared with that of other study periods (112–142%). Mixing of waters from the subsurface, where relatively large $\delta^{18}O$ are seen, would significantly enrich mixed-layer $\delta^{18}O$. In order to examine this, the influence of vertical mixing was quantified following Sarma et al. (2005). These results suggest that vertical mixing had less influence during 01–02 August 2004 (by 20–40 mg^{-1} on $\delta^{18}O$) compared with the other study periods (50 to 200 mg^{-1} on $\delta^{18}O$) due to a relatively stable water column. In addition to this, the influx of oxygen equilibrated and brought it close to the atmospheric value. However, influx of oxygen was found to be lower during August 2004 (840 mmol O_2 m^{-2} d) compared with other periods (>1,200 mmol O_2 m^{-2} d). Therefore, enrichment in $\delta^{18}O$ during 01–02 August 2004 compared with other study periods could possibly be due to dominant respiration over production and low influx of oxygen from the atmosphere.

Short-term variations in $^{17}\Delta$ anomaly in the water column—The $^{17}\Delta$ anomaly is computed following Angert et al. (2003):

$$^{17}\Delta = \ln(^{17}R/^{17}R_{\text{ref}}) - 0.518 \ln(^{18}R/^{18}R_{\text{ref}}) \quad (5)$$

where

$$\ln(^{17}R/^{17}R_{\text{ref}}) = \ln(\delta^{17}O/1{,}000 + 1) \quad (6)$$

and

$$\ln(^{18}R/18R_{\text{ref}}) = \ln(\delta^{17}O/1{,}000 + 1) \quad (7)$$

where ^{17}R refers to the isotope ratio $^{17}O^{16}O/^{16}O_2$, ^{18}R refers to the isotope ratio $^{18}O^{16}O/^{16}O_2$, and the subscript "ref" stands for the reference. The constant 0.518 represents the slope of $\delta^{17}O$ versus $\delta^{18}O$ 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 (Luz and Barkan 2005).

The vertical distribution of the $^{17}\Delta$ anomaly is depicted in Fig. 3f–j. In contrast to Chl *a*, the $^{17}\Delta$ anomaly displayed mid-depth maximum in the Sagami Bay. The lower $^{17}\Delta$ anomaly at the surface was primarily due to the dilution by atmospheric $^{17}\Delta$ anomaly. The subsurface maximum in $^{17}\Delta$ anomaly is due to production of oxygen and reduced influence of atmospheric oxygen exchange (Sarma et al. 2005), which leads to accumulation of $^{17}\Delta$ anomaly in the subsurface layers. The mixed-layer $^{17}\Delta$ anomaly showed large diurnal variations during the entire study period. Both the distribution and variations in the mixed-layer $^{17}\Delta$ anomaly is largely controlled by physical processes, whereas subsurface $^{17}\Delta$ anomaly is influenced by biological processes in the Sagami Bay. For instance, during 29 August 2003, the mixed-layer $^{17}\Delta$ anomaly was $\sim$75 mg^{-1} at 04:00 h, then sharply decreased to $\sim$20 mg^{-1} at 12:00 h, followed by an increase to $\sim$70 mg^{-1} at 20:00 h (Fig. 3f). This distribution pattern is contrary to the phytoplankton production, as the peak in production generally occurs midday. The magnitude of $^{17}\Delta$ anomaly in the mixed layer depends mainly on influx of oxygen from the atmosphere and oxygen production by phytoplankton. These two processes drive $^{17}\Delta$ anomaly in the opposite directions (Luz and Barkan 2000; Sarma et al. 2005). As discussed above, wind speed, and thus influx of oxygen, was increased in the study region from 10:00 to 16:00 h during 29 August 2003 (Fig. 2p). As a result of the high flux of oxygen from the atmosphere, surface waters equilibrated with the atmosphere and led to a decrease in the $^{17}\Delta$ anomaly from 10:00 h to 16:00 h. The values of $\delta^{18}O$ were also close to the atmospheric value when wind speeds were higher than 10 m s^{-1} (Figs. 2p, 3a). This suggests that mixed-layer $^{17}\Delta$ anomaly is strongly governed by influx oxygen when wind speed is higher. On the contrary, the changes in $^{17}\Delta$ anomaly in the subsurface layers is associated with Chl *a* distribution (Fig. 2k). For instance, subsurface Chl *a* maximum (1.8 to >3 mg m^{-3}) during 18–19 June from 12:00 h to 20:00 h (Fig. 2lm) was associated with high $^{17}\Delta$ anomaly (Fig. 3g,h) and could possibly be due to higher production at this depth.

The average mixed-layer $^{17}\Delta$ anomaly showed an inverse relationship with wind speed (Fig. 4), especially during 29 August 2003 ($p = 0.001$) and 18–19 June 2004 ($p \leq 0.01$), which suggests significant influence of air-sea exchange of atmospheric oxygen. On the contrary, $^{17}\Delta$ anomaly showed no significant relation with wind speed during 01–02 August 2004 when wind speeds were relatively stable. This suggests that the influence of biological oxygen production overcame the air-sea exchange effect during 01–02 August 2004. It is unlikely to find one-to-one correlation between wind speed and $^{17}\Delta$ anomaly because of the influence of both oxygen production and air-sea exchange as they drive $^{17}\Delta$ anomaly in opposite directions. This relation is further complicated by horizontal advection of water masses with different $^{17}\Delta$ anomaly to the study region and vertical mixing with subsurface waters, which have higher $^{17}\Delta$ anomaly as the result of production and reduced air-sea exchange effect.

Influence of horizontal and vertical mixing on mixed layer $^{17}\Delta$ anomaly—Horizontal advection of water masses, either from high-productive coastal regions or low-productive open ocean regions, would strongly affect time-series variations in the $^{17}\Delta$ anomaly structure in the study region. The time-series temperature and salinity structure suggests that significant changes in the water mass structure especially during 18–19 June 2004. In order to examine gradients of $^{17}\Delta$ anomaly in the neighborhood of the study

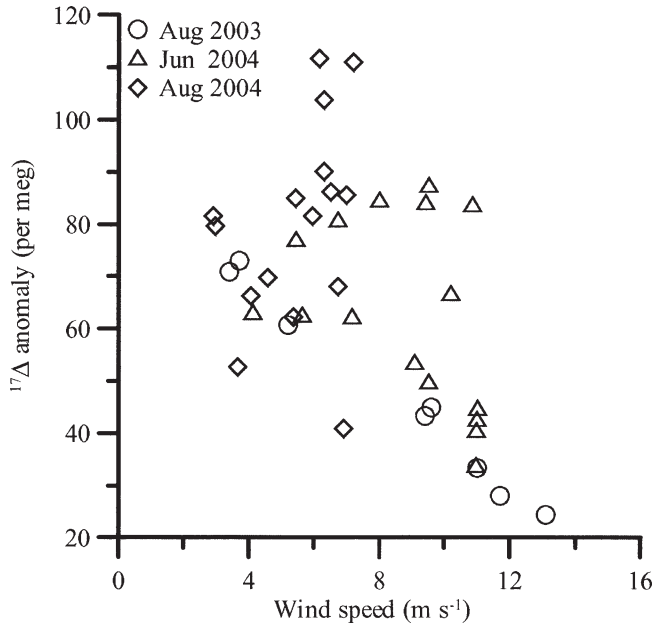

Fig. 4. Relationship between wind speed and $^{17}\Delta$ anomaly during different seasons in the Sagami Bay.

site, we have collected samples in four directions and 16.09 km away from our time-series station (Fig. 1). Fig. 5a–i depicts the temperature, salinity, and Chl *a* distribution at four stations during August 2003, June 2004, and August 2004. This survey was made at night in order to avoid the differences in $^{17}\Delta$ anomaly caused by diurnal variability driven by changes in productivity (Sarma et al. 2005). During the four-stations survey, wind speeds were relatively low (<5 m s^{-1}) and a shallow mixed layer ($\sim$8 m) was observed. The vertical distribution in temperature and salinity showed strong variability at four stations (Fig. 5). For instance, during August 2003, temperature in the mixed layer was uniform at 4 stations (Fig. 5a), whereas relatively lower salinity was found at an eastern station compared with the other three stations (Fig. 5b). The subsurface maximum in Chl *a* was evident at western and southern stations, and it was absent at the other two stations (Fig. 5c). The lowest DO concentrations were observed at the eastern station (data not shown). This further suggests that physical and biochemical properties are significantly different at eastern stations compared with the other three stations surrounding our time-series station (S3) during August 2003. The vertical distribution of $^{17}\Delta$ anomaly (Fig. 6a) suggests that the highest $^{17}\Delta$ anomaly was at the east station, whereas the variations in the average mixed-layer $^{17}\Delta$ anomaly at the other three stations were within ± 10 mg^{-1} during August 2003. Similar results were found during June and August 2004 as well (Fig. 6b,c), and this suggests that horizontal advection may not have a significant influence on mixed-layer $^{17}\Delta$ anomaly at the time-series station S3 under calm weather conditions (wind speed <5 m s^{-1}). However, at higher wind speeds (>10 m s^{-1}), which were encountered during the study period, the spatial variability in $^{17}\Delta$ anomaly was unknown. However, we assumed that the physical processes are uniform on the basin scale and that no corrections were made on our data for horizontal advection.

The vertical structure of $^{17}\Delta$ anomaly evidences occurrence of subsurface maximum in association with Chl *a* maximum in the study region due to phytoplankton production and reduced gas exchange (Sarma et al. 2005). Mixing of surface with subsurface waters would, therefore, increase the mixed-layer $^{17}\Delta$ anomaly. In order to examine this, changes in depth of mixed layer was monitored at 2-h intervals in the study region (Fig. 2a–e). This suggests that depth of the mixed layer varied from 6 to 13 m during August 2003, whereas it was 9 to 20 m and 6 to 15 m during June and August 2004, respectively (Fig. 2a–e). In order to account for the changes in mixed-layer $^{17}\Delta$ anomaly due to vertical mixing, the $^{17}\Delta$ anomaly modified as the result of vertical mixing was computed following the Sarma et al. (2005) equation

$$^{17}\Delta_{\mathrm{m}} = (^{17}\Delta_{\mathrm{d}} - {}^{17}\Delta_{\mathrm{s}}) \times \delta Z / Z_{\mathrm{m}} \qquad (8)$$

where $^{17}\Delta_{\mathrm{m}}$ is change in the $^{17}\Delta$ anomaly by mixing; $^{17}\Delta_{\mathrm{d}}$ and $^{17}\Delta_{\mathrm{s}}$ are the $^{17}\Delta$ anomaly below and above the mixed layer, respectively; δZ is the change in mixed-layer depth; and Z_{m} is the depth of the mixed layer. During the residence time of oxygen, if the mixed layer deepened, it was likely that $^{17}\Delta$ maximum would mix up with surface layers and could be quantified using Eq. 8. Based on this equation, the vertical mixing increased mixed-layer $^{17}\Delta$ anomaly by 5–18 mg^{-1} during the study period. Thus, influence of vertical mixing is very important on the mixed-layer $^{17}\Delta$ anomaly, and thus computation of GOP by phytoplankton.

Relationship of $^{17}\Delta$ anomaly with DO and Chl a—The $^{17}\Delta$ anomaly displayed positive relation with both DO and Chl *a* in the mixed layer during different seasons (Fig. 7a,b). Although these relations are significant ($p < 0.001$; $n = 35$), large scatter in the relation can be observed, which is mainly caused by several influencing parameters such as wind speeds, respiration, and grazing on the $^{17}\Delta$ anomaly, DO, and Chl *a*. For instance, although wind speeds did not vary significantly during August 2004, large scatter in the $^{17}\Delta$ anomaly was mainly caused by respiration. Respiration changes DO concentrations but has no effect on $^{17}\Delta$ anomaly because of mass dependent fractionation of triple oxygen isotopes (Sarma et al. 2005). Because the $^{17}\Delta$ anomaly relationship with DO and Chl *a* is controlled by more than one factor, better correlations than those observed are unlikely.

GOP by FRRF and comparison with $^{17}\Delta$ anomaly and ^{18}O incubation–based production—GOP by FRRF displayed strong diurnal variability in the upper 40 m with low production in the morning (06:00 h), and the peak in production was found at 12:00–14:00 h and then decreased in the evening (18:00 h) during the entire study period (Fig. 8a–e). This pattern is consistent with the PAR, which showed peak between 12:00 h and 14:00 h (data not shown). The depth of the euphotic zone (at a depth where

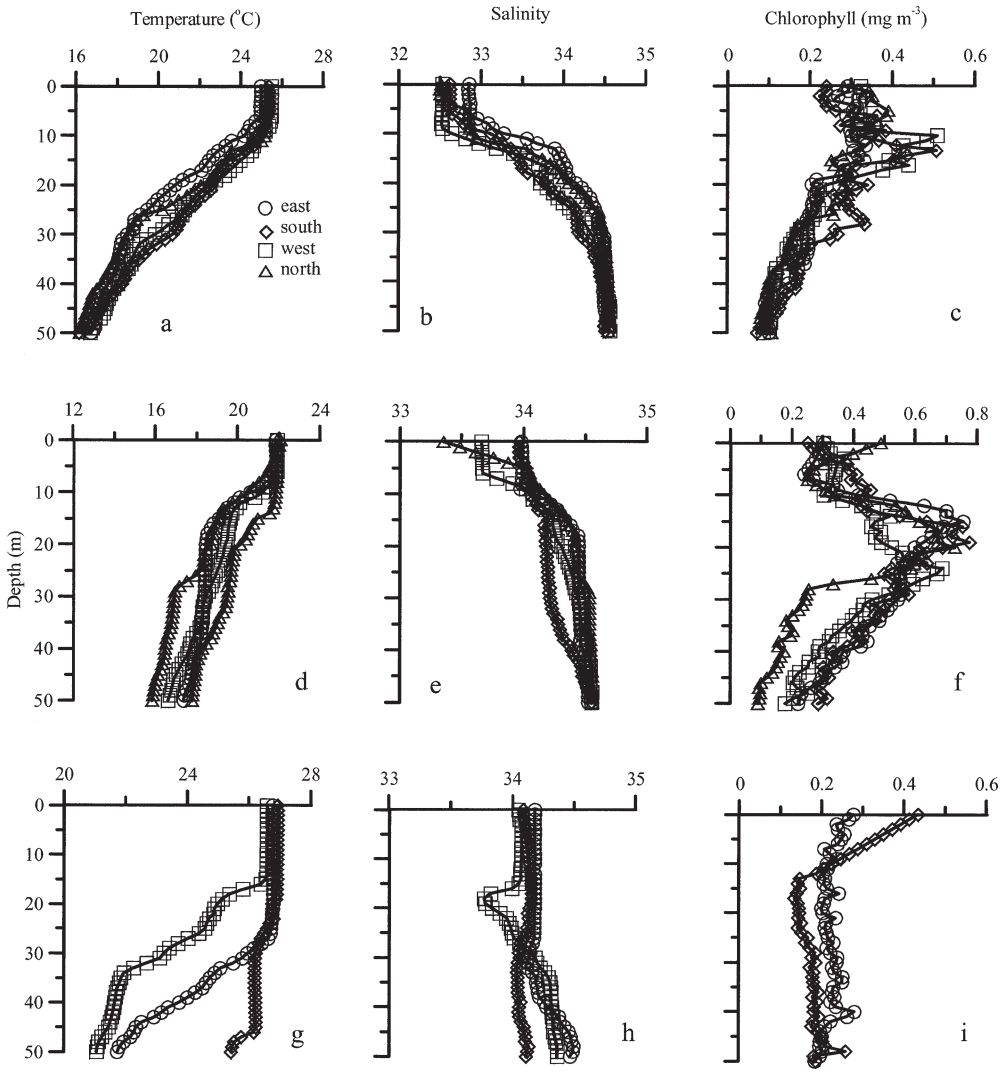

Fig. 5. Vertical distribution of temperature, salinity, and Chl *a* (mg m^{-3}) at eastern, western, northern, and southern stations surrounding the time-series station S3 in the Sagami Bay during (a) August 2003, (b) June 2004, and (c) August 2004.

~1% of PAR reaches) is relatively deeper during August 2003 (40–47 m) compared with June and August 2004 (30–32 m). However, a significant amount of production occurs (>0.2 mmol O_2 m^{-3} h^{-1}) in the upper 20 m in the Sagami Bay. The subsurface maximum in GOP occurs in the Sagami Bay (Fig. 8a–e), and it is consistent with the $^{17}\Delta$ anomaly (Fig. 3f–j) and Chl *a* maxima (Fig. 2k–o). Relatively higher GOP was observed during 19 June 2004 and was consistent with Chl *a* distribution. On the contrary, the lowest production was found during 29 August 2003 and 01–02 August 2004, and no obvious relation was found with Chl *a* distribution, suggesting GOP in the study region is governed by other factors than Chl *a* concentrations. The variations in daily-integrated production were found to be consistent with integrated PAR over daylight period. For instance, the higher mixed-layer GOP was found on 19 June 2004 (180.3 mmol O_2 m^{-2} d^{-1}) when PAR was the highest (12.7×10^4 μW m^{-2}), whereas the lowest GOP (68.6 and 82.1 mmol O_2 m^{-2} d^{-1}) and PAR (10.5 and 10.6×10^4 μW m^{-2}) was found during 29 August 2003 and 02 August 2004, respectively, in the Sagami Bay. Daily variability in mixed-layer production was also strongly related to the intensity of PAR. For instance, relatively lower GOP (125.8 mmol O_2 m^{-2} d^{-1}) and PAR (11.7×10^4 μW m^{-2}) was found during 18 June compared with 19 June 2004 (180.3 mmol O_2 m^{-2} d^{-1} and 12.8×10^4 μW m^{-2}). This suggests that the intensity of light has a strong influence of mixed-layer GOP in the study region.

In order to compare measured GOP by FRRF with the $^{17}\Delta$ anomaly-based technique, mixed-layer $^{17}\Delta$ anomaly, collected at 2-h intervals, were converted to the GOP using the Luz and Barkan (2000) model (Eq. 1) after applying corrections for vertical mixing (Fig. 8). The influx of oxygen from atmosphere through air-water interface is one of the important factors in determining mixed-layer GOP using the $^{17}\Delta$ anomaly. In order to make a precise estimation of influx of oxygen, sea surface temperature,

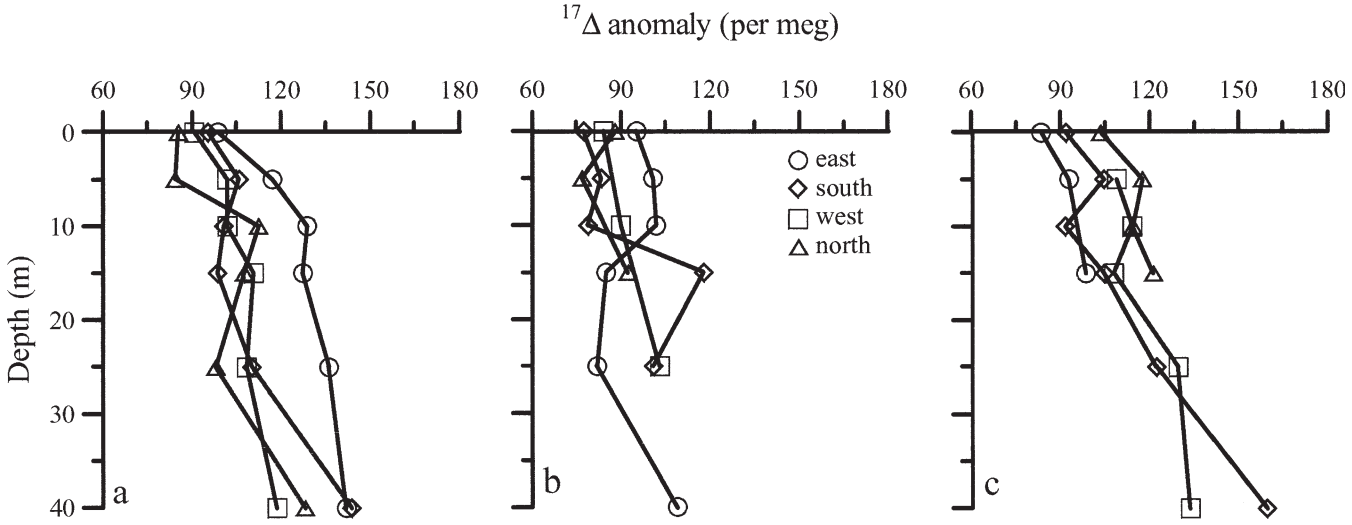

Fig. 6. Vertical distribution of $^{17}\Delta$ anomaly (mg^{-1}) at the eastern, western, northern, and southern stations surrounding the time-series station S3 in the Sagami Bay during (a) August 2003, (b) June 2004, and (c) August 2004.

salinity, DO, and dissolved nitrogen gas concentrations were measured at 5-min intervals using a gas tension device-CTD couple (McNeil et al. 1995) during the entire study period. Based on piston velocities, computed using the formulation of Wanninkhof (1992), and oxygen saturation concentrations, estimated following Garcia and Gordon (1999) equations, influx of oxygen was computed. Hourly average flux data were used to estimate hourly GOP in the Sagami Bay. We then compared GOP estimated from the $^{17}\Delta$ anomaly with that obtained in situ within the same water mass by FRRF during the day (Fig. 8f–j).

The comparison of hourly scale GOP by $^{17}\Delta$ anomaly and FRRF are possible if residence time of DO in the mixed layer is short (i.e., <2 d), as the former method stores past productivity signal. Therefore, it may be possible to examine diurnal variability in GOP based on $^{17}\Delta$ anomaly at higher wind speed (>8 m s^{-1}) when residence time is shorter.

Figure 8f–j shows that GOP estimated by mixed-layer $^{17}\Delta$ anomaly, after vertical advection correction, agreed with each other in the Sagami Bay during different seasons. Diurnal variations in productivity are well represented by the $^{17}\Delta$ anomaly–based productions as well. However,

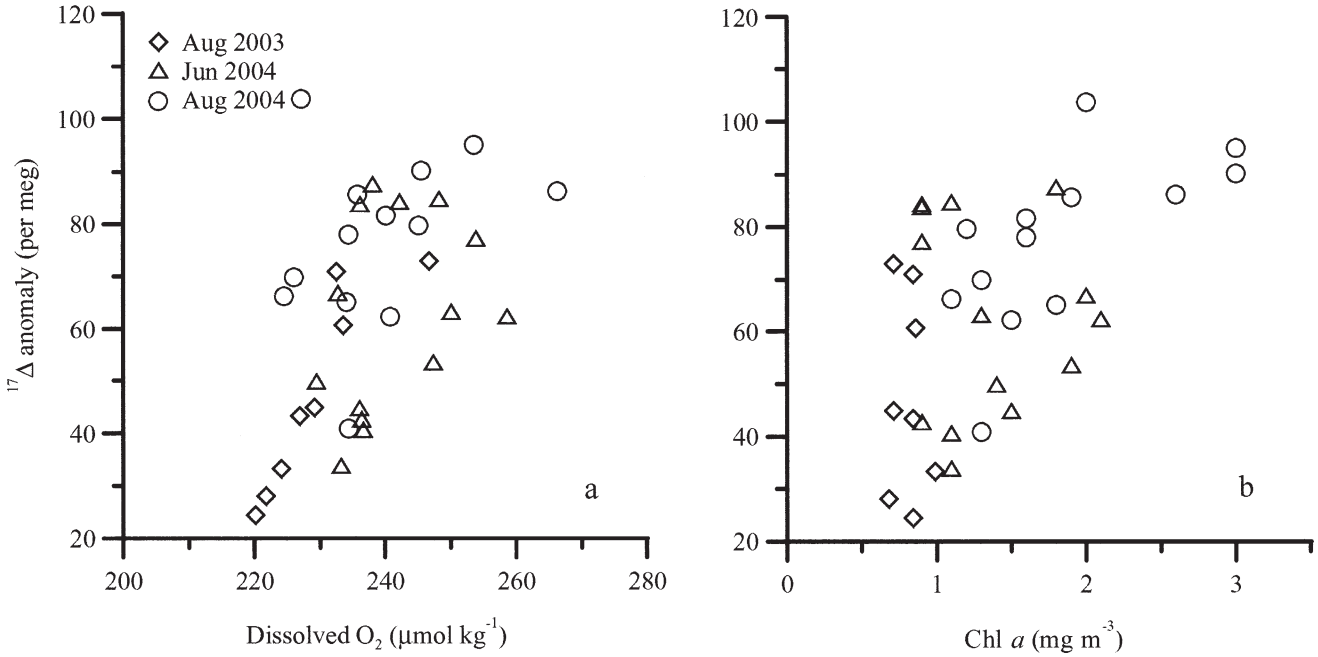

Fig. 7. Relationship of $^{17}\Delta$ anomaly with (a) dissolved oxygen and (b) Chl *a* in the Sagami Bay during different months.

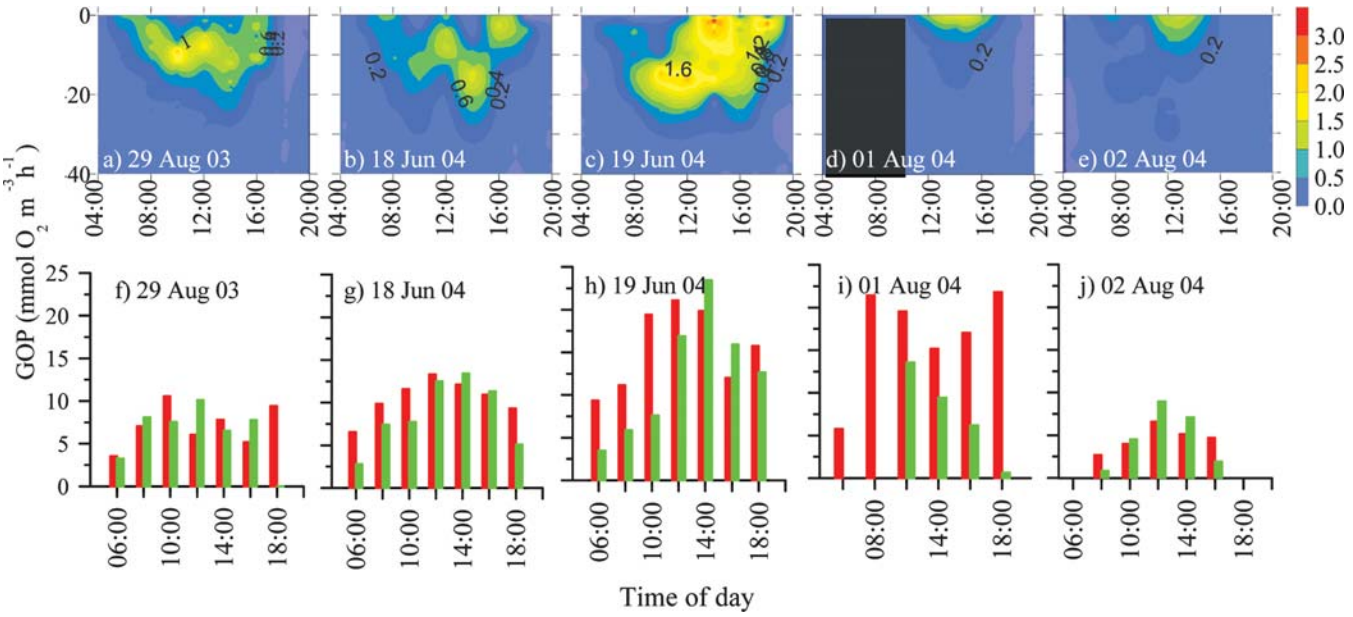

Fig. 8. (a–e) Gross oxygen production measured by FRRF at 2-h intervals and (f–i) comparison of GOP by $^{17}\Delta$ anomaly (red bars) and FRRF (green bars) in the Sagami Bay during different months.

relatively higher production rates were found by $^{17}\Delta$ anomaly–based productions in the early and late daylight periods compared with FRRF-measured GOP during the entire study period. This was mainly caused by storing of average production signal in the $^{17}\Delta$ anomaly over residence time of oxygen in the mixed layer (Luz and Barkan 2000; Sarma et al. 2005). The residence time of oxygen in the mixed layer mainly depends on air-sea flux of oxygen, which is again a function of wind speeds and gross oxygen production. With the exception of August 2004, wind speeds were relatively higher (6–12 m s^{-1}) during other study periods. Based on FRRF GOP and flux of oxygen to the mixed layer, the residence time of oxygen was found to be ~1 to 1.5 d during 29 August 2003 and June 2004, whereas it was ~3 d during August 2004. Thus, the influence of production occurring during the previous day on the $^{17}\Delta$ anomaly is possible. Therefore, small deviations in GOP based on $^{17}\Delta$ anomaly from those obtained from FRRF are likely. However, these differences are not significant, based on two-way analysis of variance (ANOVA), because of large errors associated with the $^{17}\Delta$ anomaly (~28–35%) and FRRF measurements (~20%). Although the previous day's signal influences the triple isotope estimate of GOP but not that obtained by FRRF, over the long term these two measures should still give the same answer. Nevertheless, the daily production by $^{17}\Delta$ anomaly-based, in situ FRRF-measured production and in vivo incubation, obtained by incubation of ^{18}O–enriched water, agree well with one another in the Sagami Bay during different seasons (Table 1). On the contrary, GOP by the ^{18}O spike incubation method showed relatively higher production by 40 mmol O_2 m^{-2} d^{-1} during 18 June 2004, which could be due to large variations in water mass structure (Fig. 2b,g). As ^{18}O incubation technique yields the GOP of the water mass sampled whereas FRRF measures GOP at the location of the instrument moored, these differences are likely. Moreover, small variations among the methods are expected due to associated large errors in the $^{17}\Delta$ anomaly-based technique and uncertainties in the piston velocity of oxygen computations and assumptions involved in the FRRF technique. Therefore, this study suggests that it is possible to evaluate short-term to daily-integrated GOP rates using $^{17}\Delta$ anomaly in the mixed layer when wind speeds are higher than 8 m s^{-1}.

Triple oxygen isotopes showed strong diurnal and daily variability in the Sagami Bay. Vertical mixing of O_2 from deep waters seems to be significant and should be corrected for accurate rates of GOP to be obtained. The influence of horizontal advection on $^{17}\Delta$ anomaly is found to be negligible in the Sagami Bay. GOP estimated on an hourly

Table 1. Comparison of daily mixed-layer production by different methods in the Sagami Bay (mmol O_2 m^{-2} d^{-1}).

Date	GOP by $^{17}\Delta$ anomaly	GOP by FRRF	GOP by ^{18}O spike
29 Aug 03	80.4±24	68.6±14	72.2±0.8
18 Jun 04	147.5±44	125.8±19	169.3±2
19 Jun 04	217.4±65	180.3±34	———
01 Aug 04	100.8±30	———	116.0±1.5
02 Aug 04	92.6±27	82.1±16	78.5±0.8

basis on the $^{17}\Delta$ anomaly was relatively higher than that obtained by FRRF because of storing of past productivity signal in the former technique. Daily integrated production by $^{17}\Delta$ anomaly, FRRF, and ^{18}O spike incubation techniques showed good agreement with one another within the errors involved in the techniques. This suggests that hourly to daily scale GOP can be estimated using $^{17}\Delta$ anomaly in the mixed layer.

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